The impact of heavy metal contamination on the morphology and physiology of small mammals

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Declaration

I declare that this Dissertation, “The impact of heavy metal contamination on the morphology and physiology of small mammals”, is my own, unaided work. It is being submitted for the Degree of Masters of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

Signature

_2nd_day of ___November___ 2018 in __Johannesburg__

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Abstract

Anthropogenic changes have resulted in a number of natural ecosystems being destroyed. One of the most destructive industries worldwide is mining, as it is responsible for habitat fragmentation, biodiversity loss, acid mine drainage and land use change, amongst others. While several authors have studied the interaction between heavy metals and plants, as well as larger animal species, only a few have investigated the effects of heavy metals on small mammals, particularly not in South Africa. The aim of this study was to investigate the impacts of heavy metal contamination on the morphology and physiology of small mammals in the Krugersdorp Game Reserve. Four sites within the reserve were sampled between September 2016 and January 2017 and heavy metals (Fe, U, As, Cd, Cu, Pb, Zn, Ni) known to occur in the reserve at high levels due to the nearby mine formed the basis for all heavy metal testing. Soil and vegetation samples were taken from each site and tested for heavy metal accumulation using ICP-OES. Rodents (in particular, *Mastomys natalensis*), were trapped using Sherman live traps and morphological measurements (body mass, body length, tail length and hind foot length) were taken for each trapped individual. Fifteen *Mastomys* individuals were euthanased and dissected to determine heavy metal concentrations in the spleens, livers and kidneys of these animals using ICP-OES. A number of heavy metals in the soil and vegetation exceeded recommended daily exposure levels for mammals and humans. No significant differences were found when adult morphological characteristics were compared across the sites in the reserve. Body length in *Mastomys* juveniles was significantly negatively correlated with all heavy metals measured in the soil and the vegetation in the reserve (U, As, Pb and Zn), except for Fe in the soil and Cu and Fe in the vegetation, which were positively correlated with the body length of juveniles. The same trend was found for body mass in the juveniles, with Pb in the vegetation also showing a positive correlation with body mass. The average morphological features of adult *Mastomys* individuals in this study were lower than the average body mass, body length, tail length and hind foot length of individuals not exposed to heavy metals contamination. No significant relationships were found between heavy metal concentrations in the soil and vegetation and the organs of the dissected individuals. Patterns indicating a link between high levels of heavy metals in the vegetation and high levels in the spleens of *Mastomys* individuals, as well as high levels of heavy metals in the soil and high levels in the kidneys and livers suggest that dietary intake and direct skin contact may be resulting in the bioaccumulation of heavy metals in the tissues of the rodents studied. High levels (above recommended maximum exposure limits) of heavy metals in the vegetation and soil, as well as physical impacts to internal organs of studied *Mastomys* individuals suggest that rodents in the reserve may be at risk of liver and kidney disease and necrosis. Although a limited data set was available for this study, based on the results obtained and comparison to other literature, it can be suggested that small mammals within the Krugersdorp Game Reserve are at risk of heavy metal toxicity and a number of associated diseases.

Keywords: Heavy metal contamination, mining, small mammals, rodents, morphology

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CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

Human activities severely affect animals in a multitude of ways. Habitat loss or degradation, resource depletion and utilisation for activities such as trophy hunting or traditional medicinal practices are just some of the threats to wildlife (Ripple *et al.* 2014). Rapid deforestation and the fragmentation of pristine areas of land lead to a great loss of biodiversity and sometimes even the extinction of a number of species (Gibson *et al.* 2013). The expansion of urban areas in the form of commercial, industrial and housing developments has resulted in disturbance to a number of plant and animal communities. The building of roads in relatively pristine areas, or in areas bordering natural sites often results in significant habitat disruption for wildlife and an overexploitation of natural resources, as the surrounding land is transformed through development (Laurance *et al.* 2014).

One industry that contributes significantly to land transformation and development is mining. Disturbances to land surrounding mines include the encroachment of the mine onto buffer zones near rivers or protected areas of land (Farrington 2005). This type of encroachment can affect the distribution and migration patterns of a number of species living near the site. Some avian species may also be affected, for example golden eagles (*Aquila chrysaetos*) and turkey vultures (*Cathartes aura*) in the northern hemisphere, which may be directly impacted by mountaintop removal mining as it changes the habitats directly below their flight paths. The land-use change caused by mountaintop removal mining and development can have a direct impact on the thermal uplift, which these birds use to assist them when flying (Bohrer *et al.* 2012).

Although a number of these examples exist, many countries are still dependent on mining to fuel their economies. Conflicts between mines and protected areas in several countries have increased, with the realisation that the costs to repair ecological damage caused by mining activities will likely exceed the profits made by the mines themselves (Farrington 2005).

1.1 Impacts of human activities on the environment

Human population growth and the consequent increased levels of urbanisation have resulted in many land use changes around the world (Nagendra *et al.* 2013). Habitat loss, reduction of patch sizes, increased distances between home ranges and the formation of new habitats may all result from anthropogenic changes to the natural environment, such as the conversion of pristine land for agricultural use (Kasso and Bekele 2014). Pollution is another significant factor resulting from human activities. These pollutants may affect the structure, composition and function of entire ecosystems through shifts in dominant plant species or an altered vegetation structure (Grimm *et al.* 2008).

Fragmentation of natural ecosystems often occurs when urban developments, parks and roads are established in once pristine areas (Garden *et al.* 2010). Many species are lost with the continued alterations of pristine land for agriculture and industry. These activities have resulted in habitat fragmentation, and subsequently a high level of biological erosion (Pichancourt *et al.* 2006). Other human disturbances, such as the introduction of cars and loud noises, may also have negative effects on animals, as they may respond to these disturbances as they would to a predator. This behaviour in turn redirects time and energy from fitness-enhancing actions such as feeding, parental care or mating displays (Frid and Dill 2002).

Habitat destruction is considered to be one of the most influential drivers of species extinction worldwide (Pimm and Raven 2000) and occurs when a natural habitat is altered so much that it is no longer able to support the species that originally inhabited the land (Laurance 2010). The process of habitat fragmentation or loss often destroys or displaces multiple plant and animal populations. This is often due to the lower genetic diversity and limited gene flow caused by an inability to move between different habitats (Frankham *et al*. 2010; Sato *et al.* 2014). Lower genetic diversity can result in inbreeding of populations as well as reduced immunology and evolutionary potential of the population, all of which contribute to a high risk of extinction (Lacy 1997; Frankham *et al*. 2010; Sato *et al.* 2014).

While the abovementioned anthropogenic changes all result in harm to the environment, mining activities and processes pose perhaps the greatest risk to biota, as mining results in much of the destruction mentioned (Schueler *et al.* 2011). Habitat degradation, deforestation, fragmentation and pollution are just some examples of the negative impacts mining has on the environment (Weiersbye and Witkowski 2007; Tutu *et al.* 2008). Damage to plants and animals due to mining activities is of particular concern due to the interconnectedness of food webs and the relationships of different species to one another (Dudka and Adriano 1997; Yule *et al.* 2010).

1.2 Impacts of mining on the environment

There are multiple mining processes in use today, with different methods being used based largely on which mineral is being mined (Hustrulid 1982; Durucan *et al.* 2006). Gold mining, one of the most common mining processes in South Africa, has changed over the years and each method has brought a number of environmental challenges with it. Initially, gold was extracted from coarsely crushed ore using mercury, and the remaining waste was deposited in large sand dumps (Tutu *et al.* 2008). This method lead to high levels of mercury in plants, soils, animals and humans, which resulted in changes in fish alimentary habits as well as human health impacts such as kidney and neurological damage (Malm 1998). When pyritic ore was encountered, this process became inefficient and was replaced by cyanide extraction, which requires finer milling and results in tailings (waste) being pumped to slimes dumps (Tutu *et al.* 2008). One source of pollution often occurs when the buffering capacity of the tailings is insufficient to neutralise the acid and acidification of the water occurs. This is known as acid mine drainage (AMD) and can result in increased mobility and toxicity of heavy metals from the mining process (Webster *et al*., 1994; McGregor *et al*., 1998; Tutu *et al.* 2008). Other primary sources of AMD include the leaching and seepage of heavy metals from overburdened rehabilitation facilities, underground and open-cast mining workings and discharged underground water flowing through mines (Akcil and Koldas 2006). These sources also result in the mobility of heavy metals and pollution of groundwater surrounding mines (Akcil and Koldas 2006; Tutu *et al.* 2008).

Mining is a destructive process that generally results in high levels of ecological damage (Chauhan 2010). For example, mining activities in India have resulted in deforestation, habitat destruction and biodiversity loss (Chauhan 2010). In Alberta, Canada, open-pit sand mining at one site resulted in the destruction of more than 29500 hectares of peatland habitat (Rooney *et al.* 2012). In Ghana, small mammals such as the Allen's wood mouse (*Hylomyscus alleni*), Tullberg's soft-furred mouse (*Praomys tullbergi*), Cansdale's swamp rat (*Malacomys cansdalei*) and Temminck's striped mouse (*Hybomys trivirgatus*) were reported to be negatively affected by mining, as species richness and diversity were low after the rehabilitation of altered habitats (Attuquayefio *et al.* 2017). Unfortunately, as many environmental factors have not been quantified, it is difficult to provide true estimates of biodiversity and habitat loss (Rooney *et al.* 2012).

Changes in land cover due to mining activities have been shown to result in the destruction of a number of environmental features (including vegetation and microhabitats) and increase the risk of deforestation, erosion of ecosystems, agricultural intensification and land degradation (Schueler *et al.* 2011). Mining activities that occur near protected areas are known to disturb threatened species, destroy ecosystems and disrupt animal migration routes (Farrington 2005). Larger mammals are often more visibly affected by mining activities, with migratory behaviour and survival rates in areas near mines differing significantly from those observed in more undisturbed landscapes (Blum 2014). For example, mule deer (*Odocoileus hemionus*) individuals in Nevada that have to make use of habitat that has been disturbed by mining activities often expend more energy trying to find migratory routes that are less disturbed than they would in undisturbed habitat (Blum 2014).

While these damages occur during and after the mining process, a number of threats to the environment exist purely due to the presence of mines. For example, large scale rock deposits and wastelands are dangerous for animals due to the increased risk of landslides and dust erosion which are harmful to the health of animals inhabiting the site (Broemme and Stolpe 2007). Additionally, deep pits that are created for excavation often trap or kill wild animals that fall in (Eisler and Wiemeyer 2004). Habitat destruction, land use change and deforestation are some other negative effects caused by the establishment of mines (Tutu *et al.* 2008; Maya *et al.* 2015). Alterations to natural environments for mining also results in the loss of species due to restrictions in gene flow and death by vehicles and heavy machinery on site (Spreadbury *et al.* 1996).

1.3 The South African Mining Industry

George Harrison's discovery of the Main Reef in 1886, which led to the gold rush in South Africa, is considered to be the beginning of mining in South Africa (Adler *et al.* 2007; Tutu *et al.* 2008; SAHO 2011). The West Rand region of Gauteng has hosted a large number of mines since then (Durand 2012; Lusilao-Makiese *et al.* 2013). The discovery of gold in Johannesburg quickly led to the Witwatersrand becoming the most densely populated region of South Africa (Durand 2012). This led to the establishment of a number of mines, industries and settlements in the area, most of which lacked the potential for long-term sustainability in terms of factors such as water availability for industrial use, drinking and sanitation (Durand 2012). It has been estimated that 105 million tonnes of mine waste have been generated per annum by gold mining activities in the Witwatersrand Basin, which includes more than 270 tailings storage facilities covering a surface area of approximately 400 km^2 (AngloGold Ashanti 2004; Oelofse *et al.* 2007).

While South Africa is most famous for its gold mining, it also mines large quantities of coal, diamonds, platinum, palladium and chromium, to name a few (Chamber of Mines of South Africa 2013). All of these processes produce a number of by-products, although not all carry the same impacts or risks of contamination (Maya *et al.* 2015). Gold mines in particular are associated with a significant number of wildlife deaths, as cyanide-bearing mine waste solutions and tailings from these gold mines are thought to result in death by cyanide toxicity (Donato *et al.* 2007).

In the gold mining process, tailings storage facilities (TSFs) are used to store diluted sodium cyanide, with the aim of minimising environmental and social impacts and aiding in the water recovery process (Lötter 2005). Unfortunately, flooding, degradation and failure of these storage facilities often causes leaching of heavy metals into surface and groundwater, causing large-scale environmental degradation (Bempah *et al.* 2013). Degradation of TSFs is sometimes caused by the oxidation of sulphide minerals in mine tailings, which in turn triggers the release of arsenic and other sulphide-bearing minerals such as pyrite into the surrounding soil and groundwater (Kim *et al*. 2000; Lim *et al.* 2009; Bempah *et al.* 2013).

Different types of materials, including native gold, uranium oxides and sulphide minerals, are contained within the gold-bearing reefs (Naicker *et al.* 2003). An abundance of pyrite, which is the greatest contributor to AMD, can also be found in many of these reefs (Naicker *et al.* 2003). Pyrite is an iron sulphide that is present in most of the waste rock found in the hardrock mining process, and is therefore found in large quantities in many areas surrounding mines (Roberts 2016). Although the pyrite-containing waste rock is sometimes left in large chunks, the rock is most often milled down into a fine powder, which is known as mine tailings (Roberts 2016).

Environmental deterioration caused by mining is extremely common due to the large amounts of waste produced (Kitula 2006). Despite the 1990s seeing the cessation of all underground mining activities in the area, including the pumping of water from the mine voids in the West Rand Goldfield (Coetzee *et al.* 2009), the shallow ground water in the Witwatersrand basin has been contaminated with heavy metals and mining waste (Weiersbye and Witkowski 2007).

1.4 Effects of mining related pollution on the environment

Of all impacts, pollution is possibly the most significant, as it can have far reaching effects, is often not easily detected, and may have consequences that are unknown (Duruibe *et al.* 2007; Adham *et al.* 2011). While pollution encompasses a large number of harmful processes and categories, AMD is perhaps the most well-known and possibly most dangerous consequence of mining activities. As reactions that occur in the AMD process are self-sustaining, pollution of water sources around mines occurs over the long term (Pulles 2003; Tutu 2012). In South Africa, AMD is considered to be the single most significant threat to the environment (Younger 2001; Ochieng *et al.* 2010). This is because it is the largest source of pollution and is responsible for a significant portion of environmental and socio-economic damage (Oelofse *et al.* 2007). Human and animal health risks are key examples of this, as are political unrest and unemployment due to mine closures (Adler and Rascher 2007; Oelofse *et al.* 2007), as the rebound of water levels after the closure of mines can lead to the discharge of contaminated groundwater (Johnson and Hallberg 2005). This is due to the fact that a number of freshwater resources and systems in the country are contaminated by AMD, which limits the amount of usable water available for domestic, agricultural, and industrial practices (Wamsley and Mazury 1999; Younger 2001).

Heavy metals are found in a number of places, including soils, water, vegetation and sediment (Glasby and Szefer 1998; Szefer *et al.* 1998; Dube *et al.* 2001). Processes such as acid rain, leaching, weathering and flooding can all transport heavy metals through the environment (Dube *et al.* 2001; Fujimori and Takigami 2014). While these and other anthropogenic processes are responsible for a large amount of heavy metal pollution in the environment, mining is considered to be one of the most significant contributors to heavy metal pollution worldwide (Liu *et al*. 2005; Fryer *et al.* 2006; Acosta *et al.* 2011; Li *et al.* 2014). Mine tailings from storage facilities as well as AMD have been identified as some of the most destructive pollutants to the environment (Abdul-Wahab and Marikar 2012).

Many mining sites around the world are responsible for negative impacts on species richness, abundance and diversity through the release of heavy metals and metalloids such as cadmium, zinc, copper, lead and arsenic (Espinosa-Reyes *et al*. 2014). Heavy metal particles from mining activities, including smelting, are often absorbed by plants and soils in surrounding areas, and subsequently by animals (Beyer *et al.* 1985). Heavy metals can be toxic to animals when ingested, as bioreactions and bodily functions may be hindered when these metals bind to biomolecules within the animal's body (Duruibe *et al.* 2007). The corrosion and leaching of heavy metals to soil and groundwater can also result in the contaminations of soils and water bodies, along with the runoff from erosion of mine wastes and dusts produced during crude ore transportation (Nagajyoti *et al*. 2010).

Heavy metals are commonly introduced into the aquatic environment through mining effluent (Li *et al.* 2014; Yuan *et al.* 2015). Although less visible than other aquatic pollutants, their overall toxicity and ability to accumulate in aquatic organisms result in both intensive effects (those which are not affected by the amount of matter present) and extensive effects (effects which depend on the amount of matter present) on the ecosystem (Edem *et al.* 2008). These effects can cause extreme harm to wildlife through direct skin contact and absorption through food. For example, algal exposure to heavy metals in aquatic environments can result in cell damage, inhibition of photosynthesis and even death (Zhou *et al.* 2008). Protozoa, invertebrates and fish are subsequently affected by these heavy metals when they feed on the algae and organisms below them in the food chain (Zhou *et al.* 2008).

Heavy metals are present in the water and sediment of aquatic systems such as rivers, lakes and oceans (Singh *et al*. 2005; Schwarzenbach *et al.* 2006). In Montana, studies showed that the growth, survival rates and fecundity of fish in contaminated waters are negatively affected due to high levels of copper, zinc, cadmium, arsenic, iron, and lead in the water passing over the fish's gills during respiration and being absorbed into the system (Phillips and Lipton 1995; Neuman *et al.* 2014). The absorption of these heavy metal particles into the body resulted in slowed growth as well as lower fecundity and survival rates in the fish from contaminated waters compared to those from uncontaminated waters (Phillips and Lipton 1995; Neuman *et al.* 2014). The activity patterns and swimming abilities of a number of fish exposed to heavy metals have also been proven to be different from their unaffected counterparts (Lanctôt *et al.* 2016).

Mine water, which is often heavily polluted with heavy metals such as aluminium, zinc, iron, manganese, cobalt, and cadmium, often degrades the quality of many surface water sources and may have an impact on industrial, agricultural and domestic users (Wamsley and Mazury 1999; Ochieng *et al.* 2010). AMD pollution from mining activities often leads to very serious contamination of agricultural lands, as well as the disrupted growth of aquatic plants and animals (Ochieng *et al.* 2010). Aquatic biodiversity in areas surrounding contaminated mines could also decline significantly due to the accumulation of metals in soils and water bodies (Tarras-Wahlberg *et al.* 2001). This has happened in the Puyango River basin in southern Ecuador, where gold mining resulted in the discharge of cyanide, mercury and metal-rich tailings into the rivers, and subsequently resulted in various fish being exposed to toxic levels of heavy metals (Tarras-Wahlberg *et al.* 2001).

Pollution from tailings storage facilities is also a significant negative consequence of the mining process (Durand *et al.* 2010). Run-off from deposits and slag often has harmful effects on water, soil and sediment surrounding mines (Espinosa-Reyes *et al.* 2014). When mining takes place in an area, ecosystem service flows are disrupted as air pollution from dust or water pollution from runoff begin to develop (Schueler *et al.* 2011). The pollution of mining sites and surrounding areas can have detrimental effects to both humans and wildlife, as the toxic effects from heavy metals are generally extremely harmful (Schueler *et al.* 2011; Espinosa-Reyes *et al.* 2014).

Many urban and rural areas in countries such as South Africa are reliant on surface water from these bodies for agriculture and irrigation, among other uses (DWAF 2004). The distribution of heavy metals is often facilitated by acidified water because it pollutes lakes, rivers and streams and aids in the mobilisation of heavy metals. A number of contaminants and heavy metals are spread to crops and vegetation further away from the initial site of contamination through irrigation systems and natural flooding (Ochieng *et al.* 2010; Liao *et* *al.* 2016). These heavy metals are absorbed by the soil and vegetation, which subsequently results in animals and humans ingesting the (often dangerous) heavy metals, despite not being near the contaminated water source (Liao *et al.* 2016). This could result in illnesses associated with these toxic substances, causing harm to these animals and humans, as large amounts of heavy metals are known to have negative effects on many species (Boularbah *et al.* 2006; Ochieng *et al.* 2010).

Water bodies, particularly freshwater sources such as rivers and streams, are vital to the survival of a number of species, as they provide pathways for aquatic organisms to obtain energy (Shieh *et al.* 2002). Therefore, any pollution to these waters, such as the addition of heavy metals, could decrease primary production and subsequently have an impact on secondary production and trophic links (Yule *et al.* 2010). Through the process of releasing runoff into the environment, mining not only has impacts on natural vegetation surrounding the mine itself, but also increases the silt levels of affected dams and rivers. This affects the gills of fish and makes the habitat undesirable for species such as freshwater otters (Mason and Macdonald 1988). Even low heavy metal concentrations often found in mining effluent that is discharged into aquatic environments are toxic to fish and other animals in the food chain (Ellias 2009).

While concentrations of these heavy metals in the water can vary hourly, sediment usually maintains high concentrations of heavy metals for decades (Neuman *et al.* 2014). Sediment plays a number of important roles in the environment, including providing shelter and nutrients to microorganisms and supporting vegetation (Holguin *et al.* 2001). Many organisms also use sediment for shelter and hunting prey. As such, heavy metal contamination of sediments can result in potential risk to ecological factors, including water, species living in the contaminated environment and humans (Maanan *et al.* 2015). In estuaries, Bryan and Langston (1992) found that clams (*Scrobicularia plana*) living in contaminated sediment had accumulated heavy metals such as tin, copper and iron due to processes such as mobility of the heavy metals and the uptake of nutrients.

While severe environmental damage is often caused by mining pollution, some vegetation is able to grow in contaminated areas. A number of grass species, such as tufted grass (*Deschampsia cespitosa*) and redtop (*A. gigantea* Roth) have become heavy metal tolerant, but more often than not, large areas of land that have been exposed to mining activities, such as Sudbury in Ontario, Canada, remain barren with very little hope of producing any viable organisms (Dudka and Adriano 1997). The uptake and distribution of heavy metals in plants is species-dependent and controlled by different mechanisms (Kukier *et al*. 2004; Li *et al.* 2004; Bakkaus *et al.* 2005). For example, excessive accumulation of heavy metals can cause reduction in photosynthetic rates (Kitao *et al.* 1997), necrosis of leaves (Wu 1994) and reduced meristem mitotic activity due to their inability to remobilise contaminants through the phloem to other organs after reaching the leaves (Loneragan 1988; Nagajyoti 2010). It has been shown that the regeneration potential and predation rates of *Acacia* sp. are lower on heavy metal contaminated sites than uncontaminated sites. (Weiersbye and Witkowski, 2007).

Some plant species, including members of the Asteraceae family in South Africa, are capable of hyperaccumulation. This process is often seen in species living on or near contaminated land, and involves plants being able to store large amounts (usually approximately 1000 $\mu g \cdot g^{-1}$) of a particular heavy metal and thriving in these conditions (Turnau and Mesjasz-Przybylowicz 2003). In most cases, metals are accumulated in the shoots of such plants (Schwartz *et al.* 2001). While some plants such as those of the *Brassica* species are capable of removing large amounts of heavy metals such as zinc from the environment, damages such as reduced root and shoot dry weight, as well as leaf chlorosis often result when they are placed in areas of high contamination (Ebbs and Kochian 1997).

Mining sites are often dominated by grasses, legumes and shrubs, as these low height plants are capable of tolerating higher metal concentrations due to their ability to absorb nutrients present in contaminated soils (Anawar *et al*. 2013).

While heavy metal contamination from mining sources is most commonly associated with water pollution, many terrestrial organisms are negatively affected by this pollution (Koldas 2000). An example of this can be seen in a study by Sánchez-Chardi *et al.* (2009a), where greater white toothed shrews (*Crocidura russula*) in a nature reserve in Spain showed evidence of increased liver-body ratios, focal necrosis and signs of apoptosis in hepatocytes. This occurred after a spillage from a pyrite mine allowed acidic water carrying a number of heavy metals such as lead, cadmium, mercury, zinc, antimony and thallium to flow into a river and subsequently a protected wetland. A number of dangerous chemicals and heavy metals are disposed of in tailings storage facilities, and these substances often negatively affect surrounding plants and animals by poisoning cells and even resulting in death (Mudder and Botz 2004; Donato *et al.* 2007). The reproductive activity, including seed production, nutrient content, metal content, viability and germinability of vegetation surrounding mines is often negatively impacted by runoff and AMD (Weiersbye and Witkowski 2007). Higher levels of heavy metals, including manganese, iron, cobalt, nickel, copper and zinc, have been found in vegetation surrounding tailings storage facilities (Weiersbye and Witkowski 2003) and are extremely toxic to animals in the vicinity (Souren 2000; Sang and Li 2004).

Toxic wastes or tailings produced by mining activities often affect both aquatic and terrestrial organisms, and consequently entire ecosystems (Ekpo *et al.* 2013). Mammals that rely on fish for food are also affected by water contaminated with heavy metals such as arsenic, titanium, cadmium, and mercury, as these are toxic to fish (Mason and Macdonald 1988). Metals such as calcium, aluminium, iron, magnesium, manganese, copper, zinc, cobalt, nickel, uranium and lead are often found in environments near mines and are absorbed by organisms within the ecosystem (Durand 2012). Pollution from these heavy metals has also been found to limit both the range and the food supply of a number of species within the environment (Mason and McDonald 1988). Herbivores and plants are affected by this pollution, which results in an upheaval of food chains and ecosystems and in turn affects a number of other organisms such as lions, leopards, pumas and other large carnivores that have high food requirements and usually respond poorly to a loss of habitat through pollution and contamination (Ripple *et al.* 2014). The distribution of the Eurasian otter may be restricted in some areas due to acidification of water sources caused by mining processes (Mason and Macdonald 1988).

Species such as shrews (particularly *Sorex araneus*) (Wijnhoven *et al.* 2008), various farm animals (Durand *et al.* 2010) and plants such as phreatophyes (Weiersbye and Witkowski 2007) have been shown to be significantly affected by pollution and groundwater contamination in many countries. For example, while Mongolia’s low human population density, remoteness and traditional nomadic culture have likely aided in preserving the high levels of biodiversity in the country, the rapid expansion of its mining industry has begun to have an impact on a number of the nation’s natural resources (Farrington 2005). While there are many suspected cases of animal deaths due to heavy metal toxicity, AMD and other impacts associated with the mining process, little proof exists for a distinctive cause-effect relationship between these two factors (du Toit 2006; Durand *et al.* 2010). This may be due to the fact that few scientific data on heavy metal contamination related deaths exist for wild (non-domestic) animals, which are the ones often affected in protected areas (Winde and Stoch 2010).

1.5 Small mammal responses to contamination

Small mammals are known to be particularly affected by pollution and environmental degradation due to their small body sizes, diet and habitat use (Fritsch *et al.* 2010). The specific traits of certain small mammals may cause these organisms to absorb heavy metals such as uranium and cadmium so that the pollutants accumulate in the organs of the animal (Winde 2010; van den Brink *et al.* 2011). Rodents have however shown the ability to successfully adapt to changing environments, and evolve to survive challenges associated with polluted, degraded and variable environments (Auffray *et al.* 2009). Due to the ability to live in impacted habitats, rodents can be used as surrogates for other small mammals living in contaminated areas to evaluate the risks of metal exposure (Espinosa-Reyes *et al.* 2010).

The sensitivity of rodents to their environment allows for the impacts of heavy metal exposure on other mammal species to be observed, making this group of animals a useful bio-indicator in impacted landscapes (Espinosa-Reyes *et al*. 2010). Murid rodents tend to have shorter generation times than most other rodents, with lifespans generally not exceeding four to five years (Gorbunova *et al.* 2008). As many members of the Muroidae, such as rats and mice, are known to produce multiple litters throughout the year, it can be expected that faster rates of adaption and mutation may be exhibited by genera such as *Acomys* (spiny mice), *Rhabdomys* (striped mice), and *Aethomys* (rock rats or rock mice) due to their faster reproductive rates (see Spradling *et al.* 2001; Schradin and Pillay 2003).

Sensitivity to heavy metals in rodents and small mammals has previously manifested itself physically in decreased body weight (Nakayama *et al*. 2007), kidney dysfunction (Leffler and Nyholm 1996) and reduced skeletal development (Milton *et al.* 2003). Certain heavy metals, such as cadmium, lead and arsenic, have an effect on male reproductive function in rodents (Wirth and Mijal 2010). Decreased sperm concentration and motility, testicular atrophy, changes in the weights of accessory glands, alterations in semen quality and changes in the percentage of sperm with morphological abnormalities are just some of the known impacts of exposure to these heavy metals (Laskey *et al.* 1984; Thoreux-Manley *et al.* 1995; Wirth and Mijal 2010). Chronic exposure to coal dust from mines is also suspected to lead to genotoxicity and DNA damage in rodents such as mice living in contaminated areas (Caballero-Gallardo and Olivero-Verbel 2016).

Rodents from heavily contaminated mining and remediation sites have exhibited lower body conditions as well as lower organ masses of kidneys, livers and lungs (Drouhot *et al.* 2014). Sánchez-Chardi *et al*. (2009b) elaborate on this further, suggesting that apoptosis, necrosis, and inflammation of the kidneys are also possible consequences of exposure to heavy metal contamination. Although it is generally agreed that heavy metal contamination is the overall cause of these correlations, it is suggested that diet and habitat use can determine the amount of exposure that a rodent experiences (Drouhot *et al.* 2014).

1.6 The Krugersdorp Game Reserve

As a number of mines are already in existence near reserves and protected areas in South Africa, it seems necessary to explore the possible effects that these mines and the associated communities have on the morphology and physiology of mammals within these reserves. One such reserve, the Krugersdorp Game Reserve, is located approximately 40km north-west of Johannesburg in the Gauteng Province of South Africa (Figure 1). The reserve hosts several game, birds, and mammals. Some of the animals that can be found in the reserve include blesbok (*Damaliscus pygargus phillipsi*), guinea fowl (*Numida meleagris f. domestica* and *Guttera pucherani*), squirrels (*Xerus inauris*), and rodents (van Niekerk 2010; Chapman *et al.* 2012; Nöthling *et al.* 2014).

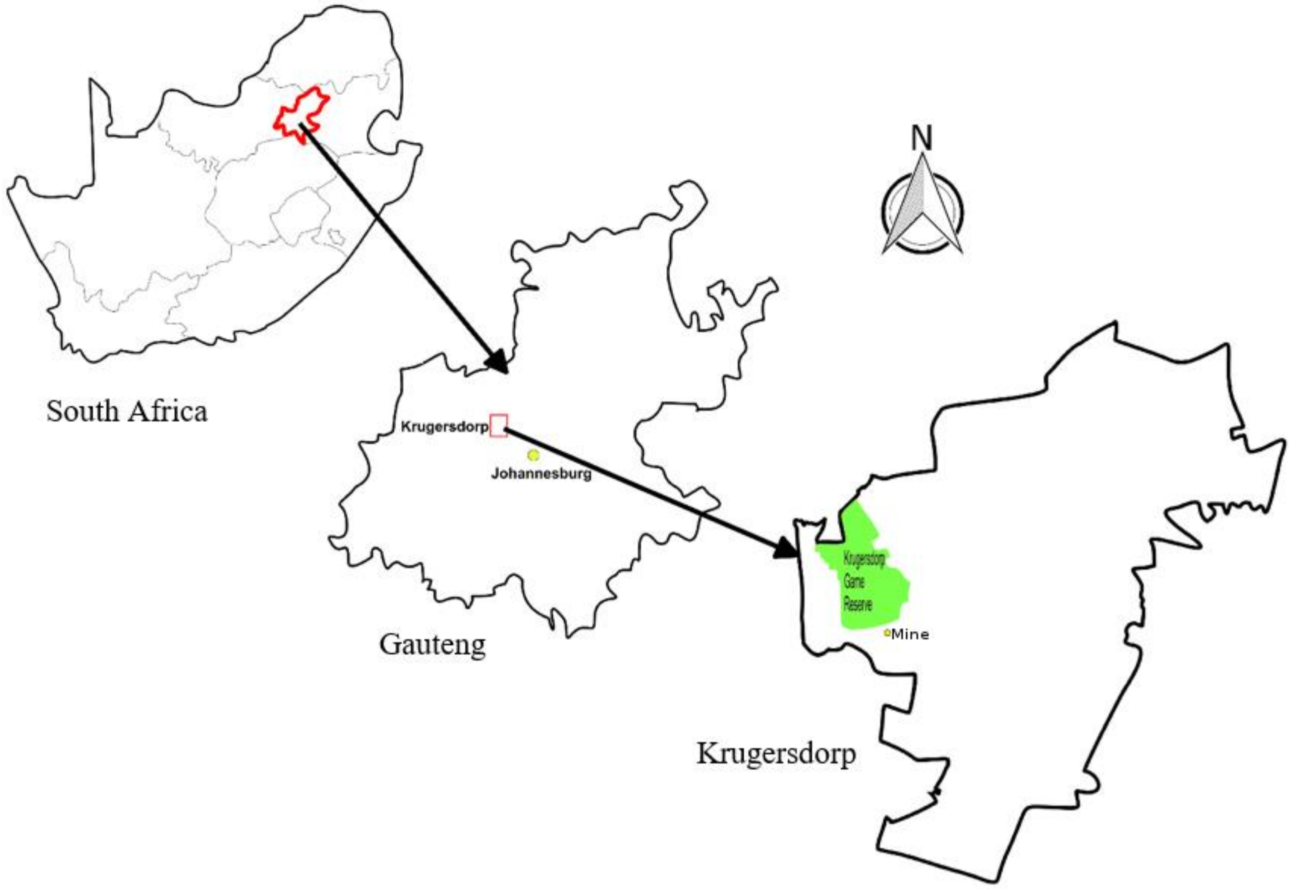

Figure 1. Location of the study site in Krugersdorp, Gauteng, South Africa. Krugersdorp Game Reserve (green) and the neighbouring mine shown within the borders of Krugersdorp (Google Earth 2015).

Throughout South Africa, protected areas and nature reserves have been established to protect the country's fauna and flora. Unfortunately, due to the country's dependence on mining, a number of these protected areas are located close to active or abandoned mines (Winde and Stoch 2010; Lusilao-Makiese *et al.* 2013). The Krugersdorp Game Reserve is one such protected area. Located just less than 1 km from an active mine, the fauna and flora within the reserve have experienced the negative effects of AMD and heavy metal contamination for a number of years (Lusilao-Makiese *et al.* 2013). For example, the water and sediment within the reserve's rivers and dams are significantly contaminated by mercury

(Lusilao-Makiese *et al.* 2013), sulphates and AMD-associated decant (Coetzee *et al.* 2003; Coetzee *et al.* 2009). Scott (1995) and Ochieng *et al.* (2010) found that contamination of the groundwater by heavy metals had reached the Krugersdorp Game Reserve and moved further north due to untreated acid mine water flooding underground mine tunnels and escaping into the environment.

Mining contaminants have flowed into the Tweelopie Spruit and through the Krugersdorp Game Reserve for several years (Coetzee *et al.* 2009). The potential impact of the decant was first realised in 2002, when the underground mine tunnels in the West Rand flooded. AMD became a concern in the area and the destructive effect of this phenomenon on the ecology of the region has increased since then (Hobbs and Cobbing 2007). The contaminants in the water and soil are thought to have been the cause of a number of fish and mammal deaths already, although these deaths have not been conclusively linked to mining runoff (Fourie 2005; Winde and Stoch 2010).

The runoff from the abandoned mine situated within 1 km of where the Tweelopie Spruit enters the Krugersdorp Game Reserve is thought to have resulted in pH levels of as low as 3 in the water within the reserve (Booyse *et al.* 2015). A pH level of 3 is considered to be extremely acidic and harmful to organisms in the environment (Kloke *et al.* 1984). Previous studies have shown that heavy metal levels in the reserve are known to be high (Du Toit 2006). While it is possible that uraniferous rocks in the reserve may also influence the heavy metal (particularly U) levels within the environment of the reserve, it has not been conclusively proven that this is so (Nöthling *et al.* 2014). Most studies conducted have shown that increased levels of heavy metals began appearing shortly after the decant from the mine first reached the reserve (Du Toit 2006; Winde 2010). The mining activities and associated runoff which polluted the Tweelopie Spruit resulted in the sickness and death of a number of animals in the Krugersdorp Game Reserve (Du Toit 2006).

1.7 Research gap

While the impacts of plant exposure to high levels of heavy metal contamination derived from mining pollution are well documented (see Weiersbye and Witkowski 2002; Lokeshwari and Chandrappa 2006; Weiersbye *et al.* 2006; Weiersbye and Witkowski 2007), the effects on morphology and physiology of small mammals are not as well documented. However, a previous study by Nunes *et al*. (2001) on the Algerian mouse (*Mus spretus*) in Portugal suggests that growth rates, organ and body weights of rodent species in their study were inhibited and much lower than of animals that have not been affected by heavy metals. The study found that the masses of organs such as the spleen and kidneys, as well as body mass, were lower in contaminated sites, as poorer environmental quality likely had an impact on the morphology and physiology of the organism (Nunes *et al*. 2001).

Sánchez-Chardi *et al*. (2009b) studied the effects of leachate from a landfill and found that the greater white-toothed shrew (*Crocidura russula*) and the wood mouse (*Apodemus sylvaticus*) in the region presented with severe apoptosis and necrosis of the liver, while the mice also showed signed of kidney damage. Other studies have focused on the negative impacts to reproductive health and success in male rats (Chowdhury 2009; Wirth and Mijal 2010), as well as decreases in population densities of Microtine rodents along a pollution gradient from a smelter (Kataev *et al.* 1994).

As mentioned, the Krugersdorp Game Reserve is severely impacted by heavy metal contamination from the mining processes that occurred nearby (Oelofse *et al.* 2007). Studies conducted in the area have focused on water quality, plant response to contamination and behavioural ecology of animals (Winde 2010; Chapman *et al.* 2012; Lusilao-Makiese *et al.* 2013; Van Deventer and Cho 2014), but only a limited amount of literature specifically focuses on the impact of heavy metal contamination in the reserve on animal health, and these studies usually focus on impacts on large mammals.

1.8 Aim and objectives

In light of the limited research on the relationship between heavy metal contamination and the morphological and physiological features of small mammals, the aim of this study was to investigate the impacts of heavy metal contamination on the morphology and physiology of small mammals in the Krugersdorp Game Reserve. Specifically, the objectives were (1) to assess the levels of heavy metal contamination in the soil and vegetation of the Krugersdorp Game Reserve, (2) to assess the levels of heavy metal accumulation in small mammals in the Krugersdorp Game Reserve, (3) to compare several morphological and physiological characteristics (i.e. the body mass, body length, tail length, hind foot length, and organ masses of individuals) between rodents exposed to different levels of soil and plant contamination, and (4) to discuss possible risks to small mammals in the reserve based on data and literature.

CHAPTER 2: MATERIALS AND METHODS

2.1 Study sites

The study was conducted at the Krugersdorp Game Reserve, a 1500ha nature reserve located west of the town of Krugersdorp in the province of Gauteng (S 26° 6' 21.218" E 27° 43' 26.094"; Figure 1, Chapter 1). The reserve is located near a number of mines and is known to be heavily affected by pollution resulting from these mines (Hobbs and Cobbing 2007).

Four sites, in both higher lying land and flat areas near water sources, were used as trapping locations (Figure 2). Site 1 was situated near the entrance to the reserve. This site consisted almost entirely of grasses, and was approximately10m from the bank of the river. Dominant grass species included *Bothriochloa radicans* (stinking grass), *Eragrostis curvula* (weeping love grass) and *Alloteropsis semialata* (black seed grass). Site 2 was located on a slope overlooking the Tweelopies Spruit. The terrain at this site was extremely rocky, with vegetation consisting mostly of *Acacia* trees, small shrubs, and scattered clumps of grass, including *Hyperthelia dissoluta* (yellow thatching grass) and *Sporobolus pyramidalis* (giant rats tail grass). The site itself was shielded by thorn trees and vegetation and not exposed to much human activity. Site 3 consisted predominantly of grasses and reeds, and traps were set up approximately 5-10m from the river bank. This site was relatively flat, and used by visitors on horseback in the reserve. Site 4 was the most secluded site, at the far end of the reserve, which is very rarely travelled to by visitors to the reserve. Site 4 consisted of a variety of vegetation types, although grasses and small trees were dominant in the uneven rocky terrain. *Themeda triandra* (red grass), *Cenchrus ciliaris* (foxtail buffalo grass), *Eragrostis curvula* (weeping love grass) were common at this site.

Although the sites differed in vegetation structure and species composition, the main aim of this study was to investigate the link between levels of heavy metal contamination and possible morphological and physiological changes in rodents, therefore the slight differences in vegetation structure at each of the four sites should not have affected the results. Scott (1995) found evidence of groundwater contamination extending into the Krugersdorp Game reserve, a concern which was re-evaluated later by Coetzee *et al.* (2009) and found to be valid due to the severe pollution of groundwater from previous mining operations. As the groundwater throughout the reserve is polluted, the differences in vegetation structure at each site are therefore not deemed a determining factor in the effect of contamination on the small mammals in this study.

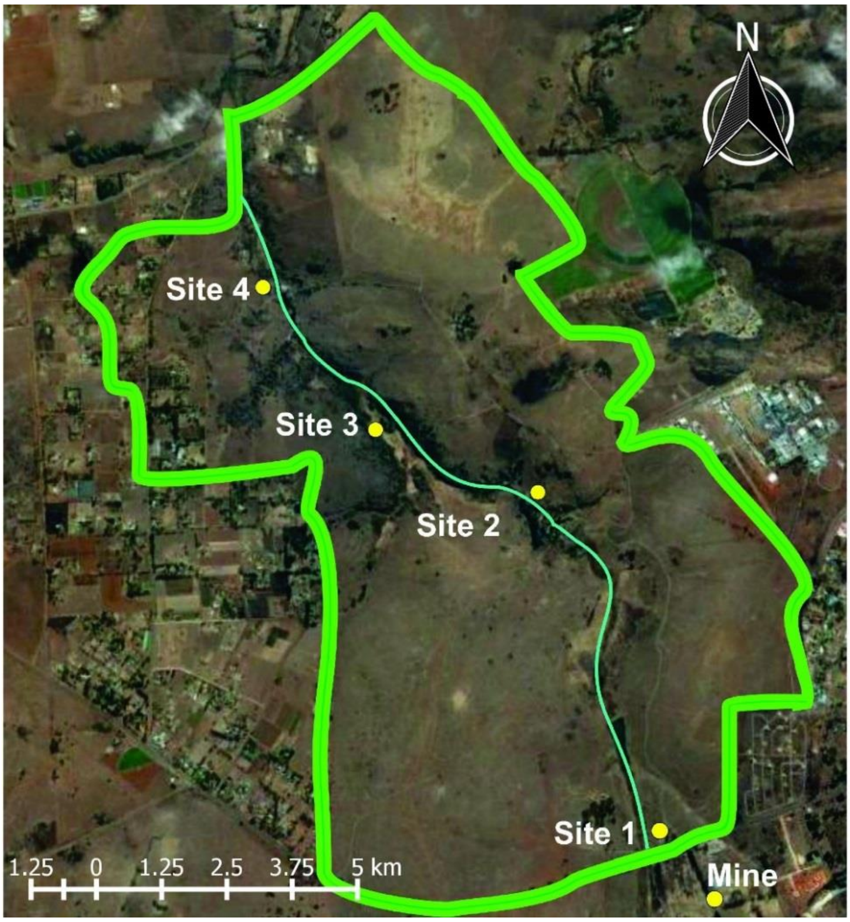

Figure 2. The four study sites chosen at Krugersdorp Game Reserve (green boundary) with Tweelopies Spruit (blue) indicated (Google Earth 2015).

2.2 Soil and vegetation sampling

A number of heavy metals are known to occur in the water at Krugersdorp Game Reserve (Lusilao-Makiese *et al.* 2013; Nöthling *et al.* 2014). Based on this, concentrations of uranium (U), arsenic (As), cadmium (Cd), copper (Cu), iron (Fe), lead (Pb), and zinc (Zn) were determined for in soil and vegetation samples collected at each location using inductively-coupled plasma optical emission spectroscopy (ICP-OES). Soil samples were collected using an auger, which was twisted into the ground at each trap site. Each soil sample bore 30cm into the ground, as most smaller burrowing rodents do not exceed depths of 35cm (Davies and Jarvis 1986; Bennett *et al.* 1988). Samples were frozen at -20°C in the laboratory until needed for further analysis (as per Sany *et al.* 2013). A soil sample from each site was sieved through a 2mm sieve, placed in a petri-dish covered with pierced Parafilm and freeze-dried at approximately -80°C for three days. One soil sample from each site, containing a mixture of soil from a depth of 0-30cm was sent to the Council for Scientific and Industrial Research (CSIR) in Stellenbosch, South Africa for analysis. A weak acid extraction of the soil was used to analyse each sample. The leachate was filtered and analysed using ICP-OES against a blank (0ppm) and a set of calibration standards (between 0.1ppm and 1ppm).

Vegetation samples were also collected at each of the four sites. Grasses were used as sample species, as many rodents (for example *Otomys irratus* and *Mastomys natalensis*) feed on grass stems and rhizomes (Coetzee 1975; Curtis and Perrin 1979). Samples of the most dominant grass species were collected at each site. Plants were sampled by removing the whole structure from the ground. Vegetation samples were placed in large plastic bags in cooler boxes during transport and frozen to below -20°C once in the lab. The roots and shoots of these samples were chopped up into 0.1cm pieces using a ceramic knife, in order to avoid any contamination from a metal blade. The samples were then freeze-dried in a plastic container covered in Parafilm at -80°C for approximately 3 days. Vegetation samples were then sent to the CSIR, where ICP-OES analysis was done to test for heavy metals.

2.3 Small mammal trapping and tissue sampling

Animal ethics clearance (2016/05/25/B) and trapping permits (CPF6 permit no. 0190) were obtained from the University of the Witwatersrand and the Gauteng Department of Agriculture and Rural Development (GDARD) respectively, before trapping began. Sherman live traps (26cm x 8cm x 8cm) were set out for 14 consecutive nights at each site over four sessions between September 2016 and January 2017. At each site, twenty traps were placed in a 2 by 10 (sites 1 and 3) or 4 by 5 (sites 2 and 4) configuration, with a spacing of approximately 5-7m between traps. At site 1, traps were set up on a relatively flat piece of land, about 60m from a road leading to a recreational area (approximately 600m away) within the reserve. Although relatively secluded, some traps at site 2 were set up approximately 20m from a relatively busy road and 50m from the lodge occasionally housing guests. At site 4, the closest traps to any motor vehicles were set up approximately 40m from a relatively frequently used road. Traps were baited with a mixture of rolled oats, sunflower seeds and peanut butter (Mills *et al.* 1995; Nupp and Swihart 1996; Lancaster and Pillay 2010). Brown bread soaked in beer was also used as bait, as rodents are known to respond well to this mix, likely due to the strong smell and nutritious benefits of the ingredients (Pastoriza *et al*. 2015). The Sherman live traps were covered with wooden boards and grass to protect captured animals from rain and heat. Cotton wool balls were placed in the traps to allow the individuals captured to keep warm and nest overnight.

Traps were checked twice daily, in the early morning and late afternoon. Empty traps were returned to their initial positions and re-covered, and all traps were re-baited every second day using alternating bait mixtures (i.e. the peanut butter mixture was replaced with bread and beer every second day). Captured animals were identified to species level, weighed and measured to determine body mass and length, tail length, hind foot length, sex and sexual maturity. These measurements, along with the point of capture, were recorded for each trapped animal (Nupp and Swihart 1996; Wijesinghe and Brooke 2005; Espinosa-Reyes *et al*. 2014). Captured animals were marked with non-toxic hair dye in order to identify them should they be recaptured (as per Schradin and Pillay 2004). Four different markings were created for each of the sites, and all individuals were marked using the symbol assigned to the site where they were captured. The recapture status of individuals was also recorded before release. Photographs of individuals were taken, especially in cases where immediate species identification was difficult. Traps were washed thoroughly between trap sessions in order to remove any odours associated with previous captures.

Blagojević *et al.* (2012) suggest that, in order to compare the impact of heavy metal contamination across sites or seasons, one must make use of only the oldest individuals, as these should have accumulated the greatest amount of heavy metals over time, making a study more valid. This, in conjunction with the fact that *Mastomys natalensis* was the only species trapped at all four sites, is why only adult *Mastomys natalensis* were used for tissue sampling during this study.

A total of 15 adult *Mastomys natalensis* were euthanased using a mixture of 80% carbon dioxide and 20% oxygen (as recommended by the Animal Ethics Committee of the University of the Witwatersrand), which was administered from a pressurised gas bottle. Four rodents each were euthanased at sites 1, 2, and 4, and three rodents were euthanased at site 3. Animals were placed in a dark plastic bag during the gassing procedure, and were held in the bag for a minimum of one minute after they had ceased moving (Artwohl *et al*. 2006). These individuals were then placed in sealed plastic bags and immediately cooled to -4°C in a cooler box. The animal samples were then removed from the cooler as soon as a particular fieldwork session had been completed, with care being taken to avoid any contamination of the samples. The liver, kidneys and spleen of the animals were dissected out, weighed and freeze-dried at approximately -80°C. After freeze-drying was completed, dry weights for the organs were recorded.

Samples were sent to the CSIR in Stellenbosch for analysis of heavy metal concentrations. Due to the small sample sizes and weights of some of the organs, inductively coupled plasma mass spectrometry (ICP-MS) was used to determine heavy metal concentrations in these samples. Results were then compared with recommended limits from the Department of Environmental Affairs (2010) and Govind and Madhuri (2014) in order to determine if the levels small mammals within the reserve are exposed to are above or below what is considered safe.

2.4 Data analysis

Statistical analyses of all data were done using Statistica 13 (StatSoft Inc. 2015). A Generalized Linear Model (GLZ) with a Poisson distribution and log link function was run to determine whether sampling session or site were predictors for any of the measured variables and therefore whether or not data could be pooled. A GLZ was also used to assess whether maturity may have been a predictor for heavy metal concentrations in order to decide whether data sets for these two categories of maturity could be combined.

As the data were not normally distributed, non-parametric statistical tests were used. Differences in heavy metal concentrations, as well as differences in morphological characteristics of the captured individuals were compared across various heavy metal concentration levels in soil and vegetation from each of the four sites using a Kruskal-Wallis H test. Spearman rank correlations were used to identify any relationships between heavy metal levels and morphological and physiological features of the captured rodents.

Ratios of organ mass to total body mass were calculated for each of the euthanased individuals using both the wet and dry masses of organs (Pereira *et al*. 2006). These ratios were then divided by the amount of each heavy metal present in the organ of the individual rodents and correlated with heavy metal concentrations in the vegetation using Spearman rank correlations in order to identify any relationships between heavy metal levels ingested by rodents and their physiology. Significance levels for all tests were set at $p<0.05$.

CHAPTER 3: RESULTS

3.1 Soil and vegetation analysis

Iron (Fe) was found in the highest concentration across all sites in both the soil and the vegetation (Table 1). Zinc (Zn) in the soil and vegetation, as well as Cu and Pb in the soil were also present in relatively high concentrations compared to other heavy metals in the Krugersdorp Game Reserve (Table 1). Concentrations of Pb, As and Cu in the soil in the reserve are already exceeding recommended safe levels for oral and dermal intake by humans (DEA 2010; Govind and Madhuri 2014). No strong relationships existed between any of the concentrations of heavy metals tested for in the soil and their counterparts or other heavy metals in the vegetation in the Krugersdorp Game Reserve ($p>0.05$). No significant differences were found between sites in terms of heavy metal contamination levels in the soil ($p>0.05$), or in the vegetation ($p>0.05$).

Heavy metals in the vegetation were highest at site 2 (As, Cu, Pb and Zn) and 4 (Fe), while the majority of the heavy metals tested were highest in the soil at site 3 (U, As, Cu, Zn) (Table 1). The majority of heavy metals tested for exceeded the maximum recommended exposure levels as recommended by DEA (2010), Govind and Madhuri (2014) and ATSDR (2016), particularly those from sites 2 and 3.

Table 1. Heavy metal concentrations (mg·kg^{-1}) during the dry season in soil and vegetation samples from four sample sites in the Krugersdorp Game Reserve with comparison to maximum recommended daily exposure in soil and vegetation for humans and animals (DEA 2010; Govind and Madhuri 2014; ATSDR 2016).

Heavy Metal (mg·kg^{-1})	Site				Govind & Madhuri (µg per day)	DEA (mg·kg^{-1})	ATSDR (mg·kg^{-1}·day^{-1})
	1	2	3	4			
Uranium in soil	9.60	2.60	17.61	1.50			0.20
Uranium in vegetation	0.19	0.50	0.40	0.14			
Arsenic in soil	18.00	11.00	44.00	8.00	15.00		0.0003
Arsenic in vegetation	0.70	2.70	0.80	0.60			
Cadmium in soil	0.50	0.50	0.50	0.50	5.00	7.50	0.0002
Cadmium in vegetation	0.10	0.10	0.10	0.10			
Copper in soil	60.00	30.00	82.00	30.00	2500.00	16.00	
Copper in vegetation	8.20	12.00	8.80	8.90			
Iron in soil	40900.00	14750.00	23350.00	62850.00			
Iron in vegetation	2100.00	4010.00	614.00	5550.00			
Lead in soil	27.00	12.00	22.00	20.00	10.00	20.00	
Lead in vegetation	1.63	4.35	0.62	1.81			
Zinc in soil	149.00	63.00	314.00	95.00		240.00	0.30
Zinc in vegetation	50.00	64.00	35.00	29.00			

3.2 Small mammal species occurrence and morphology

Three small mammal species were captured across the four sites between September 2016 and January 2017: the Angoni vlei rat (*Otomys angoniensis*), four-striped mouse (*Rhabdomys pumilio*), and multimammate mouse (*Mastomys natalensis*). *Rhabdomys pumilio* and *O. angoniensis* were found only at some sites, and only a small number of these two species were captured. A total of three *Rhabdomys* individuals were caught at sites 1, 3 and 4, and a total of five *Otomys* individuals were captured at sites 3 and 4. *Mastomys natalensis* was found at all four sites and made up the majority of the sample. Therefore, all statistical analyses and comparisons of morphological data across sites were applied only to data related to the 21 juvenile and 39 adult individuals of this species. Juveniles were found at only three of the four sites (sites 1, 3 and 4).

A GLZ showed that neither trapping session nor site were predictors for morphological measurements or heavy metal concentrations. Accordingly, data from the two sessions were pooled for further analysis. A significant difference was found between the body mass (Wald $\chi^2(1)=4.1638$, $p=0.0412$), body length (Wald $\chi^2(1)=5.2502$, $p=0.02195$) hind foot (Wald $\chi^2(1)=5.1036$, $p=0.0239$) and tail lengths (Wald $\chi^2(1)=5.4427$, $p=0.0197$) of adult and juvenile *Mastomys*, and between maturity and heavy metal levels in the soil and vegetation (U in soil: Wald $\chi^2(1)=0.8367$, $p=0.3603$; U in veg: Wald $\chi^2(1)=0.0017$, $p=0.9674$; As in soil: Wald $\chi^2(1)=0.9735$, $p=0.3238$; As in veg: : Wald $\chi^2(1)=0.5324$, $p=0.4656$; Cu in soil: Wald $\chi^2(1)=0.8423$, $p=0.3587$; Cu in veg: Wald $\chi^2(1)=0.5741$, $p=0.4486$; Fe in soil: Wald $\chi^2(1)=0.0057$, $p=0.9399$; Fe in veg: Wald $\chi^2(1)=0.4543$, $p=0.5003$; Pb in soil: Wald $\chi^2(1)=0.3853$, $p=0.5348$; Pb in veg: Wald $\chi^2(1)=1.3081$, $p=0.2527$; Zn in soil: Wald $\chi^2(1)=1.2334$, $p=0.2667$; Zn in veg: Wald $\chi^2(1)=0.5006$, $p=0.4793$). Therefore, data for juveniles and adults were analysed separately. Comparisons of morphological features for juveniles showed no significant difference across the three sites they were found at (Figures 3 and 4). While features such as average juvenile body mass, body length, and tail length appear to increase at sites furthest from the mine, no significant difference was found between the sites for body mass (H_2=1.0213, p=0.6001), body length (H_2=4.5298, p=0.1038), tail length (H_2=4.4071, p=0.1104) or hind foot length (H_2=4.0280, p=0.1335).

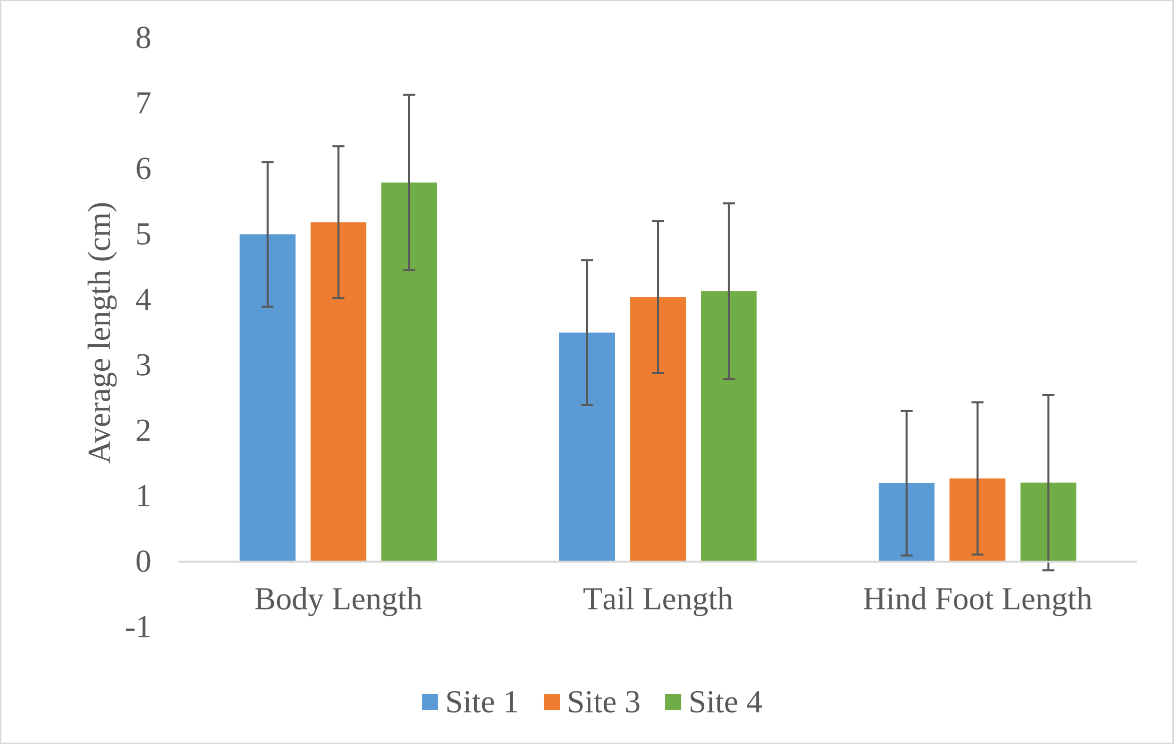

Figure 3. Mean (±SE) body length, tail length, and hind foot length for juvenile *Mastomys* at sites in Krugersdorp Game Reserve.

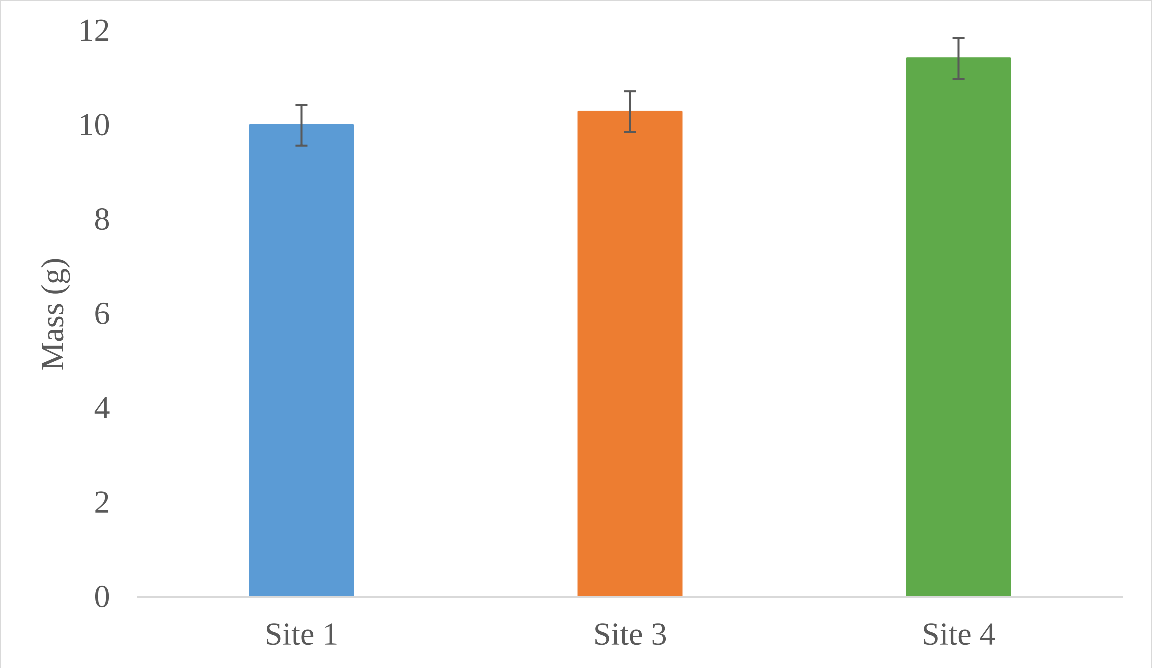

Figure 4. Mean (±SE) body mass for juvenile *Mastomys* at sites in Krugersdorp Game Reserve.

Figures 5 and 6 show the average measurements for the morphological characteristics of adult *Mastomys* individuals at each of the four study sites. When comparing only the adult captured *Mastomys* individuals at each site, the average body mass as well as hind foot, tail and body length of individuals at site 3 was higher than at the other three sites (Figures 5 and 6). The average measurements for all morphological characteristics of *Mastomys* at site 4 were also higher than those of individuals at sites 1 and 2 (Figures 5 and 6). There was however no significant difference between the body mass (H_3=2.6283, p=0.4525), body length (H_3=2.3105, p=0.5105), tail length (H_3=5.2584, p=0.1538) and hind foot length (H_3=4.5637, p=0.2067) of adult *Mastomys* at each of the four study sites.

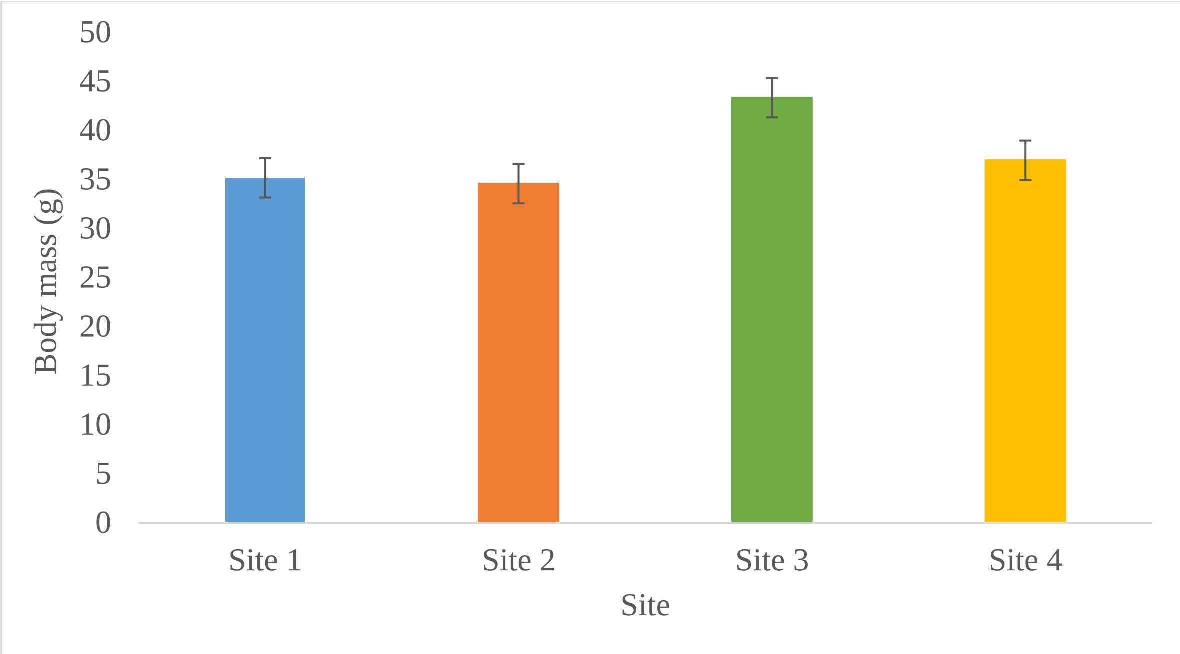

Figure 5. Mean (±SE) body mass for adult *Mastomys* at four sites in Krugersdorp Game Reserve.

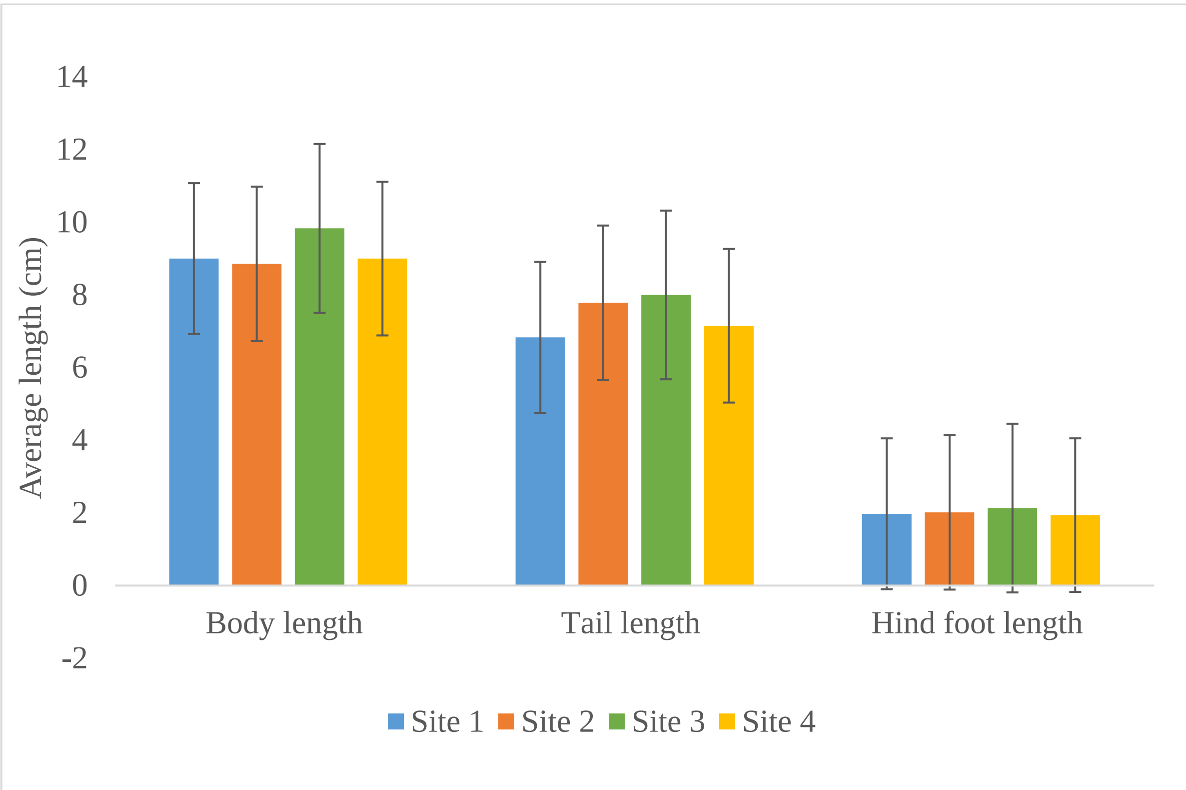

Figure 6. Mean (±SE) body length, tail length, and hind foot length for adult *Mastomys* at four sites in Krugersdorp Game Reserve.

Body length in *Mastomys* juveniles was significantly correlated with all heavy metals (U, As, Cu, Fe, Pb and Zn) measured in the soil and the vegetation in the reserve (Table 2). Body mass decreased with an increase in U, As, Cu, Pb, and Zn in the soil, as well as U, As, and Zn in the vegetation (Table 2). Cu, Fe and Pb in the vegetation, and Fe in the soil correlated positively with an increase in body length as concentrations of these heavy metals increased (Table 2). The levels of U and As in the vegetation were positively correlated with tail length, while Fe in the soil was negatively correlated with this morphological characteristic (Table 2).

Table 2. **Correlation values for heavy metals and morphological characteristics of *Mastomys* juveniles at Krugersdorp Game Reserve**

Medium	Body feature	Heavy metal	r	p
Soil	Body length	Uranium	-0,4455	0,0429
		Arsenic	-0,4455	0,0429
		Copper	-0,4455	0,0429
		Iron	0,4455	0,0429
		Lead	-0,4734	0,0302
		Zinc	-0,4455	0,0302
Vegetation	Body length	Uranium	-0,4455	0,0429
		Arsenic	-0,4455	0,0429
		Copper	0,4455	0,0429
		Iron	0,4455	0,0429
		Lead	-0,4455	0,0429
		Zinc	-0,4734	0,0302
Soil	Body mass	Uranium	-0,4455	0,0429
		Arsenic	-0,4455	0,0429
		Copper	-0,4455	0,0429
		Lead	-0,4734	0,0302
		Iron	0.4455	0.0429
		Zinc	-0,4455	0,0429
Vegetation	Body mass	Uranium	-0,4455	0,0429
		Arsenic	-0,4455	0,0429

Vegetation	Body mass	Copper	0,4734	0,0302
		Iron	0,4455	0,0429
		Lead	0,4455	0,0429
		Zinc	-0,4734	0,0302
Vegetation	Tail length	Uranium	0.3279	0.0416
		Arsenic	0.3279	0.0416
Soil	Tail length	Iron	-0.3279	0.0416

3.3 Tissue samples

Despite relatively high average concentrations of tested heavy metals in question being found in the spleens of the rodents compared to the other organs used in this study (Figures 7-10), no significant relationships were discovered between heavy metals in the soil or vegetation and heavy metal concentrations in the spleens or kidneys of *Mastomys sp.* in the reserve. No significant differences in heavy metal levels were found across the four sites for any of the heavy metals tested for in the soil or vegetation (U in soil: Wald $\chi^2(1)=0.5188$, $p=0.8198$; U in veg: Wald $\chi^2(1)=0.0776$, $p= 0.7806$; As in soil: Wald $\chi^2(1)=0.0011$, $p=0.9733$; As in veg: Wald $\chi^2(1)=0.1589$, $p=0.6901$; Cu in soil: Wald $\chi^2(1)=0.0749$, $p=0.7843$; Cu in veg: Wald $\chi^2(1)=0.0138$, $p=0.9066$; Fe in soil: Wald $\chi^2(1) =0.4048$, $p=0.5246$; Fe in veg: Wald $\chi^2(1)=0.3424$, $p=0.5585$; Pb in soil: Wald $\chi^2(1)=0.1034$, $p=0.7478$; Pb in veg: Wald $\chi^2(1)=0.1335$, $p=0.7149$; Zn in soil: Wald $\chi^2(1)=0.0212$, $p=0.8843$; Zn in veg: Wald $\chi^2(1)=1.1032$, $p=0.2935$). In the case of heavy metals in the tissues of rodents, no significant difference was found between any of the four sites when heavy metal levels in the spleen (As: Wald $\chi^2(1)=0.4361$, $p= 0.5090$; Cd:Wald $\chi^2(1)=0.4361$, $p= 0.5090$; Pb: Wald $\chi^2(1)=0.0311$, $p=0.8599$; U: Wald $\chi^2(1)=0.4361$, $p=0.5090$; Ni: : Wald $\chi^2(1)=0.4141$, $p=0.5199$; Cu: Wald $\chi^2(1)=0.0824$, $p=0.7741$; Zn: Wald $\chi^2(1)=0.0910$, p=0.7629), liver (As: Wald $\chi^2(1)=2.0472$, p= 0.1525; Cd: Wald $\chi^2(1)=3.8389$, p=0.0501; Pb: Wald $\chi^2(1)=2.3984$, p=0.1215; U:Wald $\chi^2(1)=1.1614$, p=0.2812 0.5090; Ni: Wald $\chi^2(1)=0.0010$, p=0.9743; Cu: : Wald $\chi^2(1)=0.0092$, p=0.9234; Zn: Wald $\chi^2(1)=0.2763$, p=0.5991), or kidneys (As: Wald $\chi^2(1)=1.2109$, p= 0.2712; Cd: Wald $\chi^2(1)=1.2196$, p= 0.2694; Pb:Wald $\chi^2(1)=2.3211$, p=0.1276; U:Wald $\chi^2(1)=0.8014$, p= 0.3707; Ni: Wald $\chi^2(1)=0.5056$, p=0.4771; Cu: Wald $\chi^2(1)=0.0682$, p=0.7940; Zn: Wald $\chi^2(1)=0.0074$, p=0.9317) were compared.

While no statistical relationships were found between levels of heavy metals in the soil and vegetation, or site and heavy metal levels in the tissue samples, the average heavy metal levels in the spleens of rodents at site 2 are higher than all other sites (Figures 7-10). At sites 1 and 4, the highest levels of these heavy metals were also detected in the animals' spleens. Site 3 however exhibited the highest concentrations of heavy metals, particularly Zn, in the livers and kidneys studied individuals (Figure 9). This trend is mirrored by heavy metal concentrations in the soil and vegetation (Table 1), as peaks in levels in the vegetation correspond to the high levels of heavy metals found in the spleens of individuals from sites 1, 2 and 4, while the Zn and Cu levels in the soil correspond to a peak in Zn and Cu in the kidneys of mice from site 3.

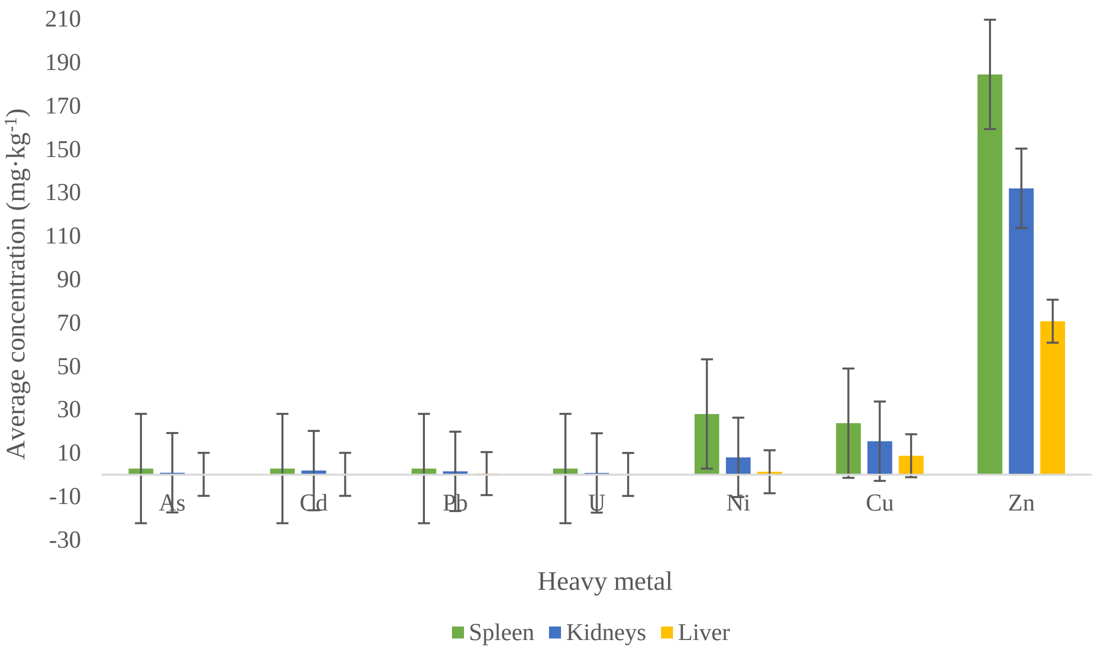

Figure 7. Average (±SE) heavy metal concentrations in spleen, liver, and kidneys of rodents from site 1 in Krugersdorp Game Reserve.

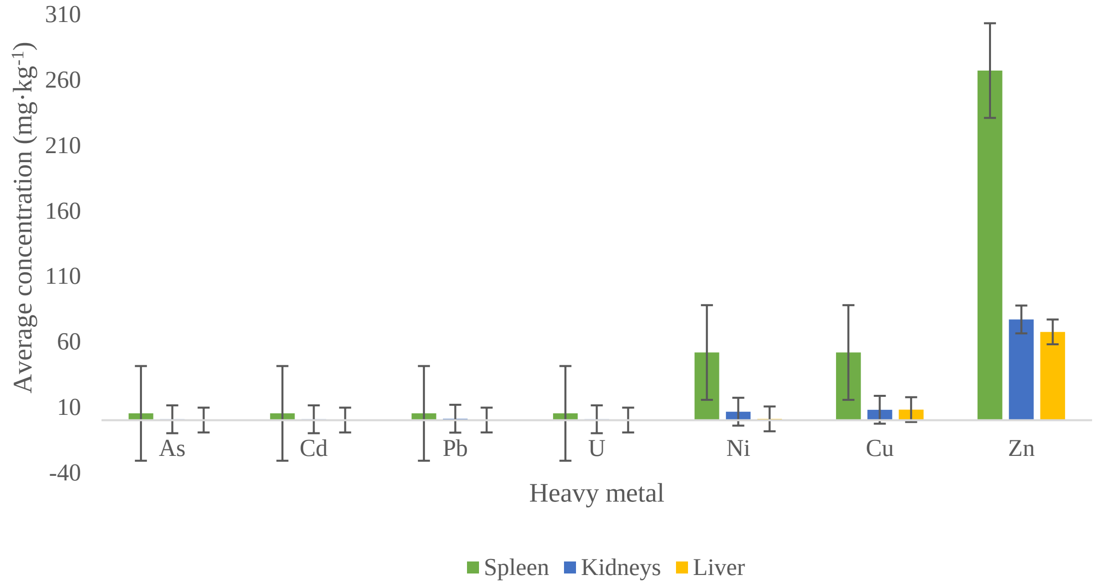

Figure 8. Average (±SE) heavy metal concentrations in spleen, liver, and kidneys of rodents from site 2 in Krugersdorp Game Reserve.

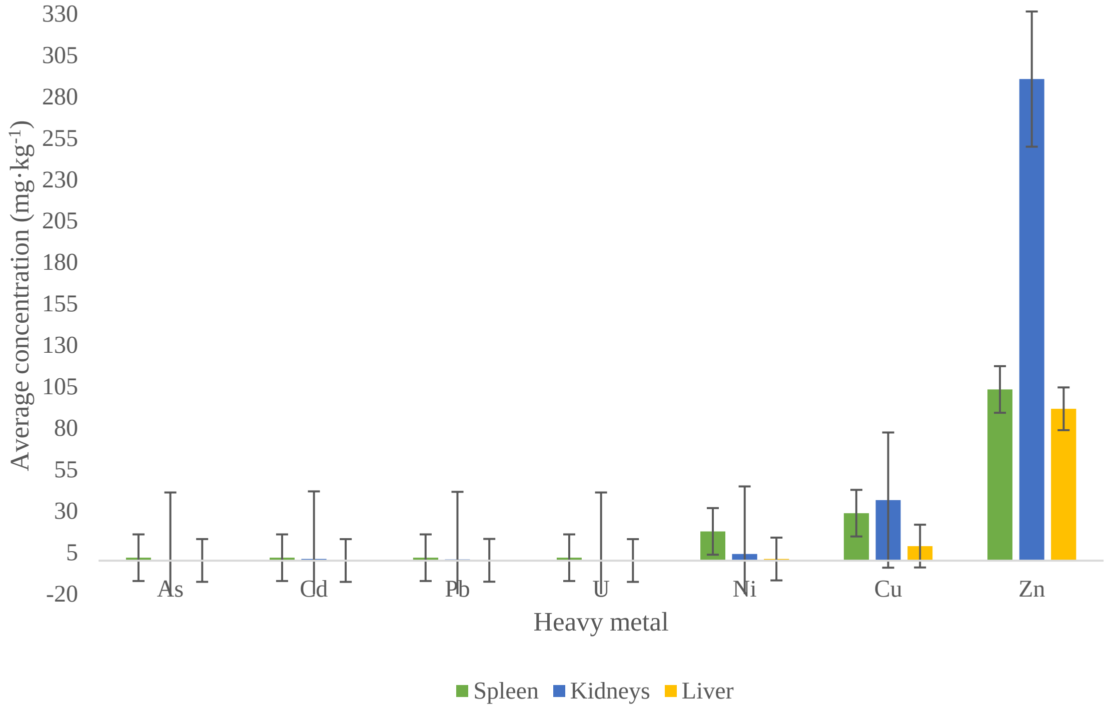

Figure 9. Average heavy metal concentrations in spleen, liver, and kidneys of rodents from site 3 in Krugersdorp Game Reserve.

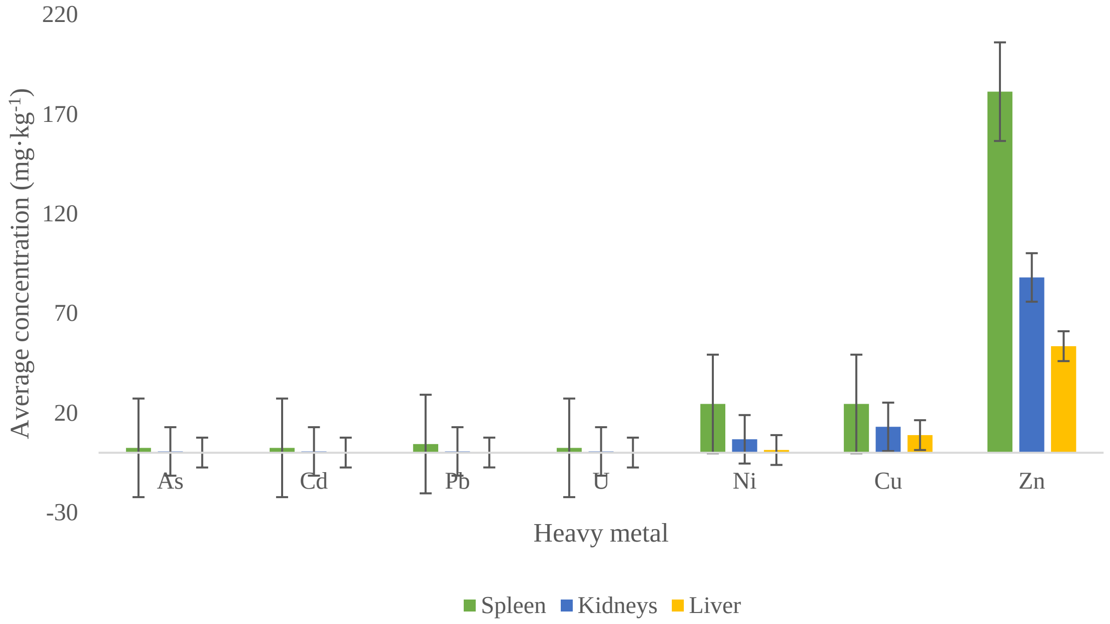

Figure 10. Average heavy metal concentrations in spleen, liver, and kidneys of rodents from site 4 in Krugersdorp Game Reserve.

3.4 Observations

Observations made during the dissection of the organs from the fifteen euthanased individuals revealed additional physical impacts. While one individual from site 1 did exhibit an extremely soft and pulpy right kidney, most noticeable organ abnormalities were found in individuals from site 3. The livers of two individuals from site 3 were clearly irregular, based on previous rodent dissections and comparisons to other individuals in the study. One individual's liver had a slight yellow colouration and was speckled in appearance, while another individual's liver had white spots on it. Although the four sites were not significantly different statistically, the observations made during the dissection stage suggest that rodents at site 3 encountered these additional physical ailments. As heavy metals in the soil (U, Zn, As and Cu), as well as Zn levels in the livers and kidneys were highest at this site and above recommended maximum exposure levels (Table 1), it could be suggested that toxicity from these may have resulted in liver disease and necrosis. Externally, several of the trapped rodents across all four sites exhibited growths on their skin, usually on their backs and stomach areas.

Figure 11. Site 3 individual with fur missing and small skin lesions

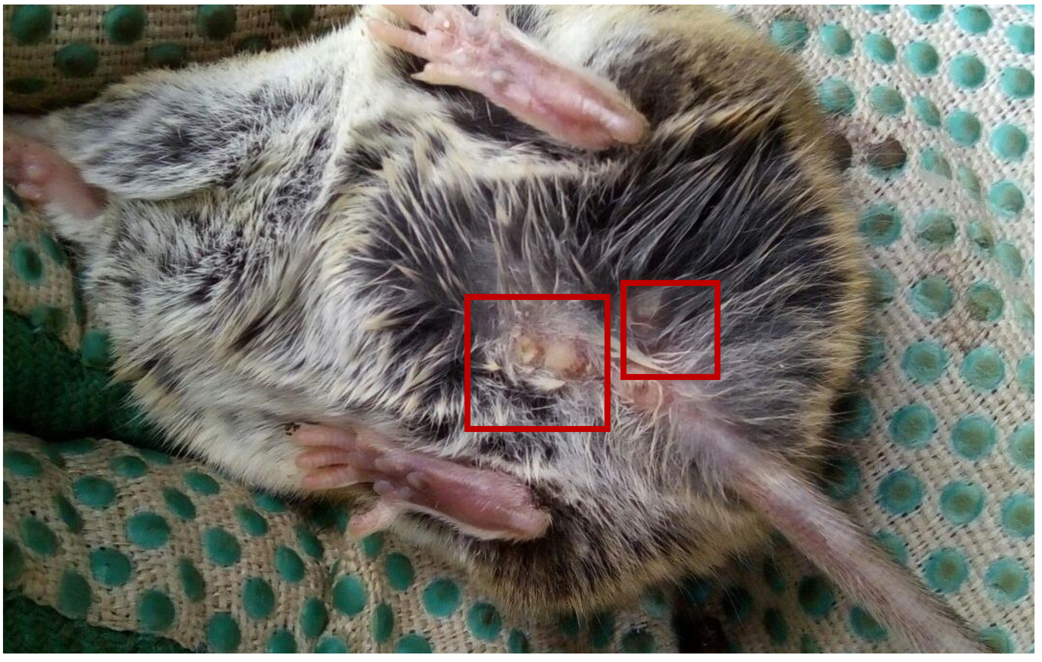

Figure 12. *Mastomys* individual with abdominal growths indicated.

CHAPTER 4: DISCUSSION

Mining and associated activities have a significant impact on the health of a variety of components of an environment. Primary contamination of air, water, soil and vegetation can lead to further contamination of the environment and negative impacts on the health of animals living in the ecosystem (Sánchez-Chardi and Nadal 2007; Peng *et al.* 2009). Small mammals are known to be strongly affected by polluted and contaminated environments, as their small body sizes, habitat use and often specialised diets allow for heavy metals to be absorbed through ingestion and burrowing processes in relatively high quantities compared to other animals (Fritsch *et al.* 2010; van den Brink *et al.* 2011). Despite being sensitive to environmental conditions (Espinosa-Reyes *et al*. 2010), rodents possess the ability to adapt to changing environments, have shorter generation times than other small mammals and are able to survive in damaged ecosystems (Gorbunova *et al.* 2008; Auffray *et al.* 2009; Espinosa-Reyes *et al*. 2010). All of these traits make rodents ideal bioindicators for studies on the effect of heavy metal contamination on small mammals.

This study aimed to investigate the impacts of heavy metal contamination on the morphology and physiology of small mammals in the Krugersdorp Game Reserve. This was achieved by comparing several morphological characteristics, as well as organ masses and characteristics between rodents from sites throughout the reserve, along a contaminated water source.

A number of heavy metals occur naturally within the environment (Alloway 2013), but anthropogenic activities such as mining have resulted in a massive increase in the concentrations of heavy metals in many areas around the world (Bhattacharjee *et al.* 2016). Although the presence of heavy metals such as Fe, Ni, Zn and Cu are important for the healthy function of an environment, an excess or shortage of these metals could result in negative effects on the function of food webs, ecosystems and natural resources (Lokeshwari and Chandrappa 2006). As there are a number of ways that heavy metals can accumulate in soils and vegetation (for example, through aerial particles from gasses, or through AMD mobilising them into water and sediment sources) (Clemens and Ma 2016), one cannot always be certain as to what the origins of the total heavy metal content in an organism are.

4.1 Soil and vegetation patterns

Due to their presence in the atmosphere, metals such as Ni, Cd, Zn and Pb are often found in soils and vegetation samples (Lagerwerff and Specht 1970). Copper, along with Zn and Cd, are more easily taken up by plants than other heavy metals such as Pb (Kabata-Pendias 2004; Angelova *et al.* 2010). The composition and amount of heavy metals in the vegetation are usually determined by their concentration within the soil, as well as the ability of plants to absorb these metals (Alloway 1995; Angelova *et al.* 2010). Studies have shown that trees as far away as 20km from a smelter contained high concentrations of Zn (4500 ppM) in their dried foliage (Buchauer 1973). Although Pb is relatively immobile, and not readily absorbed by plants and animals, pollution by this heavy metal is particularly widespread (Alloway 1995; Kesler and Simon 2015). During mining processes, small particles of lead oxides and sulphates are formed. These are more soluble than Pb itself, and are able to penetrate deeper into soils through precipitation (Kesler and Simon 2015). It is therefore possible that the high levels of Pb, Zn, and Cu in the soil and vegetation in the Krugersdorp Game Reserve exist due to the high availability of these metals in the atmosphere and the ability for soils and plants to absorb them.

In the Krugersdorp Game Reserve, extremely high levels of several heavy metals were found in some of the vegetation tested. In particular, levels of U, As, Cu and Zn in the soil at site 3 were much higher than their counterparts at other sites. Levels of Fe in the soil and vegetation were particularly high at site 4, while Pb in the soil was highest at site 1. This suggests that grasses from the reserve may be accumulating large amounts of heavy metals such as Fe and Zn, which are known to negatively affect the health of a number of mammal species through poisoning (Rascio and Navari-Izzo 2011). While plants are known to accumulate heavy metals naturally through a variety of processes, including atmospheric absorption (Cataldo and Wildung 1978), soil remains one of the primary ways in which vegetation accumulates high levels of heavy metals. As plants are capable of absorbing nutrients, sulphides, metals and other substances from water and soil, it stands to reason that the high levels of heavy metals found in the vegetation within the reserve are likely a result of the environmental contaminants originating from a combination of these features (Cataldo and Wildung 1978; Nazir *et al.* 2015; Thakur *et al.* 2016).

4.2 Impact of heavy metals on rodent morphology

Exposure to high levels of heavy metals has an impact on a number of morphological features of small mammals. Different heavy metals also have different effects on these animals. For example, while increased levels of lead in the environment have been found to result in increased body mass by increasing fat reserves (Klosinska 1996; Phelps and McBee 2010), exposure to other heavy metals such as cadmium and arsenic results in decreased body mass (Perugini *et al*. 2014). While no strong relationship existed between heavy metals and the body mass of *Mastomys* individuals in the Krugersdorp Game Reserve, adults from sites further away from the mine tended to have higher body weights than those closer to the source of contamination (Figure 7).

It is likely that, in *Mastomys* individuals, stronger relationships are seen between heavy metal concentrations in the soil and morphological characteristics due to the ability of heavy metals to leach into the soil. Although rodents are usually exposed to heavy metals primarily through eating vegetation, the captured individuals in this study also burrow (Massawe *et al.* 2008; Meliyo *et al.* 2014), which means that they are directly exposed to high concentrations of heavy metals through contact with the skin, as well as through ingestion when grooming themselves (van den Brink *et al.* 2010; Gall *et al.* 2015). This method of exposure could explain why heavy metals in the soil correlate more strongly with *Mastomys* morphological features than contaminants in the vegetation. This relationship has been studied by Latha and Basha (2015), who found that earthworm species that burrow in heavy metal contaminated soils suffer heavy metal toxicity, resulting in impaired burrowing functionality, and by Bryan and Uysal (1978), who found that ingestion of heavy metals in soils by the burrowing bivalve (*Scrobicularia plana*) could provide an indication of estuary health. Some burrowing mammals such as the European mole (*Talpa europaea*) (Godfrey and Crowcroft 1960; Haeck 1969) and the common vole (*Microtus arvalis*) (Lange *et al*., 2004) create tunnels and hillocks of contaminated substrate which, along with absorption into their own bodies, is then distributed to other areas where it can result in toxicity to more animals (Wijnhoven *et al.* 2006).

Animals exposed to high levels of heavy metal contamination and pollution are generally expected to have smaller morphological features such as body mass and body length than those from less contaminated sites (Sánchez-Chardi *et al*. 2007). As there was no significant difference between the heavy metal levels across the four sites in the reserve, it is not surprising that morphological results did not differ much across captured individuals in the reserve either. It is possible that the rodents in the study are able to tolerate higher levels of contamination (Nunes *et al.* 2001), resulting in less obvious morphological differences between individuals from sites containing higher levels of contamination. Despite no literature focusing on the specific relationship between heavy metal contamination and body or tail length of rodents, some authors have studied the impact of heavy metal contamination on the early development of different species, including birds and fish, and have found that metals associated with mining often affect the development of embryos and wings in animals living near to the source of contamination (Dauwe *et al.* 2006; Jezierska *et al.* 2009).

The average body mass of *Mastomys* individuals between the ages of 1-6 months (weaning to fully grown) ranges from 20-54g, depending on sex (Jackson and van Aarde 2003). Average body length ranges from 90-120mm, with tail length ranging between 80-100mm and hind foot length measuring between 19-23mm for individuals during this period. Adult individuals in this study averaged between 34.57-43.33g for body mass, 8.86-9.83cm for body length, 6.83-8.00cm for tail length and 1.94-2.13cm for hind foot length. While these morphological measurements are within the average growth rate limits for the species, they are lower than the average adult measurement for individuals from uncontaminated sites (Jackson and van Aarde 2003). This suggests that individuals from the Krugersdorp Game Reserve are generally smaller than *Mastomys* individuals that have not been exposed to heavy metal contamination, a finding echoed by Sánchez-Chardi *et al*. (2009b) and Drouhot *et al.* (2014) with reference to other rodents and study locations.

While no significant relationships were discovered between the contamination levels of heavy metals in the soil or vegetation at any of the four study sites, nor between site, soil and vegetation levels, certain heavy metals were found in relatively high concentrations. For example, levels of As and Pb in the soil at the Krugersdorp Game Reserve exceeded the recommended safe oral, topical, mucosal and dermal intake levels for animals according to Govind and Madhuri (2014), which means the soil contains levels of these heavy metals in excess of what a small mammal should be exposed to. High levels of U, As, Cu and Zn in the soil were found at site 3, while site 2 showed high levels of As, Pb, and Zn in the vegetation. These trends are echoed by the tissue data in most cases, with average levels for all heavy metals tested being the highest in the spleen at site 2, and average heavy metal levels in the kidneys proving highest at site 3.

The high levels of heavy metals in the vegetation at sites 1, 2 and 4 have shown a link to heavy metal concentrations in the spleens of individuals from these sites, likely because dietary intake is the most common means of heavy metal accumulation in the body (Demirbaş 1999; Komarnicki 2000). It is suggested that animals ingest contaminated food sources (grass) and heavy metals accumulate in the organs regardless of the biological needs of the animal (Yilmaz 2003). Komarnicki (2000) found that there was a correlation between Pb levels in the spleens of moles (*Talpa europaea* L.) and the levels present in their diet.

As the kidneys and livers are known to be severely affected by heavy metal contamination (Sánchez-Chardi *et al.* 2009a; Sánchez-Chardi *et al.* 2009b), it is not surprising that results from this study show high levels of heavy metals in these organs, particularly at site 3. As the soil and vegetation levels for the majority of the heavy metals tested for in the reserve peak at this site, it is likely that the individuals from this site have bioaccumulated large amounts of the heavy metals into their systems through grooming, feeding and suckling (Hunter and Johnson 1982; Gall *et al.* 2015). Previous studies have found that while the peaks in heavy metal concentrations in the organs of small mammals correlate to a degree with soil and vegetation data, the analyses of soil and vegetation does not reflect the real bioavailability of the heavy metals (Hunter and Johnson 1982; Wren 1986; Folkeson *et al*. 1990; Shore 1995; Komarnicki 2000). It is therefore possible that heavy metals are not statistically correlated with rodent organs in this study, as some of the available heavy metals have been lost through excretion and filtration processes.

4.3 Adult and juvenile morphology

The morphology of the rodents in this study showed strong correlations with heavy metal contamination. In particular, tail length and hind foot length were most significantly correlated with the heavy metal levels in the soil and vegetation. Body length in juveniles was strongly correlated with As, Cu, Fe, Pb, Zn, and U, although this may be because young mice absorb and retain more heavy metals than adult mice (Sánchez-Chardi and Nadal 2007). No significant relationships were found between any heavy metals and body mass of *Mastomys natalensis*, although average body mass was higher at sites further away from the mine than at sites closest to the mine.

Heavy metal contamination in small mammals manifests itself differently in various species, and across age classes (Gall *et al.* 2015). The type of heavy metal that an animal is exposed to also plays a large role in how different age groups respond to the contamination (Sánchez-Chardi and Nadal 2007; Blagojević *et al.* 2012). In some cases, juveniles may be more prone to adverse effects of heavy metals, while in other cases adults will be prone to more toxic effects, as the pollutant has accumulated in their system over a number of years (Sánchez-Chardi and Nadal 2007). For example, heavy metals such as Pb and Cd are known to have a greater impact on adult rodents due to their ability to build up in the animal's system and accumulate to a level of toxicity over time. On the other hand, metals such as Cr are known to be high in juvenile but low in adult wood mice, as they absorb less of this heavy metal as they age (Sánchez-Chardi and Nadal 2007; Gall *et al.* 2015). As juvenile wood mice have a higher energy requirement than adults, they accumulate larger amounts of metals through the intake of food (Lopes et al. 2002; Sánchez-Chardi and Nadal 2007; Gall *et al.* 2015).

In this study, morphological features of juvenile *Mastomys natalensis* were strongly correlated with many more heavy metals than morphological features of adult *Mastomys* individuals. Almost all heavy metals tested for in both the soil and vegetation (U, As, Cu, Pb, Zn, and Fe) were significantly correlated with the body length, tail length and hind foot length as well as body mass of juveniles, while only Fe, As, and U were correlated with adult tail length. This suggests that juvenile *Mastomys natalensis* may be ingesting and storing a larger amount of heavy metals, while adults are able to excrete larger amounts of these metals, such as Cu and Ni (Blagojević *et al.* 2012). There are however a variety of explanations for the trends exhibited by juveniles, for example, placental transfer during pregnancy, grooming, suckling and skin absorption could all contribute to the high levels of heavy metals juveniles are exposed to (Hunter and Johnson 1982; van den Brink et al. 2010; Gall *et al.* 2015).

4.4 Emerging patterns

Despite high levels of heavy metals being found in the spleens of rodents at the reserve, no significant relationship was found between heavy metal concentrations in the spleens of euthanased *Mastomys* individuals and any other tested variables. This is likely due to the low weight of these organs and the small sample size of the study, as in all cases the wet mass of the extracted spleen was below 1g and the mass of the freeze-dried organ was below 0.15g. Although ICP-OES and ICP-MS are capable of detecting trace elements using samples of 50mg (Packer *et al.* 2007), the efficiency of the test could still be compromised due to low sample weights (S. Brown *pers. comm.*). In this study, the kidneys and livers of rodents from site 3 in particular showed high concentrations of all heavy metals tested for, while sites 1, 2 and 4 showed greater heavy metal accumulation in the animals' spleen. When compared with levels of heavy metals such as U, As, Cu and Zn in the soil, it can be seen that there is a possible connection between heavy metal concentrations in the soil and those in the organs within the reserve, particularly at site 3. Hultman *et al.* (1994) showed that the accuracy of determining heavy metal concentrations from the spleens of mice is greatly reduced compared to that of kidneys, due to the small mass of the spleens. Therefore, although levels appear to be high in the spleens of these animals, it is difficult to state anything conclusively about the effect that heavy metals could have on these organs, or on the overall health of the *Mastomys* individual being studied, due to the small samples used for the test.

The acidic nature of the digestive tract is known to provide easy transport for Cd ions in food to animals (Ryu *et al.* 2004; Duca *et al.* 2013), resulting in the uptake of large amounts of this heavy metal and possible associated health issues such as necrosis, apoptosis, proliferation, growth inhibition or even cancer (Duca *et al.* 2013). The mechanisms leading to high levels and prolonged exposure to cadmium (Cd) are known to result in kidney and liver damage, lesions and hepatic necrosis (Andersen *et al.* 1988). In particular, kidneys are negatively affected by cadmium toxicity (Johri *et al.* 2010), and often contain the highest levels of Cd in the animal's body, compared to other organs (Saygi *et al*. 1991). The kidneys of euthanased *Mastomys* individuals from within the reserve, particularly those from site 1, exhibited this trend when compared to the liver (Figures 8 to 11).

Although not statistically significant, Pb levels were generally high in the livers and kidneys of *Mastomys* individuals within the Krugersdorp Game Reserve. This is not surprising, as the target organs (i.e. liver and kidneys) of many small ground-dwelling mammals from sites contaminated by heavy metals usually show increased levels of Pb and Cd (Ma *et al*. 1991). As food is the primary means through which many mammals accumulate heavy metals in their systems (Ma *et al.* 1991), vegetation in particular can play a significant role in the accumulation of heavy metals in rodents. Lead poisoning is a toxicological problem that is most frequently diagnosed by veterinary professionals (Madejón 2002). As previously mentioned, the small particle sizes of Pb oxides and sulphates may contribute to the presence of Pb in a number of organs (Kesler and Simon 2015). Dieter *et al.* (1993) found that Pb in more soluble forms (lead oxide and lead acetate) being fed to rats resulted in higher concentrations of the heavy metal remaining in organs such as the liver and kidneys. In a study conducted on wild rats in Ghana by Bortey-Sam *et al. (*2016), it was found that Pb levels in the kidneys could cause structural and functional kidney damage in 6% of the individuals and cause intra-nuclear inclusion bodies and karyocytomegaly in the proximal tubular cells in 29%.

Nickel concentrations in vegetation and the livers of *Mastomys* adults in the reserve were positively correlated ($r=0.05901$; $p=0.0206$). It is possible that higher levels of Ni in the vegetation lead to increased levels of Ni in the liver. In excess, Nickel is an extremely toxic substance and is known to be carcinogenic and result in changes to the structure of plants and environments (Eisler 1998).

As the main route of exposure of small mammals to heavy metals is through food (Ma *et al.* 1991), vegetation samples were expected to be largely correlated with tissue samples from the organs of *Mastomys* individuals within the Krugersdorp Game Reserve. This was however not the case in this study, as no significant relationships were found between heavy metal levels in vegetation samples and the organs of dissected individuals. It is possible that this is due to *Mastomys* sp.'s diet, as a number of studies have shown that herbivorous small mammals are less likely to accumulate heavy metals than carnivorous ones (Ma *et al.* 1991; Hamers *et al.* 2006; Gall *et al.* 2015). Heavy metal levels in the vegetation were particularly high at sites 1, 2 and 4. This trend is echoed in the contamination levels of the dissected spleens from individuals at these sites (Figures 7, 8 and 10). As such, it is possible that these heavy metals are accumulating in the spleens of rodents in the reserve, as was the case in rats studied by Buchman *et al.* (2001), where splenic concentrations of heavy metals after long term parenteral nutrition were higher than in other organs tested, such as the liver.

Based on observations made during trapping sessions and the dissection of euthanased individuals, it is likely that a number of rodents in the Krugersdorp Game Reserve suffer from liver disease, necrosis and skin lesions (Damek-Poprawa and Sawicka-Kapusta 2003; Sánchez-Chardi *et al*. 2009b). These results suggest that while heavy metal levels seem to have the greatest impact on the spleens of small mammals closest to the mine, individuals throughout the Krugersdorp Game Reserve are also strongly influenced by the contaminants in the environment. During the dissection of the euthanased rodents, the organs of individuals from site 3, one of the sites furthest from the source of contamination, exhibited abnormal physiological characteristics. As heavy metal levels in the soil were particularly high at this site, it is possible that the individuals from this site have accumulated higher amounts of heavy metals than those elsewhere in the reserve through direct contact with highly contaminated soil and are therefore more obviously affected by the high levels of heavy metals in the environment in the form of growths and skin lesions. High accumulation of heavy metals in the livers and kidneys at site 3 (Figure 9) could be reason for the discolouration and distorted appearance of these organs, possibly indicating disease due to exposure to heavy metals (Andersen *et al.* 1988).

4.5 Potential risks to small mammals in Krugersdorp Game Reserve

As the sample size for this study was relatively small, literature was used to provide additional insight into possible risks that the small mammals within the reserve could be facing. Although limited data were acquired during this project, some trends were evident and will be discussed further in context of previous studies and other literature.

The sites studied in the Krugersdorp Game Reserve were all contaminated by heavy metals in both the soil and the vegetation. The entire reserve is therefore treated as an area contaminated with heavy metals such as Pb, Zn, Ni, U, Fe, Cu, Cd and As. The presence of these heavy metals in the soil, vegetation and organs of rodents in high levels within the reserve (Table 1), means that heavy metals from the mine close to the reserve are indeed affecting the environment and therefore likely the small mammals within the reserve as well (Buchauer 1973; Jordan and Lechevalier 1975; Beyer *et al.* 1985).

Zinc, Cu and Fe are considered to be essential in the function and survival of living organisms, unless found in high concentrations, when they can then become responsible for producing toxic effects (Angelova *et al.* 2004). As Zn concentrations were particularly high in the soil, vegetation and organ samples collected during this study, it is likely that small mammals are particularly at risk of being negatively affected by this heavy metal. Examples of mammalian diseases associated with Zn include cancer (Costello *et al.* 1999; Taylor *et al.* 2008) and kidney damage and necrosis (Damek-Poprawa and Sawicka-Kapusta 2003). The small mammals in the Krugersdorp Game Reserve, having been exposed to high concentrations of Zn, are likely to be affected by or are at risk of developing chronic illnesses such as these. Cellular damage and inflammation are also a high risk due to the presence of toxic heavy metals in the environment within the reserve (Gojova *et al*. 2007). The highest levels of zinc in the vegetation and soil were at site 2 and 3 respectively. This pattern is echoed by high levels of Zn in the spleens at site 2, as well as the liver and kidneys at site 3 (Table 1, Figure 9).

While some heavy metals are necessary for normal growth and function, others such as Pb and Cd are considered to be toxic elements (Shore and Rattner 2001). Cadmium and lead toxicity has been proven to cause histopathological changes in rodents (Damek-Poprawa and Sawicka-Kapusta 2003). Lead is known to be particularly harmful to the renal systems of small mammals (Nolan and Shaikh 1992), although liver cirrhosis, damage to the nervous system and anaemia are also side-effects of lead poisoning (Sheffield *et al.* 2001). As relatively high levels of Pb were found in the soil and vegetation samples taken from the reserve (Table 1), and strong correlations existed between this heavy metal and a number of morphological features of the rodents studied (Table 2), it can be suggested that the small mammals within the Krugersdorp Game Reserve are at risk of experiencing some of these health issues. Tissue samples collected from *Mastomys* individuals within the reserve did however not yield significant correlations to Pb, possibly due to the small sample size, a low accumulation ratio of Pb based on shorter duration of exposure to the element (Fowler *et al.* 1980; Hirsch 1973), or lower levels of calcium in their diet (Bogden *et al.* 1991; Nolan and Shaikh 1992), as higher levels of calcium in a rat's diet have been shown to increase the harmful effects of lead toxicity (Bogden *et al.* 1991). Despite no statically significant data supporting the definite presence of lead toxicity in small mammals within the reserve, observations made during dissections suggest that some of the individuals in the study may have suffered from liver cirrhosis. In particular, individuals from site 1 were likely impacted by Pb, as a high concentration of this heavy metal in the soil at this site may be connected to high levels of Pb in the livers and kidneys of *Mastomys* individuals. As with other heavy metals in the reserve, Pb peaks in the spleens of rodents at site 2, suggesting that Pb in the vegetation is bioaccumulated by the animals studied. In the livers and kidneys of individuals from site 1, Pb accumulation is higher than other sites (Figure 7).

Damek-Poprawa and Sawicka-Kapusta (2003) showed that rodents from the wild were more sensitive to heavy metals than those that were involved in laboratory experiments. This is likely because wild populations have higher energy requirements due to the expenses incurred by searching for food, providing food for offspring, defending territories, and thermoregulation (Shore and Rattner 2001). It is suggested that less energy is possibly devoted to detoxification in the field due to these actions (Damek-Poprawa and Sawicka-Kapusta 2003). As the Krugersdorp Game Reserve is considered to be a natural environment, rodents in the reserve are expected to conform to this trend, as they face the same tasks requiring high energy use. Therefore, it is possible that the small mammal populations within the reserve could be at risk of the effects of severe heavy metal toxicity due to their inability to detoxify and excrete high levels of heavy metals from their system.

The apparent link between soil and vegetation results and the high levels of heavy metals in the internal organs of rodents in the reserve suggest that the small mammals are accumulating excessive amounts of heavy metals through dietary intake and direct exposure to soil. Although studies have shown that levels of heavy metals in soil and vegetation samples do not directly reflect the accumulation of heavy metals in organs (Hunter and Johnson 1982; Wren 1986; Folkeson *et al*. 1990; Komarnicki 2000), the fact that peaks in soil and vegetation correspond to peaks in tissue results in this study suggests that animals in the reserve are at risk of heavy metal toxicity and organ disease, given observations made during the dissection process, as well as patterns found between soil and vegetation data and heavy metal presence in tissue samples.

4.6 Conclusion

This study showed that rodents further from the source of heavy metal pollution (the mine near the game reserve in this case), were not significantly less impacted by these contaminants internally than those closest to the mine. As different heavy metal concentrations were found in the soil, vegetation, and organs from rodents in the reserve taken from sites varying distances from mine, it must be considered that the resulting heavy metal pollution may still be accumulating in the surroundings at further sites, or that the species composition with regards to riparian vegetation at these sites is allowing for lower levels in the soil and animals by absorbing a large amount of heavy metals, so that lower levels of these heavy metals are found in the soil and so that these particles are not readily absorbed by the terrestrial plants (Turnau and Mesjasz-Przybylowicz 2003).

Various remediation efforts have been made to try to reverse the contamination and AMD caused by the mining activities, although the AMD problem in the reserve remains severe (Cobbing 2008). It is recommended that these efforts be monitored closely, and that regular evaluations of water, soil, and vegetation quality be done in order to ensure that animals within the Krugersdorp Game Reserve are not exposed to more harmful pollutants. While it is recognised that remediation efforts are expensive and usually lengthy, the monitoring is an essential way of ensuring that the animals within the reserve are not exposed to contaminants that may cause disease and death.

4.7 Limitations to study

A small sample size existed for the majority of the study sites due to a low capture rate. A number of possibilities exist for this, including; rodents avoiding traps altogether or after being caught once, study area being too small, high death or emigration rates of rodents and extreme temperatures and drought (Krebs *et al.* 1996; Kovats 2003). During the sampling period, very little rain was experienced. As drought could contribute to a low trap rate and the ability of populations to grow and thrive, it is possible that this influenced the final sample size (Lunney *et al.* 1987).

Unfortunately, due to budget restraints, only limited heavy metals could be tested for within the soil, vegetation, and organ samples that were submitted for analysis Initially, it was expected that soil and vegetation from sites furthest from the mine would contain the lowest levels of heavy metals and the sites closest to the source of contamination would test significantly higher for all heavy metals, forming a contamination gradient through the reserve along the Tweelopies Spruit. As this was not the case and no statistical gradient was present, limitations to conclusions that can be drawn about contaminated and minimally contaminated sites existed, and literature had to be used to compare contamination levels. Patterns which emerged in this study regarding contamination levels and possible links between environmental components and tissue samples were related back to literature as no statistically significant correlations were found between these factors and across sites.

REFERENCES

Abdul-Wahab, S. and Marikar, F. 2012. The environmental impact of gold mines: pollution by heavy metals. *Open Engineering*, 2(2): 304-313.

Akcil, A. and Koldas, S. 2006. Acid Mine Drainage (AMD): causes, treatment and case studies. *Journal of Cleaner Production*, 14(12-13): 1139-1145.

Acosta J.A., Faz A., Martinez-Martinez S., Zornoza R., Carmona D.M. and Kabas S. 2011. Multivariate statistical and GIS-based approach to evaluate heavy metals behaviour in mine sites for future reclamation. *Journal of Geochemical Exploration*, 109: 8–17.

Adham, K. G., Al-Eisa, N. A. and Farhood, M. H. 2011. Impact of heavy metal pollution on the hemogram and serum biochemistry of the libyan jird, Meriones libycus. *Chemosphere*, 84(10): 1408-1415.

Adler R. and Rascher, J. 2007. A Strategy for the Management of Acid Mine Drainage from Gold Mines in Gauteng. *CSIR report Number CSIR/NRE/PW/ER/2007/0053/C*. Pretoria, South Africa.

Adler, R. A., Claassen, M., Godfrey, L. and Turton, A. R. 2007. Water, mining, and waste: an historical and economic perspective on conflict management in South Africa. *The economics of peace and security journal*, 2(2): 32-41.

Agency for Toxic Substances and Disease Registry (ATSDR). 2016. Pp. 1-347 *in* Minimal Risk Levels (MRLs) for Hazardous Substances. ATSDR. Washington, DC.

Akabzaa, T. M. 2001. *Boom and dislocation: The environmental and social impacts of mining in the Wassa West District of Ghana*. Third World Network-Africa, Accra, Ghana.

Alloway, B. J. 1995. Heavy Metals in Soils., 1995. *Blackie Academic and Professional, London*, 368.

Alloway, B. J. 2013. Heavy metals and metalloids as micronutrients for plants and animals. Pp. 195-209 *in Heavy metals in soils.* Springer, Netherlands.

Anawar, H. M., Canha, N., Santa-Regina, I. and Freitas, M. C. 2013. Adaptation, tolerance, and evolution of plant species in a pyrite mine in response to contamination level and properties of mine tailings: sustainable rehabilitation. *Journal of soils and sediments*, 13(4): 730-741.

Andersen, O., Nielsen, J. B. and Svendsen, P. 1988. Oral cadmium chloride intoxication in mice: effects of dose on tissue damage, intestinal absorption and relative organ distribution. *Toxicology*, 48(3): 225-236.

Angelova V., Ivanova R., Delibaltova V. and Ivanov K. 2004.Bio-accumulation and distribution of heavy metals in fiber crops (flax, cotton, and hemp). *Industrial Crops and Products*, 19: 197.

Angelova, V. R., Ivanova, R. V., Todorov, J. M. and Ivanov, K. I. 2010. Lead, cadmium, zinc, and copper bioavailability in the soil-plant-animal system in a polluted area. *The Scientific World Journal*, 10: 273-285.

AngloGold Ashanti. 2004. Case studies. Woodlands project — good progress being made with phytoremediation project. Environment – AngloGold Ashanti Report to Society.

Artwohl, J., Brown, P., Coming, B. and Stein, S. 2006. Report of the ACLAM Task Force on Rodent Euthanasia. *Journal of the American Association for Laboratory Animal Science*, 45(1): 98-105.

Attuquayefio, D. K., Owusu, E. H. and Ofori, B. Y. 2017. Impact of mining and forest regeneration on small mammal biodiversity in the Western Region of Ghana. *Environmental monitoring and assessment*, 189(5): 237.

Auffray, J. C., Renaud, S. and Claude, J. 2009. Rodent biodiversity in changing environments. *Kasetsart Journal, Natural Sciences*, 43(1): 83-93.

Bakkaus, E., Gouget, B., Gallien, J. P., Khodja, H., Carrot, F., Morel, J. L. and Collins, R. 2005. Concentration and distribution of cobalt in higher plants: the use of micro-PIXE spectroscopy. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms*, 231(1-4): 350-356.

Bempah, C. K., Ewusi, A., Obiri-Yeboah, S., Asabere, S. B., Mensah, F., Boateng, J. and Voigt, H. J. 2013. Distribution of arsenic and heavy metals from mine tailings dams at Obuasi municipality of Ghana. *American Journal of Engineering Research,* 2(5): 61-70.

Buchauer, M. J. 1973. Contamination of soil and vegetation near a zinc smelter by zinc, cadmium, copper, and lead. *Environmental Science & Technology*, 7(2): 131-135.

Bhattacharjee, T., Bhattacharjee, S. and Choudhuri, D. 2016. Hepatotoxic and nephrotoxic effects of chronic low dose exposure to a mixture of heavy metals–lead, cadmium and arsenic. *International Journal of Pharmaceutical, Chemical and Biological Sciences,* 6(1): 39-47.

Bennett, N. C., Jarvis, J. U. M. and Davies, K. C. 1988. Daily and seasonal temperatures in the burrows of African rodent moles. *South African Journal of Zoology*, 23(3): 189-195.

Beyer, W. N., Pattee, O. H., Sileo, L., Hoffman, D. J. and Mulhern, B. M. 1985. Metal contamination in wildlife living near two zinc smelters. *Environmental Pollution Series A, Ecological and Biological*, 38(1): 63-86.

Blagojević, J., Jovanović, V., Stamenković, G., Jojić, V., Bugarski-Stanojević, V., Adnađević, T. and Vujošević, M. 2012. Age differences in bioaccumulation of heavy metals in populations of the black-striped field mouse, *Apodemusagrarius* (Rodentia, Mammalia). *International Journal of Environmental Research*, *6*(4): 1045-1052.

Blum, M. E. 2014. Disturbance by Large Scale Gold Mining: Effects on Long Distance Migration in the Ruby Mountains of Eastern Nevada. MSc thesis. University of Nevada, Nevada, USA.

Bogden, J. D., Gertner, S. B., Kemp, F. W., McLeod, R., Bruening, K. S. and Chung, H. R. 1991. Dietary lead and calcium: effects on blood pressure and renal neoplasia in Wistar rats. *The Journal of nutrition*, 121(5): 718-728.

Bohrer, G., Brandes, D., Mandel, J. T., Bildstein, K. L., Miller, T. A., Lanzone, M., Katzner, T., Maisonneuve, C. and Tremblay, J. A. 2012. Estimating updraft velocity components over large spatial scales: contrasting migration strategies of golden eagles and turkey vultures. *Ecology Letters*, 15(2): 96-103.

Booyse, D. G., Dehority, B. A. and Myburgh, J. G. 2015. The effect of acid drinking water on rumen protozoa in the blesbok (*Damaliscus dorcas phillipsi*). *Zootaxa*, 4052(5): 577-582.

Boularbah, A., Schwartz, C. and Morel, J.L. 2006. Heavy metal contamination from mining sites in South Morocco: 2. Assessment of metal accumulation and toxicity in plants. *Chemosphere*, 63: 811-817.

Bortey-Sam, N., Nakayama, S. M., Ikenaka, Y., Akoto, O., Baidoo, E., Mizukawa, H. and Ishizuka, M. 2016. Heavy metals and metalloid accumulation in livers and kidneys of wild rats around gold-mining communities in Tarkwa, Ghana. *Journal of Environmental Chemistry and Ecotoxicology*, 8(7): 58-68.

Broemme, K., and Stolpe, H. 2007. Developing environmental concepts for Vietnamese coal mines. *International Workshop Geoecology and Environmental Technology*, 25: 218-222.

Bryan, G. W. and Langston, W. J. 1992. Bioavailability, accumulation and effects of heavy metals in sediments with special reference to United Kingdom estuaries: a review. *Environmental pollution*, 76(2): 89-131.

Bryan, G. W. and Uysal, H. 1978. Heavy metals in the burrowing bivalve *Scrobicularia plana* from the Tamar estuary in relation to environmental levels. *Journal of the marine biological association of the United Kingdom*, 58(1): 89-108.

Buchman, A. L., Neely, M., Grossie, V. B., Truong, L., Lykissa, E. and Ahn, C. 2001. Organ heavy-metal accumulation during parenteral nutrition is associated with pathologic abnormalities in rats. *Nutrition*, 17(7): 600-606.

Caballero-Gallardo, K. and Olivero-Verbel, J. 2016. Mice housed on coal dust-contaminated sand: A model to evaluate the impacts of coal mining on health. *Toxicology and Applied Pharmacology*. 294: 11-20.

Cardinale, B. J., Duffy, J. E., Gonzalez, A., Hooper, D. U., Perrings, C., Venail, P., Narwani, A., Mace, G.M., Tilman, D., Wardle, D.A. and Kinzig, A.P. 2012. Biodiversity loss and its impact on humanity. *Nature*, 486(7401): 59–67.

Cataldo, D. A. and Wildung, R. E. 1978. Soil and plant factors influencing the accumulation of heavy metals by plants. *Environmental Health Perspectives*, 27: 149-159.

Chamber of Mines of South Africa. 2013. *SA Chamber of Mines Facts and Figures 2013/2014*.

Chapman, T., Rymer, T. and Pillay, N. 2012. Behavioural correlates of urbanisation in the Cape ground squirrel *Xerus inauris*. *Naturwissenschaften*, 99(11): 893-902.

Chauhan, S. S. 2010. Mining, development and environment: a case study of Bijolia mining area in Rajasthan, India. *Journal of human ecology*, 31(1): 65-72.

Chowdhury, A. R. 2009. Recent advances in heavy metals induced effect on male reproductive function-A retrospective. *Al Ameen J Med Sci*, 2(2): 37-42.

Clemens, S. and Ma, J. F. 2016. Toxic heavy metal and metalloid accumulation in crop plants and foods. *Annual review of plant biology*, 67: 489-512.

Cobbing, J. E. 2008. Institutional linkages and acid mine drainage: The case of the Western basin in South Africa. *International Journal of Water Resources Development*, 24(3): 451-462.

Coetzee, C. G. 1965. The breeding season of the multimammate mouse *Praomys* (*Mastomys*) *natalensis* (A. Smith) in the Transvaal Highveld. *African Zoology*, 1(1): 29-39.

Coetzee, C. G. 1975. The biology, behaviour, and ecology of *Mastomys natalensis* in southern Africa. *Bulletin of the World Health Organization*, 52(4-6): 637.

Coetzee, H., Horstmann, U., Ntsume, G. and Croukamp, L. 2003. The potential environmental impact of the decant of water from Witwatersrand. *Proc, 8th International Congr on Mine Water and the Environment, Johannesburg*, 201-217.

Coetzee, H., Chirenje, E., Hobbs, P. and Cole, J. 2009. Ground and airborne geophysical surveys identify potential subsurface acid mine drainage pathways in the Krugersdorp Game Reserve, Gauteng Province, South Africa. In *11th SAGA Biennial Technical Meeting and Exhibition*.

Costello, L.C. Liu, Y., Zou, J., Franklin, R.B. 1999. Evidence for a zinc uptake transporter in human prostate cancer cells which is regulated by prolactin and testosterone. *Journal of Biological Chemistry*, 274: 17499-17504.

Clemens, S. and Ma, J. F. 2016. Toxic heavy metal and metalloid accumulation in crop plants and foods. *Annual review of plant biology*, 67: 489-512.

Curtis, B. A. and Perrin, M. R. 1979. Food preferences of the vlei rat (*Otomys irroratus*) and the four-striped mouse (*Rhabdomys pumilio*). *South African Journal of Zoology*, 14(4): 224-229.

Damek-Poprawa, M. and Sawicka-Kapusta, K. 2003. Damage to the liver, kidney, and testis with reference to burden of heavy metals in yellow-necked mice from areas around steelworks and zinc smelters in Poland. *Toxicology*, 186(1): 1-10.

Dauwe, T., Janssens, E. and Eens, M. 2006. Effects of heavy metal exposure on the condition and health of adult great tits (Parus major). *Environmental Pollution*, 140(1): 71-78.

Davies, K. C. and Jarvis, J. U. 1986. The burrow systems and burrowing dynamics of the mole-rats *Bathyergus suillus* and *Cryptomys hottentotus* in the fynbos of the south-western Cape, South Africa. *Journal of Zoology*, 209(1): 125-147.

Demirbaş, A. 1999. Proximate and heavy metal composition in chicken meat and tissues. *Food chemistry*, 67(1): 27-31.

Department of Environmental Affairs. 2010. *Framework for the Management of Contaminated Land*. Department of Environmental Affairs, Pretoria, South Africa.

Department of Water Affairs and Forestry. 2004. *Olifants Water Management Area: Internal Strategic Perspective. Report P WMA 04/0000/00/0304*. Department of Water Affairs and Forestry, Pretoria, South Africa.

Dieter, M. P., Matthews, H. B., Jeffcoat, R. A. and Moseman, R. F. 1993. Comparison of lead bioavailability in F344 rats fed lead acetate, lead oxide, lead sulfide, or lead ore concentrate from Skagway, Alaska. *Journal of Toxicology and Environmental Health, Part A Current Issues*, 39(1): 79-93.

Donato, D. B., Nichols, O., Possingham, H., Moore, M., Ricci, P. F. and Noller, B. N. 2007. A critical review of the effects of gold cyanide-bearing tailings solutions on wildlife. *Environment international*, 33(7): 974-984.

Drouhot, S., Raoul, F., Crini, N., Tougard, C., Prudent, A. S., Druart, C., Rieffel, D., Lambert, J., Tête, N., Giraudoux, P. and Scheifler, R. 2014. Responses of wild small mammals to arsenic pollution at a partially remediated mining site in Southern France. *Science of the Total Environment*, 470: 1012-1022.

Dube, A., Zbytniewski, R., Kowalkowski, T., Cukrowska, E. and Buszewski, B. 2001. Adsorption and migration of heavy metals in soil. *Polish journal of environmental studies,* 10(1): 1-10.

Duca, F. A., Swartz, T. D., Sakar, Y. and Covasa, M. 2013. Decreased intestinal nutrient response in diet-induced obese rats: role of gut peptides and nutrient receptors. *International journal of obesity*, 37(3): 375.

Dudka, S. and Adriano, D. C. 1997. Environmental impacts of metal ore mining and processing: a review. *Journal of environmental quality*, 26(3): 590-602.

Durand, J. F., Meeuvis, J. and Fourie, M. 2010. The threat of mine effluent to the UNESCO status of the Cradle of Humankind World Heritage Site.

Durand, J. F. 2012. The impact of gold mining on the Witwatersrand on the rivers and karst system of Gauteng and North West Province, South Africa. *Journal of African Earth Sciences*, 68: 24-43.

Durucan, S., Korre, A. and Munoz-Melendez, G. 2006. Mining life cycle modelling: a cradle-to-gate approach to environmental management in the minerals industry. *Journal of Cleaner Production*, 14(12-13): 1057-1070.

Duruibe, J. O., Ogwuegbu, M. O. C. and Egwurugwu, J. N. 2007. Heavy metal pollution and human biotoxic effects. *International Journal of Physical Sciences*, 2(5): 112-118.

Du Toit, S. 2006. Practical applications – Effects of mine water drainage on the Krugersdorp Game Reserve. Pp. 8 *in Procedural Conference on Mine Water Decant.* 14-15 October 2006, Randfontein, South Africa. Mine Water Division, Water Institute of South Africa.

Ebbs, S. D. and Kochian, L. V. 1997. Toxicity of zinc and copper to Brassica species: implications for phytoremediation. *Journal of Environmental Quality*, 26(3): 776-781.

Edem, C. A., Akpan, B. and Dosunmu, M. I. 2008. A comparative assessment of heavy metals and hydrocarbon accumulation in *Sphyrena afra, Orechromis niloticus* and *Lops lacerta* from Anantigha Beach market in Calabar-Nigeria. *African. Journal of Environmental Pollution and Health* 6: 61-64.

Eisler, R. and Wiemeyer, S. N. 2004. Cyanide hazards to plants and animals from gold mining and related water issues. Pp. 21-54 *in Reviews of environmental contamination and toxicology*, Springer, New York, NY.

Ekpo, F. E., Agu, N. N., and Udoakpan, U. I. 2013. Influence of heavy metals concentration in three common fish, sediment and water collected within quarry environment, Akamkpa LG Area, Cross River State, Nigeria. *European Journal of Toxicological Sciences*, 2013(3): 1-11.

Ellias, L. D. 2009. Chemical and toxicological studies on hazardous waste sites in Harris country. Doctoral dissertation, M. Sc. Thesis. Faculty of Science, Texas Solution University, USA.

Espinosa-Reyes, G., Torres-Dosal, A., Ilizaliturri, C., Gonzalez-Mille, D., Diaz-Barriga, F. and Mejia-Saavedra, J. 2010. Wild rodents (Dipodomys merriami) used as biomonitors in contaminated mining sites. *Journal of Environmental Science and Health, Part A* 45(1): 82-89.

Espinosa-Reyes, G., González-Mille, D. J., Ilizaliturri-Hernández, C. A., Mejía-Saavedra, J., Cilia-López, V. G., Costilla-Salazar, R. and Díaz-Barriga, F. 2014. Effect of mining activities in biotic communities of Villa de la Paz, San Luis Potosi, Mexico. *BioMed research international*, 2014: 1-14.

Farrington, J. D. 2005. The impact of mining activities on Mongolia's protected areas: a status report with policy recommendations. *Integrated Environmental Assessment and Management,* 1(3): 283-289.

Folkeson, L., Nyholm, N.E.I. and Tyle, G. 1990. Influence of acidity and other soil properties on metal concentrations inforest plants and animals. *Science of the Total Environment,* 96: 211-233.

Fourie, M. 2005. A rising acid tide. *Mail and Guardian* (online), 12 April. (http://mg.co.za/article/2005-04-12-a-rising-acid-tide/). Accessed on 28 August 2015.

Fowler, B. A., Kimmel, C. A., Woods, J. S., McConnell, E. E. and Grant, L. D. 1980. Chronic low-level lead toxicity in the rat: III. An integrated assessment of long-term toxicity with special reference to the kidney. *Toxicology and applied pharmacology*, 56(1): 59-77.

Frankham, R., Ballou, J.D. and Briscoes, D.A. 2010. Pp. 642 *in* Introduction to Conservation Genetics 2nd edition. Cambridge University Press, Cambridge.

Frid, A. and Dill, L. M. 2002. Human-caused disturbance stimuli as a form of predation risk. *Conservation Ecology*, 6(1): 11.

Frimmel, H. E. 2005. Archaean atmospheric evolution: evidence from the Witwatersrand gold fields, South Africa. *Earth-Science Reviews*, 70(1): 1-46.

Fritsch, C., Cosson, R. P., Cœurdassier, M., Raoul, F., Giraudoux, P., Crini, N., de Vaufleury, A. and Scheifler, R. 2010. Responses of wild small mammals to a pollution gradient: host factors influence metal and metallothionein levels. *Environmental Pollution*, 158(3): 827-840.

Fryer M., Collins C.D., Ferrier H., Colvile R.N. and Nieuwenhuijsen M.J. 2006. Human exposure modelling for chemical risk assessment: a review of current approaches and research and policy implications. *Environmental Science and Policy*, 9: 261–74

Fujimori, T. and Takigami, H. 2014. Pollution distribution of heavy metals in surface soil at an informal electronic-waste recycling site. *Environmental geochemistry and health*, 36(1): 159-168.

Gall, J. E., Boyd, R. S. and Rajakaruna, N. 2015. Transfer of heavy metals through terrestrial food webs: a review. *Environmental monitoring and assessment*, 187(4): 201.

Garden, J. G., McAlpine, C. A. and Possingham, H. P. 2010. Multi-scaled habitat considerations for conserving urban biodiversity: native reptiles and small mammals in Brisbane, Australia. *Landscape Ecology*, 25(7): 1013-1028.

Gibson, L., Lynam, A. J., Bradshaw, C. J., He, F., Bickford, D. P., Woodruff, D. S., Bumrungsri, S. and Laurance, W. F. 2013. Near-complete extinction of native small mammal fauna 25 years after forest fragmentation. *Science,* 341(6153): 1508-1510.

Glasby, G. P. and Szefer, P. 1998. Marine pollution in Gdansk Bay, Puck Bay and the Vistula lagoon, Poland: an overview. *Science of the Total Environment*, 212(1): 49-57.

Godfrey, G.K. and Crowcroft, P. 1960. The Life of the Mole. Museum Press, London.

Gojova, A., Guo, B., Kota, R. S., Rutledge, J. C., Kennedy, I. M. and Barakat, A. I. 2007. Induction of inflammation in vascular endothelial cells by metal oxide nanoparticles: effect of particle composition. *Environmental health perspectives*, 115(3): 403.

Gorbunova, V., Bozzella, M. J. and Seluanov, A. 2008. Rodents for comparative aging studies: from mice to beavers. *Age*, 30(2-3): 111-119.

Govind, P. and Madhuri, S. 2014. Heavy metals causing toxicity in animals and fishes. *Research Journal of Animal, Veterinary and Fishery Sciences*, 2(2): 17-23.

Grimm, N.B., Foster, D., Groffman, P., Grove, J.M., Hopkinson, C.S., Nadelhoffer, K.J., Pataki, D.E. and Peters, D. 2008. The changing landscape: ecosystem responses to urbanisation and pollution across climatic and social gradients. *Frontiers in Ecology and the Environment*, 6: 264-272.

Guivier, E., Galan, M., Chaval, Y., Xuéreb, A., Ribas Salvador, A., Poulle, M. L., Voutilainen, L., Henttonen, H., Charbonnel, N. and Cosson, J. F. 2011. Landscape genetics highlights the role of bank vole metapopulation dynamics in the epidemiology of Puumala hantavirus. *Molecular ecology*, 20(17): 3569-3583.

Haeck, J. 1969. Dieren in Zuidelijk Flevoland 1968. *De Levende Natuur*, 72(4):79-85.

Hamers, T., Van den Berg, J. H., Van Gestel, C. A., Van Schooten, F. J. and Murk, A. J. 2006. Risk assessment of metals and organic pollutants for herbivorous and carnivorous small mammal food chains in a polluted floodplain (Biesbosch, The Netherlands). *Environmental Pollution*, 144(2): 581-595.

Hamilton. S.J. 2004. Review of selenium toxicity in the aquatic food chain. *Science of the Total Environment* 326: 1-31.

Hendriks, A. J., Ma, W. C., Brouns, J. D., de Ruiter-Dijkman, E. M. and Gast, R. 1995. Modelling and monitoring organochlorine and heavy metal accumulation in soils, earthworms, and shrews in Rhine-delta floodplains. *Archives of Environmental Contamination and Toxicology*, 29(1): 115-127.

Hirsch, G. H. 1973. Effect of chronic lead treatment on renal function. *Toxicology and applied pharmacology*, 25(1): 84-93.

Hobbs, P. J. and Cobbing, J. E. 2007. Hydrogeological assessment of Acid Mine Drainage impacts in the West Rand Basin, Gauteng Province. *CSIR*, CSIR/NRE/WR/ER/2007/0097/C.

Holguin, G., Vazquez, P. and Bashan, Y. 2001. The role of sediment microorganisms in the productivity, conservation, and rehabilitation of mangrove ecosystems: an overview. *Biology and fertility of soils*, 33(4): 265-278.

Hultman, P., Johansson, U., Turley, S. J., Lindh, U., Eneström, S. and Pollard, K. M. 1994. Adverse immunological effects and autoimmunity induced by dental amalgam and alloy in mice. *The FASEB Journal*, 8(14): 1183-1190.

Hunter, B. A. and Johnson, M. S. 1982. Food chain relationships of copper and cadmium in contaminated grassland ecosystems. *Oikos*, 38: 108–117.

Hustrulid, W.A. *Underground mining methods handbook*. United States.

Jackson, T. P. and van Aarde, R.J. 2003. Sex- and Species-Specific Growth Patterns in Cryptic African Rodents, *Mastomys natalensis* and *M. coucha*. *Journal of Mammalogy*, 84(3): 851-860.

Jezierska, B., Ługowska, K. and Witeska, M. 2009. The effects of heavy metals on embryonic development of fish (a review). *Fish physiology and biochemistry*, 35(4): 625-640.

Johnson, D. B. and Hallberg, K. B. 2005. Acid mine drainage remediation options: a review. *Science of the total environment*, 338(1-2): 3-14.

Johri, N., Jacquillet, G. and Unwin, R. 2010. Heavy metal poisoning: the effects of cadmium on the kidney. *Biometals*, 23(5): 783-792.

Jordan, M. J. and Lechevalier, M. P. 1975. Effects of zinc-smelter emissions on forest soil microflora. *Canadian Journal of Microbiology*, 21(11): 1855-1865.

Kabata-Pendias, A. 2004. Soil–plant transfer of trace elements—an environmental issue. *Geoderma*, 122(2): 143-149.

Kasso, M. and Bekele, A. 2014. Threats to Mammals on Fragmented Habitats around Asella Town, Central Ethiopia. *International Journal of Biodiversity* 2014: 1-8.

Kataev, G. D., Suornela, J. and Palokangas, P. 1994. Densities of microtime rodents along a pollution gradient from a copper-nickel smelter. *Oecologia*, 97(4): 491-498.

Kesler, S. E. and Simon, A. C. 2015. *Mineral resources, economics and the environment*. Cambridge University Press.

Kim, M., Ahn, K. and Jung, Y. 2000. Distribution of inorganic species in mine tailings of abandoned mines from Korea. *Chemosphere*, 49: 307-312.

Kitao, M., Lei, T. T. and Koike, T. 1997. Effects of manganese toxicity on photosynthesis of white birch (Betula platyphylla var. japonica) seedlings. *Physiologia Plantarum*, 101(2): 249-256.

Kitula, A. G. N. 2006. The environmental and socio-economic impacts of mining on local livelihoods in Tanzania: A case study of Geita District. *Journal of cleaner production*, 14(3-4): 405-414.

Kloke, A., Sauerbeck, D. R. and Vetter, H. 1984. The contamination of plants and soils with heavy metals and the transport of metals in terrestrial food chains. Pp. 113-141 *in* Nriagu J.O., editor, *Changing metal cycles and human health*. Springer, Berlin, Heidelberg.

Klosinska, T. 1996. Ecological characteristics of the bank vole population in the habitat contaminated with heavy metals. *Polish Ecological Studies*, 22: 51-59.

Knight, D. 2001. Tanzanian gold mine pollution causing deaths. *The Journal of cleaner production*, 9(2): 95-189.

Koldas, S. 2000. Notes of environmental inspections in South African Mines. *Department of Minerals and Energy of South Africa*, 125.

Komarnicki, G. J. 2000. Tissue, sex and age specific accumulation of heavy metals (Zn, Cu, Pb, Cd) by populations of the mole (*Talpa europea* L.) in a central urban area. *Chemosphere*, 41(10): 1593-1602.

Kovats, R. S., Bouma, M. J., Hajat, S., Worrall, E. and Haines, A. 2003. El Niño and health. *The Lancet*, 362(9394): 1481-1489.

Kraus, J. M., Schmidt, T. S., Walters, D. M., Wanty, R. B., Zuellig, R. E. and Wolf, R. E. 2014. Cross-ecosystem impacts of stream pollution reduce resource and contaminant flux to riparian food webs. *Ecological Applications* 24(2): 235-243.

Krebs, C. J. 1996. Population cycles revisited. *Journal of mammalogy*, 77(1): 8-24.

Kukier, U., Peters, C. A., Chaney, R. L., Angle, J. S. and Roseberg, R. J. 2004. The effect of pH on metal accumulation in two Alyssum species. *Journal of Environmental Quality*, *33*(6): 2090-2102.

Lacy, R. C. 1997. Importance of genetic variation to the viability of mammalian populations. *Journal of Mammalogy*, 78(2): 320-335.

Lagerwerff, J. V. and Specht, A. W. 1970. Contamination of roadside soil and vegetation with cadmium, nickel, lead, and zinc. *Environmental Science & Technology*, 4(7): 583-586.

Lam, J. C., Tanabe, S., Lam, M. H. and Lam, P. K. 2005. Risk to breeding success of waterbirds by contaminants in Hong Kong: evidence from trace elements in eggs. *Environmental pollution*, 135(3): 481-490.

Lancaster, J. and Pillay, N. 2010. Behavioral interactions between a coexisting rodent *Micaelamys namaquensis* and macroscelid *Elephantulus myurus*. *Current Zoology,* 56(4): 395-400.

Lanctôt, C., Melvin, S. D., Leusch, F. D. L., Wilson, S. and Fabbro, L. 2016. Locomotor and behavioural responses of empire gudgeons (*Hypseleotris compressa*) exposed to coal mine wastewater. *Chemosphere,* 144: 1560-1566.

Lange, S., Stalleicken, J. and Burda, H. 2004. Functional morphology of the ear in fossorial rodents, *Microtus arvalis* and *Arvicola terrestris*. *Journal of Morphology*, 262(3): 770-779.

Laskey, J. W., Rehnberg, G. L., Laws, S. C. and Hein, J. F. 1984. Reproductive effects of low acute doses of cadmium chloride in adult male rats. *Toxicology and Applied Pharmacology,* 73: 250–255.

Latha, V. and Basha, P. M. 2015. Heavy metals (Cr and Zn) induced alterations in cast production, burrowing behaviour, surface migration and macropore formation in three ecologically different earthworm species: A comparative study. *International Journal of Environmental Sciences*, 6(2): 260-269.

Laurance, W. F. 2010. Habitat destruction: death by a thousand cuts. *Conservation biology for all*, 1(9): 73-88.

Laurance, W. F., Clements, G. R., Sloan, S., O'Connell, C. S., Mueller, N. D., Goosem, M., Venter, O., Edwards, D. P., Phalan, B., Balmford, A., Van Der Ree, R. and Burgues Arrea, I. 2014. A global strategy for road building. *Nature,* 513(7517): 229-232.

Leffler, P.E. and Nyholm, N.E.I. 1996. Nephrotoxic effects in free-living bank voles in a heavy metal polluted environment. *Ambio,* 6: 417–420.

Leirs, H., Verheyen, W., Michiels, M., Verhagen, R. and Stuyck, J. 1989. The relation between rainfall and the breeding season of *Mastomys natalensis* (Smith, 1834) in Morogoro, Tanzania. *Annales de la société Royale Zoologique de Belgique*, 119: 59-64.

León, G., Pérez, L. E., Linares, J. C., Hartmann, A. and Quintana, M. 2007. Genotoxic effects in wild rodents (*Rattus rattus* and *Mus musculus*) in an open coal mining area. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis,* 630(1): 42-49.

Li, X., Lee, S. L., Wong, S. C., Shi, W. and Thornton, I. 2004. The study of metal contamination in urban soils of Hong Kong using a GIS-based approach. *Environmental Pollution*, 129(1): 113-124.

Li, Z., Ma, Z., van der Kuijp, T. J., Yuan, Z. and Huang, L. 2014. A review of soil heavy metal pollution from mines in China: pollution and health risk assessment. *Science of the Total Environment*, 468: 843-853.

Liao, J., Wen, Z., Ru, X., Chen, J., Wu, H. and Wei, C. 2016. Distribution and migration of heavy metals in soil and crops affected by acid mine drainage: Public health implications in Guangdong Province, China. *Ecotoxicology and environmental safety*, 124: 460-469.

Liu H.Y., Probst A. and Liao B.H. 2005. Metal contamination of soils and crops affected by the Chenzhou lead/zinc mine spill (Hunan, China). *Science of the Total Environment,* 339: 153–66.

Lim, M., Han, G.C., Ahn, J.W., You, K.S. and Kim, H.S. 2009. Leachability of arsenic and heavy metals from mine tailings of abandoned metal mines. *International Journal of Environmental Research and Public Health*, 6: 2865-2879.

Lokeshwari, H. and Chandrappa, G. T. 2006. Impact of heavy metal contamination of Bellandur Lake on soil and cultivated vegetation. *Current science*, 91(5): 622-627.

Loneragan, J. F. 1988. Distribution and movement of manganese in plants. Pp. 113-124 *in Manganese in soils and plants*. Springer, Dordrecht.

Lopes, P. A., Viegas-Crespo, A. M., Nunes, A. C., Pinheiro, T., Marques, C., Santos, M. C., and da Luz Mathias, M. 2002. Influence of age, sex, and sexual activity on trace element levels and antioxidant enzyme activities in field mice (*Apodemus sylvaticus* and *Mus spretus*). *Biological trace element research*, 85(3): 227-239.

Lötter, N. 2005. Cyanide volatilisation from gold leaching operations and tailing storage facilities. Master of Metallurgical Engineering thesis. University of Pretoria, Pretoria, South Africa.

Lunney, D., Cullis, B. and Eby, P. 1987. Effects of logging and fire on small mammals in Mumbulla State Forest, near Bega, New South Wales. *Wildlife Research*, 14(2): 163-181.

Lusilao-Makiese, J. G., Cukrowska, E. M., Tessier, E., Amouroux, D., and Weiersbye, I. 2013. The impact of post gold mining on mercury pollution in the West Rand region, Gauteng, South Africa. *Journal of Geochemical Exploration*, 134: 111-119.

Ma, W. C., Denneman, W. and Faber, J. 1991. Hazardous exposure of ground-living small mammals to cadmium and lead in contaminated terrestrial ecosystems. *Archives of Environmental Contamination and Toxicology*, 20(2): 266-270.

Ma, W.C., Talmage, S., 2001. Insectivora. Pp. 123–128 *in* Shore, R.F., Rattner, B.A., editors, *Ecotoxicology of Wild Mammals, Ecological & Environmental Toxicology Series (Chapter 4)*. Wiley and Sons, New York, USA.

Maanan, M., Saddik, M., Maanan, M., Chaibi, M., Assobhei, O. and Zourarah, B. 2015. Environmental and ecological risk assessment of heavy metals in sediments of Nador lagoon, Morocco. *Ecological Indicators*, 48: 616-626.

Madejón, P., Murillo, J. M., Marañón, T., Cabrera, F. and López, R. 2002. Bioaccumulation of As, Cd, Cu, Fe and Pb in wild grasses affected by the Aznalcóllar mine spill (SW Spain). *Science of the Total Environment*, 290(1): 105-120.

Malm, O. 1998. Gold mining as a source of mercury exposure in the Brazilian Amazon. *Environmental Research*, 77(2): 73-78.

Marcheselli, M., Sala, L. and Mauri, M. 2010. Bioaccumulation of PGEs and other traffic-related metals in populations of the small mammal *Apodemus sylvaticus*. *Chemosphere*, 80(11): 1247-1254.

Mason, C.F. and Macdonald, S.M. 1988. Metal contamination in mosses and otter distribution in a rural Welsh river receiving mine drainage. *Chemosphere,* 17: 1159-1166.

Massawe, A. W., Rwamugira, W., Leirs, H., Makundi, R. H., Mulungu, L., Ngowo, V. and Machang'u, R. 2008. Soil type limits population abundance of rodents in crop fields: case study of the multimammate rat *Mastomys natalensis* Smith, 1834 in Tanzania. *Integrative Zoology*, 3(1): 27-30.

Maya, M., Sutton, M., Tutu, H., and Weiersbye, I. 2015. Mineralogy and heavy metal content of secondary mineral salts: A case study from the Witwatersrand Gold Basin, South Africa. *South African Journal of Geomatics*, 4(2): 161-173.

McGregor, R. G., Blowes, D. W., Jambor, J. L. and Robertson, W. D. 1998. Mobilization and attenuation of heavy metals within a nickel mine tailings impoundment near Sudbury, Ontario, Canada. *Environmental Geology*, 36(3-4): 305-319.

Meliyo, J., Brabers, L., Massawe, B. H., Msanya, B. M., Kimaro, D. N., Mulungu, L. S., Kihupi, N.I., Deckers, J.A., Gulinck, H. and Leirs, H. 2014. The effect of soil physical properties and soil microclimate on rodent burrows' abundance and their characteristics in Western Usambara Mountains, Tanzania. *Journal of Agriculture and Ecology Research International*, 2(2): 105-119.

Mills, J. N., Childs, J. E., Ksiazek, T. G., Peters, C. J. and Velleca, W. M. 1995. *Methods for trapping and sampling small mammals for virologic testing*. US Department of Health and Human Services, Public Health Service, Centres for Disease Control and Prevention. Atlanta, Georgia, U.S.A.

Milton, A., Cooke, J. A. and Johnson, M. S. 2003. Accumulation of lead, zinc, and cadmium in a wild population of Clethrionomys glareolus from an abandoned lead mine. *Archives of Environmental Contamination and Toxicology*, 44(3): 0405-0411.

Mudder, T. I. and Botz, M. 2004. Cyanide and society. *European Journal of Mineral Process and Environmental Protection*, 4.

Mulungu, L. S., Ngowo, V., Mdangi, M., Katakweba, A. S., Tesha, P., Mrosso, F. P., Mchomvu, M., Sheyo, P.M. and Kilonzo, B. S. 2013. Population dynamics and breeding patterns of multimammate mouse, *Mastomys natalensis* (Smith 1834), in irrigated rice fields in Eastern Tanzania. *Pest management science*, 69(3): 371-377.

Nadasan D.S., Davies T.C. and Shapi M.M. 2014. The distribution of some potentially harmful elements (PHEs) in the Krugersdorp Game Reserve , Gauteng , South Africa: Implications for wildlife health. Pp. 113–120 i*n* Křibek, B. and Davies, T. (Eds.), *IGCP/SIDA*

Projects 594 and 606, Closing workshop, Prague, Czech Republic. Prague: Czech Geological Survey.

Nagajyoti, P. C., Lee, K. D. and Sreekanth, T. V. M. 2010. Heavy metals, occurrence and toxicity for plants: a review. *Environmental chemistry letters*, 8(3): 199-216.

Nagendra, H., Mairota, P., Labadessa, R., Leronni, V., Marangi, C., Diele, F., Torri, D., Tomaselli, V. and Padoa-Schioppa, E. 2013. Developing a framework for understanding human impacts on positive and negative changes in habitat, land cover and landscape fragmentation. *BIO_SOS* Deliverble No.: D6.9 1st version.

Naicker, K., Cukrowska, E. and McCarthy, T.S. 2003. Acid mine drainage arising from gold mining activity in Johannesburg, South Africa and environs. *Environmental Pollution*, 122: 29–40.

Nakayama, S. M., Ikenaka, Y., Hamada, K., Muzandu, K., Choongo, K., Teraoka, H., Mizuno, N. and Ishizuka, M. 2011. Metal and metalloid contamination in roadside soil and wild rats around a Pb–Zn mine in Kabwe, Zambia. *Environmental pollution*, 159(1): 175-181.

Nazir, R., Khan, M., Masab, M., Rehman, H. U., Rauf, N. U., Shahab, S., Ameer, N., Sajed, M., Ullah, M., Rafeeq, M. and Shaheen, Z. 2015. Accumulation of heavy metals (Ni, Cu, Cd, Cr, Pb, Zn, Fe) in the soil, water and plants and analysis of physico-chemical parameters of soil and water collected from Tanda Dam kohat. *Journal of Pharmaceutical Sciences and Research*, 7(3): 89-97.

Neocosmos, M. 2010. *From 'foreign natives' to 'native foreigners': Explaining xenophobia in post-apartheid South Africa: Citizenship and nationalism, identity and politics*. African Books Collective, Dakar, Senegal.

Neuman, D. R., Brown, P. J. and Jennings, S. R. 2014. Metals associated with acid rock drainage and their effect on fish health and ecosystems. *Acid Mine Drainage, Rock Drainage, and Acid Sulfate Soils: Causes, Assessment, Prediction, Prevention, and Remediation*, 139-169.

Nolan, C.V., Shaikh, Z.A., 1992. Lead nephrotoxicity and associated disorders: biochemical mechanisms. *Toxicology*, 73, 127-146.

Nöthling, J. O., Du Toit, J. S. and Myburgh, J. G. 2014. Lead isotope ratios in bone ash of blesbok (*Damaliscus pygargus phillipsi*): A means of screening for the accumulation of contaminants from uraniferous rocks. *Journal of Environmental Science and Health, Part A*, 49(11): 1251-1257.

Nunes, A. C., da Luz Mathias, M. and Crespo, A. M. 2001. Morphological and haematological parameters in the Algerian mouse (*Mus spretus*) inhabiting an area contaminated with heavy metals. *Environmental pollution*, 113(1): 87-93.

Nupp, T. E. and Swihart, R. K. 1996. Effect of forest patch area on population attributes of white-footed mice (*Peromyscus leucopus*) in fragmented landscapes. *Canadian Journal of Zoology*, 74(3): 467-472.

Ochieng, G. M., Seanego, E. S. and Nkwonta, O. I. 2010. Impacts of mining on water resources in South Africa: A review. *Scientific Research and Essays*, 5(22): 3351-3357.

Oelofse, S. H. H., Hobbs, P. J., Rascher, J. and Cobbing, J. E. 2007. The pollution and destruction threat of gold mining waste on the Witwatersrand: A West Rand case study. In *10th International Symposium on Environmental Issues and Waste management in Energy and Mineral Production (SWEMP, 2007), Bangkok*, 617-626.

Oluyemi, E. A., Feuyit, G., Oyekunle, J. A. O. and Ogunfowokan, A. O. 2008. Seasonal variations in heavy metal concentrations in soil and some selected crops at a landfill in Nigeria. *African Journal of Environmental Science and Technology*, 2(5): 089-096.

Packer, A. P., Lariviere, D., Li, C., Chen, M., Fawcett, A., Nielsen, K., Mattson, K., Chatt, A., Scriver, C. and Erhardt, L. S. 2007. Validation of an inductively coupled plasma mass spectrometry (ICP-MS) method for the determination of cerium, strontium, and titanium in ceramic materials used in radiological dispersal devices (RDDs). *Analytica chimica acta*, 588(2): 166-172.

Pastoriza, S., Rufián-Henares, J. Á., and Delgado-Andrade, C. 2015. Effects of long-term consumption of standard diets including glucose–lysine model glycated compounds on the antioxidant status of adult rats. *Food chemistry*, 183: 283-290.

Pereira, R., Pereira, M. L., Ribeiro, R. and Gonçalves, F. 2006. Tissues and hair residues and histopathology in wild rats (*Rattus rattus* L.) and Algerian mice (*Mus spretus* Lataste) from an abandoned mine area (Southeast Portugal). *Environmental Pollution*, 139(3): 561-575.

Peng, J. F., Song, Y. H., Yuan, P., Cui, X. Y. and Qiu, G. L. 2009. The remediation of heavy metals contaminated sediment. *Journal of hazardous materials*, 161(2): 633-640.

Perugini, M., Visciano, P., Manera, M., Zaccaroni, A., Olivieri, V., and Amorena, M. 2014. Heavy metal (As, Cd, Hg, Pb, Cu, Zn, Se) concentrations in muscle and bone of four commercial fish caught in the central Adriatic Sea, Italy. *Environmental monitoring and assessment*, 186(4): 2205-2213.

Phelps, K. L. and McBee, K. 2010. Population parameters of Peromyscus leucopus (White-footed deermice) inhabiting a heavy metal contaminated superfund site. *The Southwestern Naturalist*, 55(3): 363-373.

Phillips, A. 2001. Mining and protected areas. *World Business Council for Sustainable Development and International Institute for environment and development, 62.*

Phillips, G. R. and J. Lipton, 1995. Injury to aquatic resources caused by metals in Montana Clark For River Basin: historic perspective and overview. *Canadian Journal of Fisheries and Aquatic Science* 82:1990-1993.

Pichancourt, J-B., Burel, F., and Auger, P. 2006. A hierarchical matrix model to assess the impact of habitat fragmentation on population dynamics: and elasticity analysis. *Comptes Rendus Biologies*, 329: 31-39.

Pimm, S. L. and Raven, P. (2000). Biodiversity: extinction by numbers. *Nature*, 403(6772): 843.

Pulles, W. 2003. *Mining Weekly*, January 24-30: 2-3

QGIS Development Team. 2016. QGIS Geographic Information System. Open Source Geospatial Foundation Project. (http://qgis.osgeo.org)

Rascio, N. and Navari-Izzo, F. 2011. Heavy metal hyperaccumulating plants: how and why do they do it? And what makes them so interesting?. *Plant science*, 180(2): 169-181.

Ripple, W. J., Estes, J. A., Beschta, R. L., Wilmers, C. C., Ritchie, E. G., Hebblewhite, M., Berger, J., Elmhagen, B., Letnic, M., Nelson, M. P., Schmitz, O. J., Smith, D. W., Wallach, A. D. and Wirsing, A. J. 2014. Status and ecological effects of the world's largest carnivores. *Science*, 343(6167): 1241484.

Roberts, K. 2016. A Legacy That No One Can Afford to Inherit: The Gold King Disaster and the Threat of Abandoned Hardrock Legacy Mines. *Journal of the National Association of Administrative Law Judiciary*, 36 (1): 361-406.

Rooney, R. C., Bayley, S. E. and Schindler, D. W. 2012. Oil sands mining and reclamation cause massive loss of peatland and stored carbon. *Proceedings of the National Academy of Sciences*, 109(13): 4933-4937.

Russo, I.M. 2009. *African Rock Rats*. (Unpublished doctoral thesis). University of Pretoria, Pretoria, South Africa.

Ryu, D. Y., Lee, S. J., Park, D. W., Choi, B. S., Klaassen, C. D. and Park, J. D. 2004. Dietary iron regulates intestinal cadmium absorption through iron transporters in rats. *Toxicology letters*, 152(1): 19-25.

SAHO. 2011. Discovery of the Gold in 1884. *South African History Organisation*, Cape Town, South Africa (http://www.sahistory.org.za/article/discovery-gold-1884). Accessed 23 June 2017.

Sala, O. E., Chapin, F. S., Armesto, J. J., Berlow, E., Bloomfield, J., Dirzo, R. and Kinzig, A. 2000. Global biodiversity scenarios for the year 2100. Science, 287(5459): 1770‑1774.

Sánchez-Chardi, A. and Nadal, J. 2007. Bioaccumulation of metals and effects of landfill pollution in small mammals. Part I. The greater white-toothed shrew, *Crocidura russula*. *Chemosphere*, 68: 703–711.

Sánchez-Chardi, A., Peñarroja-Matutano, C., Ribeiro, C. A. O. and Nadal, J. 2007. Bioaccumulation of metals and effects of a landfill in small mammals. Part II. The wood mouse, *Apodemus sylvaticus*. *Chemosphere*, 70(1): 101-109.

Sánchez-Chardi, A., Ribeiro, C. A. O. and Nadal, J. 2009a. Metals in liver and kidneys and the effects of chronic exposure to pyrite mine pollution in the shrew *Crocidura russula* inhabiting the protected wetland of Doñana. *Chemosphere*, 76: 387-394.

Sánchez-Chardi, A., Peñarroja-Matutano, C., Borrás, M. and Nadal, J. 2009b. Bioaccumulation of metals and effects of a landfill in small mammals Part III: structural alterations. *Environmental research*, 109(8): 960-967.

Sang, N. and Li, G. K. 2004. Genotoxicity of municipal landfill leachate on root tips of *Vicia faba*. *Mutation Research*, 560: 150 – 165.

Sany, S. B. T., Salleh, A., Sulaiman, A. H., Sasekumar, A., Rezayi, M. and Tehrani, G. M. 2013. Heavy metal contamination in water and sediment of the Port Klang coastal area, Selangor, Malaysia. *Environmental Earth Sciences*, 69(6): 2013-2025.

Sato, J. J., Kawakami, T., Tasaka, Y., Tamenishi, M. and Yamaguchi, Y. 2014. A few decades of habitat fragmentation has reduced population genetic diversity: A case study of landscape genetics of the large Japanese field mouse, *Apodemus speciosus*. *Mammal study*, 1(39): 1-10.

Saygi, A., Deniz, G., Kutsal, O. and Vural, N. 1991. Chronic effects of cadmium on kidney, liver, testis, and fertility of male rats. *Biological trace element research*, 31(3): 209-214.

Schradin, C. and Pillay, N. 2003. Paternal care in the social and diurnal striped mouse (*Rhabdomys pumilio*): laboratory and field evidence. *Journal of Comparative Psychology*, 117(3): 317.

Schradin, C. and Pillay, N. 2004. The striped mouse (*Rhabdomys pumilio*) from the succulent karoo, South Africa: a territorial group-living solitary forager with communal breeding and helpers at the nest. *Journal of Comparative Psychology*, 118(1): 37.

Schueler, V., Kuemmerle, T. and Schröder, H. 2011. Impacts of surface gold mining on land use systems in Western Ghana. *Ambio*, 40(5): 528-539.

Schwartz, C., Guimont, S., Saison, C., Perronnet, K. and Morel, J. L. 2001. Phytoextraction of Cd and Zn by the hyperaccumulator plant Thlaspi caerulescens as affected by plant size and origin. *South African journal of science,* 97(11-12): 561-564.

Schwarzenbach, R. P., Escher, B. I., Fenner, K., Hofstetter, T. B., Johnson, C. A., Von Gunten, U. and Wehrli, B. 2006. The challenge of micropollutants in aquatic systems. *Science*, 313(5790): 1072-1077.

Scott, R. 1995. *Flooding of Central and East Rand Gold Mines: An Investigation Into Controls Over the Inflow Rate, Water Quality and the Predicted Impacts of Flooded Mines: Report*. Water Research Commission. Report No. 486/1/95. Pretoria, South Africa.

Sharma, R. K. and Agrawal, M. 2005. Biological effects of heavy metals: an overview. *Journal of environmental Biology,* 26(2): 301-313.

Sheffield, S.R., Sawicka-Kapusta, K., Cohen, J.B. and Rattner, B.A. 2001. Rodentia and Lagomorpha. Pp. 215–314 *in*: Shore, R.F., Rattner, B.A. (Eds.), Ecotoxicology of Wild Mammals. Wiley, Chichester, New York, Weinheim, Brisbane, Singapore, Toronto.

Shieh, S. H., Ward, J. V. and Kondratieff, B. C. 2002. Energy flow through macroinvertebrates in a polluted plains stream. *Energy*, 21(4): 660-675.

Shore, R.F. 1995. Predicting cadmium, lead and fluoride levels in small mammals from soil residues and by species-species extrapolation. *Environmental Pollution*, 88: 333-340.

Shore, R.F., Rattner, B.A. (Eds.) 2001. Ecotoxicology of Wild Mammals. Wiley, Chichester, New York, USA.

Singh, K. P., Mohan, D., Singh, V. K. and Malik, A. 2005. Studies on distribution and fractionation of heavy metals in Gomti river sediments—a tributary of the Ganges, India. *Journal of hydrology*, 312(1): 14-27.

Souren, A. W. M. G. 2000. Living with cyanide. *Geochemical News*, 105: 16 – 26.

Spradling, T. A., Hafner, M. S. and Demastes, J. W. 2001. Differences in rate of cytochrome-b evolution among species of rodents. *Journal of Mammalogy*, 82(1): 65-80.

Spreadbury, B. R., Musil, R. K., Musil, J., Kaisner, C. and Kovak, J. 1996. Cougar population characteristics in southeastern British Columbia. *The Journal of wildlife management*, 962-969.

StatSoft, Inc. 2015. STATISTICA (data analysis software system), version 13. (www.statsoft.com).

Szefer, P., Glasby, G. P., Kunzendorf, H., Görlich, E. A., Latka, K., Ikuta, K. and Ali, A. 1998. The distribution of rare earth and other elements and the mineralogy of the iron oxyhydroxide phase in marine ferromanganese concretions from within Slupsk Furrow in the southern Baltic. *Applied geochemistry*, 13(3): 305-312.

Tarras-Wahlberg, N. H., Flachier, A., Lane, S. N. and Sangfors, O. 2001. Environmental impacts and metal exposure of aquatic ecosystems in rivers contaminated by small scale gold mining: the Puyango River basin, southern Ecuador. *Science of the Total Environment*, 278(1): 239-261.

Taylor, K.M., Vichova, P., Jordan, N., Hiscox, S., Hendley, R., Nicholson, R.I. 2008. ZIP7-mediated intracellular zinc transport contributes to aberrant growth factor signalling in antihormone-resistant breast cancer Cells. *Endocrinology*, 149: 4912-4920

Tembeni, B., Oyedeji, O. O., Ejidike, I. P., and Oyedeji, A. O. 2016. Evaluation of Trace Metal Profile in Cymbopogon validus and Hyparrhenia hirta Used as Traditional Herbs from Environmentally Diverse Region of Komga, South Africa. *Journal of Analytical Methods in Chemistry*, 2016.

Thakur, S., Singh, L., Ab Wahid, Z., Siddiqui, M. F., Atnaw, S. M., and Din, M. F. M. (2016). Plant-driven removal of heavy metals from soil: uptake, translocation, tolerance mechanism, challenges, and future perspectives. *Environmental monitoring and assessment*, 188(4): 206.

Thoreux-Manlay, A., Velez de la Calle, J. F., Olivier, M. F., Soufir, J. C., Masse, R. and Pinon-Lataillade, G. 1995. Impairment of testicular endocrine function after lead intoxication in the adult rat. *Toxicology,* 100: 101–109.

Turnau, K. and Mesjasz-Przybylowicz, J. 2003. Arbuscular mycorrhiza of *Berkheya coddii* and other Ni-hyperaccumulating members of Asteraceae from ultramafic soils in South Africa. *Mycorrhiza*, 13(4): 185-190.

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

Tutu, H. 2012. Mining and water pollution. *Water quality monitoring and assessment.* 347-372.

U.S. Geological Survey. 2016. Mineral commodity summaries 2016: U.S. Geological Survey, pp. 1-202, Reston, Virginia, U.S.A.

van den Brink, N., Lammertsma, D., Dimmers, W., Boerwinkel, M. C. and van der Hout, A. 2010. Effects of soil properties on food web accumulation of heavy metals to the wood mouse (*Apodemus sylvaticus*). *Environmental Pollution*, 158(1): 245-251.

van den Brink, N. W., Lammertsma, D. R., Dimmers, W. J. and Boerwinkel, M. C. 2011. Cadmium accumulation in small mammals: species traits, soil properties, and spatial habitat use. *Environmental science & technology,* 45(17): 7497-7502.

Van Deventer, H. and Cho, M. A. 2014. Assessing leaf spectral properties of *Phragmites australis* impacted by acid mine drainage. *south African Journal of science*, 110(7-8): 1-12.

Van Niekerk, J. H. 2010. Social organization of a flock of Helmeted Guineafowl (*Numida meleagris*) at the Krugersdorp Game Reserve, South Africa. *Chinese Birds*, 1(1): 22-29.

Wamsley RD, Mazury D 1999. *A management plan for the Blesbokspruit Ramsar site. Volume 2: Objectives and Management plan*. University of Pretoria, Pretoria, South Africa.

Webster, J. G., Nordstrom, D. K. and Smith, K. S. 1994. Transport and natural attenuation of Cu, Zn, As, and Fe in the acid mine drainage of Leviathan and Bryant Creeks. *Environmental Geochemistry of Sulfide Oxidation,* 17: 244-260

Weiersbye, I. M. and Witkowski, E. T. F. 2002. Seed fate and practical germination methods for 46 perennial species that colonize gold mine tailings and acid mine drainage-polluted soils in the grassland biome. *Multiple use management of natural forests and woodlands: Policy refinements and scientific progress. Department of Water Affairs & Forestry,* 221-255.

Weiersbye, I. M. and Witkowski, E. T. F. 2003. Acid rock drainage (ARD) from gold tailings dams on the Witwatersrand Basin impacts on tree seed fate, viability, inorganic content and seedling morphology. In: Armstrong, D., de Villiers, A. B., Kleinmann, R. L. P., McCarthy, T. S. & Norton, P. J. (eds.). *Mine Water and the Environment, Proceedings of the 8th International Congress on Mine Water & the Environment.* Johannesburg, South Africa.

Weiersbye, I. M., Witkowski, E. T. F. and Reichardt, M. 2006. Floristic composition of gold and uranium tailings dams, and adjacent polluted areas, on South Africa's deep-level mines. *Bothalia,* 36(1): 101-113.

Weiersbye, I. M. and Witkowski, E. T. F. 2007. Impacts of acid mine drainage on the regeneration potential of Highveld phreatophytes. *Multiple use management of natural forests and woodlands: Policy refinements and scientific progress*, 224-237.

Wijesinghe, M. R. and Brooke, M. D. L. 2005. Impact of habitat disturbance on the distribution of endemic species of small mammals and birds in a tropical rain forest in Sri Lanka. *Journal of Tropical Ecology*, 21(06): 661-668.

Wijnhoven, S., Thonon, I., Van Der Velde, G., Leuven, R., Zorn, M., Eijsackers, H. and Smits, T. 2006. The impact of bioturbation by small mammals on heavy metal redistribution in an embanked floodplain of the river Rhine. *Water, air, and soil pollution*, 177(1-4): 183-210.

Wijnhoven, S., Leuven, R. S., van der Velde, G. and Eijsackers, H. J. 2008. Toxicological risks for small mammals in a diffusely and moderately polluted floodplain. *Science of the total environment*, 406(3): 401-406.

Winde, F. 2010. Uranium pollution of the Wonderfonteinspruit, 1997-2008 Part 1: Uranium toxicity, regional background and mining-related sources of uranium pollution. *Water SA*, 36(3): 239-256.

Winde, F. and Stoch, E. J. 2010. Threats and opportunities for post-closure development in dolomitic gold mining areas of the West Rand and Far West Rand (South Africa)-a hydraulic view part 1: mining legacy and future threats. *Water SA*, 36(1): 69-74.

Wirth, J. J. and Mijal, R. S. 2010. Adverse effects of low level heavy metal exposure on male reproductive function. *Systems Biology in Reproductive Medicine*, 56(2): 147-167.

Wren, C.D. 1986. Mammals as biological monitors of environmental metal levels. *Environmental Monitoring and Assessment*, 6: 127-144.

Wu, S. H. 1994. Effect of manganese excess on the soybean plant cultivated under various growth conditions. *Journal of plant nutrition*, 17(6): 991-1003.

Younger, P.L. 2001. Passive treatment of ferruginous mine water using high surface area media. *Elsevier science Ltd*. Great Britain.

Yahaya, M. I., Mohammad, S. and Abdullahi, B. K. 2009. Seasonal variations of heavy metals concentration in abattoir dumping site soil in Nigeria. *Journal of Applied Sciences and Environmental Management*, 13(4).

Yilmaz, A.B. 2003. Levels of heavy metals (Fe, Cu, Ni, Cr, Pb, and Zn) in tissue of *Mugli cephalus* and *Trachurus mediterraneus* from Iskenderun Bay, Turkey. Environmental Research, 92(3): 277-281.

Yuan, X., Leng, L., Huang, H., Chen, X., Wang, H., Xiao, Z., Zhai, Y., Chang, H. and Zeng, G. 2015. Speciation and environmental risk assessment of heavy metal in bio-oil from liquefaction/ pyrolysis of sewage sludge. *Chemosphere*, 120: 645-652.

Yule, C. M., Boyero, L. and Marchant, R. 2010. Effects of sediment pollution on food webs in a tropical river (Borneo, Indonesia). *Marine and Freshwater Research*, 61(2): 204-213.

Zhou, Q., Zhang, J., Fu, J., Shi, J. and Jiang, G. 2008. Biomonitoring: an appealing tool for assessment of metal pollution in the aquatic ecosystem. *Analytica chimica acta*, 606(2): 135-150.