EXAMINING HEAVY METAL CONCENTRATIONS IN HAIR OF SOUTH AFRICAN VERVET MONKEYS (*CHLOROCEBUS PYGERYTHRUS*) TO ACCESS ANTHROPOGENIC IMPACTS

By

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High concentrations of heavy metals are known to have deleterious effects on the nervous, endocrine, hepatic, and immune systems of mammals. Environmental toxicology has traditionally been used to understand impacts of pollutants on human health and aquatic and marine ecosystems but has rarely been adopted by primatologists to examine the effects of toxins on nonhuman primates. I analyzed 60 vervet monkey (*Chlorocebus pygerythrus*) hair samples for concentrations of Arsenic (As), Cadmium (Cd), Lead (Pb), and Mercury (Hg) using an inductively coupled plasma mass spectrometer (ICP-MS). Hair samples were collected from anesthetized monkeys at 10 South African field sites with varying degrees of anthropogenic impact. All hair samples contained Pb and As. Some samples had Cd and Hg but at levels below the limit of quantitation (LOQ). Animals acquire heavy metals naturally via environmental particulates, or through the consumption of food and water and sometimes in unnaturally high quantities as a result of human activity. In South Africa, mining is fairly widespread with the harmful potential for exposure to increased levels of heavy metal pollution. Given our shared physiologies, nonhuman primates can act as proxies for those humans occupying polluted

ecosystems, and the data collected from examining nonhuman primate hair for pollutants may also be viewed as less controversial by public and private institutions. The data presented here demonstrate that toxicology studies can improve our understanding of nonhuman primate health and behavior especially for populations in degraded habitats.

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CHAPTER 1

INTRODUCTION

Heavy metals occur naturally throughout the Earth's geosphere and biosphere. To be precise, heavy metals are those elements with an atomic mass of 200 or greater (Baldwin and Marshall 1999). However, those metals that are considered toxic (i.e. result in an adverse health effect) to vertebrates at high concentrations are conventionally referred to as heavy metals. In terrestrial ecosystems, heavy metals make their way to the surface through soil erosion or underground water ways that emerge into surface water sources. As a result, heavy metals may become available for those animal populations living on a toxic landscape via ingestion, inhalation, or absorption. The effects of these exposures vary based on an animal's physiology, but among mammals high concentrations of heavy metals can disrupt the development and/or function of the renal, hepatic, nervous, and endocrine systems (Nordberg 2015). Consistent exposures to heavy metals at low concentrations, generally pose little to no threat to the health of an animal. From an evolutionary perspective, animals should evolve physiological and behavioral adaptations to cope with naturally occurring, toxic levels of heavy metals. However, as human populations continue to grow, expand, and modify habitats for their own means, they sometimes introduce these heavy metal toxins into the environment in an accelerated pace and increased magnitude that does not mimic a natural process. As a result, animals that experience the human influenced heavy metal exposure often aren't provided with enough time to develop a successful coping mechanism, before they are exposed to the toxins again. This can be particularly damaging to mammal and bird species that are long-lived and have very few offspring throughout their lives. This not only stresses the animal physiologically, but has the potential to negatively impact the animal's behavior as well, especially since many heavy metals directly impair the nervous and endocrine systems.

Nonhuman primates are especially prone to the exposure of these anthropogenic impacts as we inhabit and utilize many of the same areas and exploit landscapes modified by people. In South Africa, the vervet monkeys (*Chlorocebus pygerythrus*), live in close proximity to humans primarily to gain access to water and food resources. Seasonal changes requirs vervet monkeys to maintain a certain degree of plasticity in regards to diet and behavior (Cheney and Seyfarth 1990).. Considering vervet monkeys exhibit an astute understanding of their surroundings, modifying their diet and behavior to the current season is transparent (Barrett et al. 2016).

Throughout many regions in South Africa, humans practice agriculture that vervets frequently exploit (Loudon et al. 2014). Unfortunately for these vervets, this often labels them as a pest species, especially to those who are involved in agriculture (Grobler et al. 2006). The cohabitation of anatomically modern humans and nonhuman primates is not a new phenomenon, but instead a relationship that has existed for thousands of years. Because of the overlap in habitats, nonhuman primates are exposed to human practices such as industrial processes. These industrial processes are an excellent example of an anthropogenic impact, as they are often coupled with the contamination of surrounding areas by toxic chemicals, heavy metals, and other non-natural stressors. One of the primary industrial processes responsible for pollution in South Africa is the mining industry. Mining regularly introduces heavy metals and other toxins into the environment, subsequently exposing nonhuman primates to these harmful contaminants (Naidoo 2014).

The natural materials deemed unusable in the extraction of valuable minerals (gems, precious metals) and forms of energy (coal, natural gas, oil, or oil shales) are frequently left in a variety of mine waste composites on and around mine sites. Toxic discarded mine waste is readily incorporated into the surrounding environment through wind, water, and soil (Rose 2013) and this may lead to lethal levels of exposure to local populations of humans and animals. In this study, the concentrations of the heavy metals Arsenic (As), Cadmium (Cd), Leadv (Pb), and Mercury (Hg) are analyzed in South African vervet monkey hairs. At high levels, heavy metals have profoundly adverse effects on the biological systems of mammals who inhale, ingest, or engage with them directly or indirectly. Additionally, prolonged exposure to one or a combination of these heavy metals can result in delayed physical and neurological development (Fowler et al. 2015). Moreover, some heavy metals including Cd and Pb have carcinogenic properties and exposure to these elements increases the likelihood of cancer (Nordberg 2015).

South African activists and scholars have addressed the mounting hazardous conditions near mines since the early 1950's. Many existing South African water sources are contaminated from current mine waste sites that are experiencing unregulated waste management. A main concern at these sites is Acid Mine Drainage (AMD), which is an "uncontrolled discharge of contaminated water" (Naidoo 2014). This contaminated water is moved from the mine waste site to nearby water sources through one of two ways. The first is discharge from an underground water source. Rain water that falls over the mine waste collects at the base, which then leeches into local underground water sources, polluting surface water sources over an extended period of time (i.e. potentially millions of years). The second way contaminated rainwater reaches a local surface water source is by running directly into it. This happens when the contaminated rainwater either overflows from the waste site, or by some unregulated industries pumping directly into surface water sources (Naidoo 2014). Humans, livestock, nonhuman primates, and other wildlife that utilize the contaminated water sources are at risk of adverse physiological, neurological, and behavioral effects. These potentially hazardous mine waste sites are present both at active mines, and those mines that have closed for decades. Although closed mines are no longer adding to mine waste composites, the separated and crushed rock within them have the potential to continue contaminating the soil, water, and air for a prolonged period of time.

This study seeks to access the various levels of environmental toxicology by identifying heavy metal concentrations of As, Cd, Pb, and Hg in 10 populations of vervet monkeys throughout South Africa with varying degrees of anthropogenic disturbance. Heavy metal exposure in one or more vervet monkey groups can highlight the importance of necessary countermeasures to slow or cease the spread of heavy metal toxins from various anthropogenic sources including mine waste and urban development. Given our close evolutionary relationships with vervet monkeys, an understanding of their levels of heavy metals can also add to the well-documented literature regarding the adverse effects of toxins on human health. Furthermore, identifying these heavy metals in vervets can serve as a reflection of toxicity experienced by humans living alongside them. In addition, it is also important to understand the impacts of heavy metal toxicity on vervet monkey health if we seek to protect their populations. Lastly, heavy metal toxicity is a growing environmental threat to many nonhuman primate species, yet its impacts are frequently omitted in most conservation initiatives. To address this lacuna, this project documents the concentrations of heavy metals that vervet monkeys are exposed to and highlight the potential and very real detrimental effects of heavy metals in the environment.

The goal of this research project is to compare levels of heavy metal toxicity in hair samples from different vervet monkey populations, some of which are known to inhabit severely anthropogenically disturbed regions (i.e. at or near mine sites or urban cities). This research will employ trace element detection using an Inductively Coupled Plasma Mass Spectrometer (ICP-MS) to determine the parts per million (ppm) of each element within each hair sample. This noninvasive method for measuring toxicology in hair has been used to understand toxicity in other free-ranging mammalian species and humans (Poon 2004). However this approach has been infrequently employed to measure levels of heavy metal exposure in nonhuman primates (but see Engel et al. 2010 and Schillaci et al. 2013). The concentrations of As, Cd, Pb, and Hg will be compared between vervet monkey populations living in habitats with low, intermediate, and high levels of anthropogenic disturbance; to understand the relationship between anthropogenic disturbance and heavy metal concentrations. The degree to which each population is impacted by humans was assessed as low, intermediate, or high risk based on interviews with people that live among the vervet monkeys, rangers and game reserve employees who observe and interact frequently with the monkeys, and an in-depth literature review that focused on the impacts of humans at each of the 10 sites. It is expected that the populations of vervets that live in cities or inhabit landscapes in close proximity to mines will exhibit the highest concentrations of As, Cd, Pb, and Hg.

The data generated from this study can lead to future research in regards to the behavioral coping mechanisms (diet and behavior) exhibited by the various vervet monkey populations, based on their exposure to heavy metals as well as levels of toxicity in relation to their body size. Additionally, this preliminary research can provide the foundation for future, more in-depth studies of these populations and the environmental contaminants they are exposed to. These data can also be used to develop durable, long-term conservation strategies and buffer specific populations of vervet monkeys against harmful levels of heavy metal concentrations. Towards this end, these data can be applied to the conservation initiatives of other populations of nonhuman primates that live in landscapes severely altered by humans.

CHAPTER 2
BACKGROUND

South African Vervet Monkeys (Chlorocebus pygerythrus)

Vervet monkeys (*Chlorocebus*) consist of six species and are among the most widespread Old World monkeys found throughout northern, western, eastern, and southern portions of the African continent. Vervet monkeys, also commonly referred to as African green monkeys or grivets, are semi- terrestrial, and spend about half of their waking hours on the ground feeding, moving, and engaging in social behavior (Seyfarth and Cheney 1990). Vervet monkeys return to the trees each night to sleep and throughout the day use trees for safety, as sleeping sites, and for visually monitoring other groups or potential predators (Seyfarth et al. 1980). These monkeys are sexually dimorphic with males weighing 3.9-8.0kg, and females weighing 3.4-5.3kg (Napier 1981). In South Africa, vervet monkeys (*C. pygerythrus*) generally inhabit savanna to open woodland biomes accompanied by forest-grassland mosaics and always with a water source nearby (Grobler et al. 2006). Vervet monkeys are diurnal and omnivorous with an approximate life span of 17 years in the wild and up to 30 years in captivity (Fairbanks and McGuire 1986). Vervet groups can range from 20-50 individuals and are characterized by a matrilineal social structure, with males migrating out of their natal group when they reach maturity (usually 5-6 years in age) (Struhsaker 1967; Wrangham 1980). The home range of *C. pygerythrus* is ~100-175 hectares, though this size can change depending on the season as food resources become available. The primary limitations to a vervet monkey's home range are dependable water sources and trees for sleeping and protection (Wolfheim 1983). According to the International Union for the Conservation of Nature's (ICUN) Red List of Threatened Species, vervet monkeys are considered "Least Concern." This classification, coupled with a vervet monkey's small size and close evolutionary history with humans make them a suitable model species for biomedical research. In fact, vervet monkeys are among the most commonly used nonhuman primate in biomedical research in the United States (Carlsson et al. 2004).

In their native African landscapes, vervet monkeys are often considered pest primates because their habitats frequently overlap those of humans, including urban areas. Their widespread distribution sometimes results in human conflict and predation by farmers whom crops are being raided (Grobler et al. 2006). Farmers growing corn and sweet potatoes reportedly have a higher level of vervet crop raiding than those farmers growing other crops (Saj et al. 2001). In addition to human interaction, vervets are also at risk of exposure to environmental toxins due to industrialization. Vervets in South Africa are especially susceptible to industrialization through mining, given that there are over 5000 abandoned mines in South Africa (South African Auditor General 2009). The mining industry of South Africa began in the 1870s (Verhoef 1998) and has resulted in over 150 years of heavy metal (and other mining byproducts) contamination to some environments. Those vervet monkey populations living near these mining locations are at high risk for toxic levels of the following heavy metal elements Lead (Pb), Mercury (Hg), Cadmium (Ca), and Arsenic (As).

Mining in South Africa

Mining in South Africa started in the 1870s with the discovery of diamonds and gold (Verhoef 1998). Many mines have been closed or abandoned since then, and as of 2009, there are over 5000 abandoned mines in South Africa (South African Auditor General

2009) (Figure 1). Mines still under operation as of 2002, are presented in Figure 2 for comparison (Council for Geoscience 2002).

Figure 1. Map of abandoned mines in South Africa from the South African Auditor General Report of 2009, each dot represents one mine

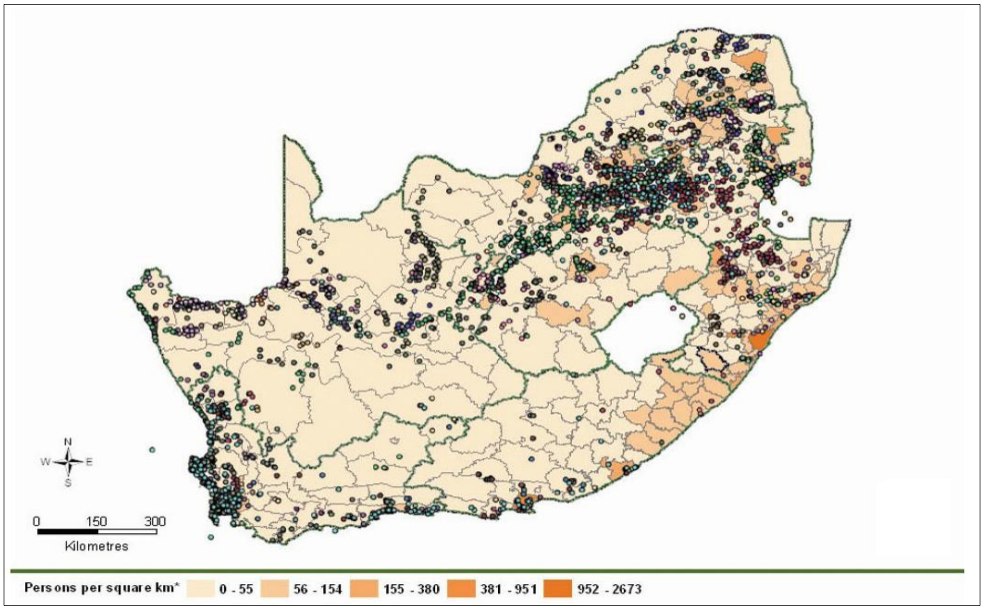
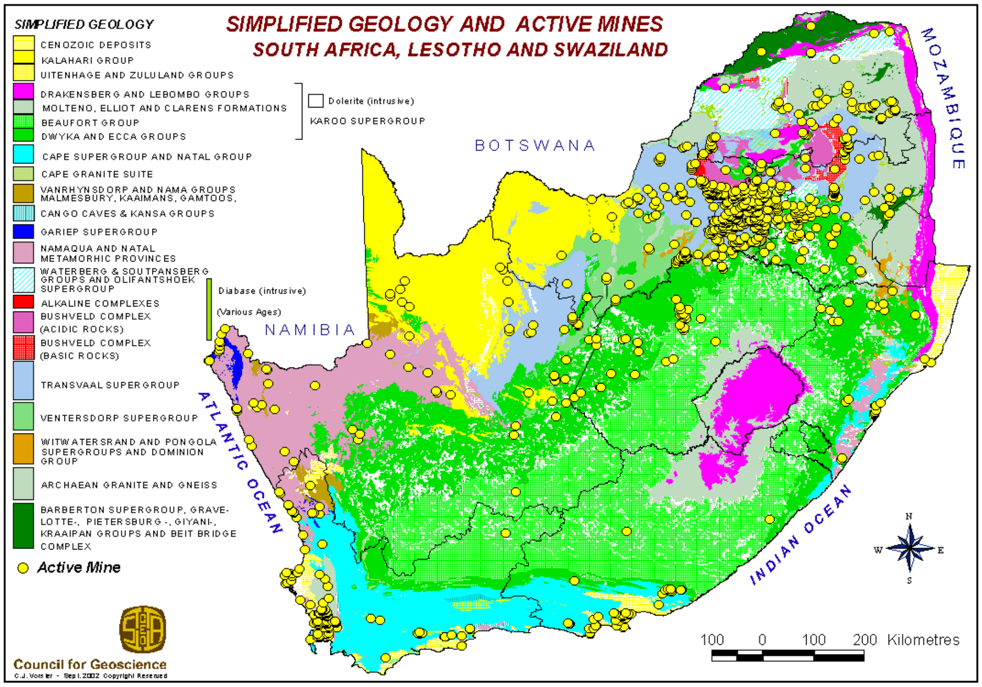


Figure 2. Map of active mines in South Africa from the Council for Geoscience as of 2002, each yellow dot represents one mine



Each of these mines, both active and abandoned, has one or more mine waste sites associated with it. At a mine waste site there are seven broad types of waste: 1) overburden, 2) tailings, 3) slags, 4) mine water, 5) water treatment sludge, 6) gaseous wastes, and 7) waste rock (Rankin 2011). Overburden waste consists of the surface soil and rock from the top levels of a mine site that are removed or disturbed to gain access to the deposits beneath. These materials are often piled on the surface of the mine or near the mine site. Tailings are finely ground rock and mineral waste byproducts that result from operations and can include toxic chemicals, heavy metals, and other industrial compounds. Tailings are often stored in holding ponds to decrease the potential of dust pollution. However, these ponds can and often do leech harmful contaminants into underground water sources. Slags are non-metallic byproducts from metal smelting processes. Slag materials are commonly converted into aggregate to be used in concrete mixtures. Mine water is produced in a variety of ways at mine sites but one common process is the wetting of otherwise dusty materials to reduce air pollution. Water management strategies at mine sites have made advancements in the recycling of mine water. The used mine water is collected, filtered, and reused at the site (Younger 2002). The filtered materials including harmful chemicals, heavy metals, and other industrial compounds are deposited into water treatment sludge that is often handled in the same manner as hazardous waste materials, because the compounds are considered poisonous. Gaseous wastes include dust particulates and sulphur oxides produced during high temperature processing such as smelting (U.S. Environmental Protection Agency 2010). Waste rock is the remaining crushed or separated rock that is considered to have no economic value and therefore is classified simply as waste. Waste rock is commonly piled on the surface of the mine or near the mine site and can be considered the most hazardous type of mine waste due to its potential for Acid Mine Drainage (AMD) (McCarthy 2011).

Acid Mine Drainage occurs when materials from the mining process that are discarded into waste rock piles containing deposits of pyrite (FeS_2). This pyrite then reacts with rainwater or purposefully sprayed water (for dust control) to produce sulphuric acid (H_2SO_4) that can break down and release heavy metals also contained in the waste rock, as it migrates to the bottom of the waste rock pile (Akcil 2004). This drainage results in pools of contaminated water at the base of the waste pile. At mines, policy indicates that the contaminated water be removed and stored in tailing ponds (described above) (Auditor General Report 2009). However, at unregulated mine sites, the contaminated water is disposed of in one of two ways. One, it simply is not disposed of, but it is left at the base of the waste pile where it eventually leeches into the ground and into underground water sources (Naidoo 2014). This is dangerous because all water sources are connected via underground sources; therefore over time (potentially decades), surrounding areas that were not otherwise effected by a mine, can become contaminated at the surface or among well water sources (Carson 1962; Winter et al. 1998) (Figure 3). Alternatively, unregulated mine industries pump the contaminated water directly into surface water sources (Naidoo 2014; Nordberg 2015). These polluted water sources contain hazardous chemicals used by the mining industry and heavy metals from the mine waste sites and are unknowingly utilized by humans, nonhuman primates, and other wildlife and livestock.

Figure 3. Groundwater contamination diagram from Pennsylvania State University

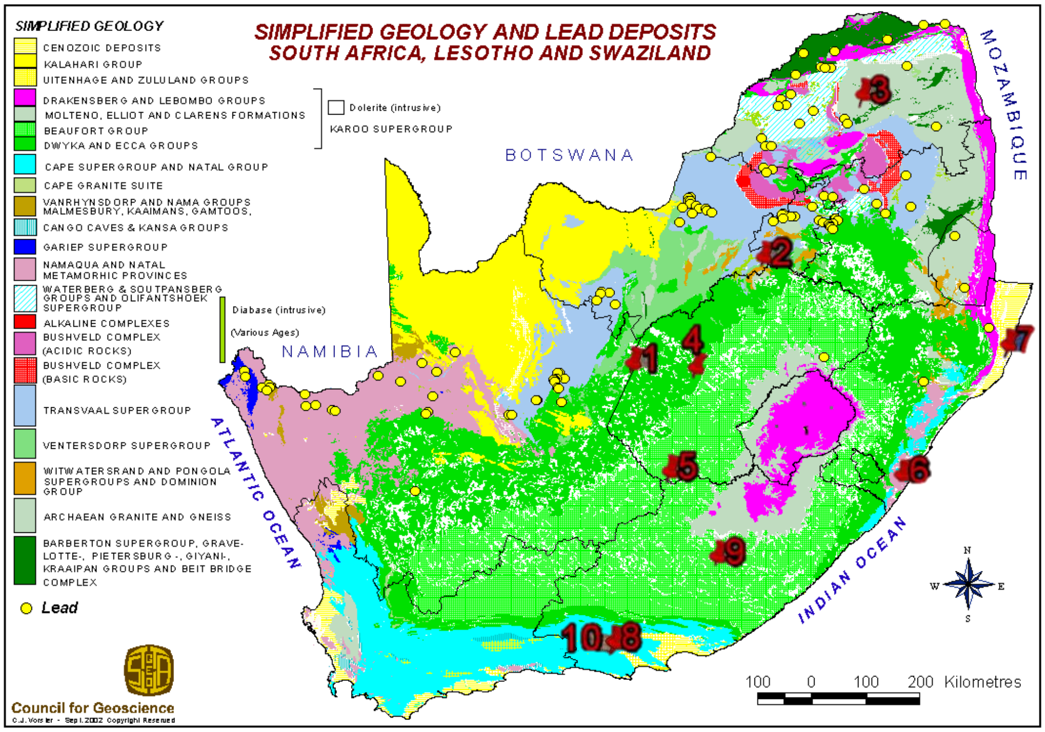
The exposure to chemical compounds and heavy metals can result in negative health risks such as increased cancer risk and, decreased neural, cognitive, and reproductive development in fetus's and young children and mammals. In adults, exposure to these pollutants can cause adverse physiological effects to the renal, hepatic, nervous, and endocrine systems (Naidoo 2014; Nordberg 2015; Kreitinger 2016).

Heavy metals

Lead (Pb)

Lead (Pb) occurs naturally within the Earth's crust. However, high concentrations of exposure to Pb is a health risk. In urban environments Pb as it is a byproduct for a variety of industrial processes including welding, urban development and construction, and mining. The most common mode of exposure to Pb is through inhalation or ingestion. Acute exposure to Pb at both high and low concentrations has the ability to impede the development and function of the skeletal, nervous, renal, circulatory, hepatic, and reproductive systems (Nordberg 2015; Engel 2010; Lee 2012). Chronic exposure to Pb results in bioaccumulation within the body and whereby Pb is slowly released over an extended period of time. This suggests that chronic exposure, even at low concentrations, is more threatening than acute exposure (Rosin 2009). On a molecular level, Pb closely mimics calcium which allows it to cross the blood brain barrier as well as be deposited in the bones and soft tissue (Sanders et al. 2009). When Pb is deposited in the bones, it can remain there for upwards of twenty years. During this period, the bones slowly release small amounts of Pb into the body. When Pb is mistaken for calcium in the body, it can be integrated into processes that largely depend on calcium, including interactions with proteins and alteration of enzymes. Utilizing Pb instead of calcium in these important processes results in identifiable adverse health effects. (Agency for Toxic Substances and Disease Registry 2005; Skerfving 2015). Pb exposure, both acute and chronic, varies in its intensity depending on the age of the individual exposed. Children and juvenile mammals are at a higher risk for Pb toxicity because on average they absorb 40% of dose lead compared to 10% in adults (Nordberg 2015). Moreover, many biological systems are still developing in children and juvenile mammals, making them more susceptible to toxicity (Rosin 2009 and Skerfving 2015). There is no reference dose for exposure to Pb because no low dose threshold has been recognized (Center for Disease Control and Prevention 2010). However, the median reference value considered to be a threshold for high levels of Pb in human hair is 11ppm (Iyengar and Woittiaz 1988). To date, there has been no reference median assessed for high hair Pb levels in nonhuman primates. Pb deposits in South Africa are presented in Figure 4 (Council for Geoscience 2002).

Figure 4. Pb deposits in South Africa (yellow dots) with population location markers of the vervet monkey populations sampled (red pins and numbers). Map adapted from the Council for Geoscience of 2002



Mercury (Hg)

Mercury (Hg) poses a serious health risk but is dependent on the form, amount, duration, and method of exposure. Elemental Hg most commonly is encountered in a traditional thermometer, but it is also used in fluorescent light bulbs, electrical switches, and mining, and other industrial processes. The most common way for elemental Hg to be released into the environment is through the burning of coal and other fossil fuels during mining processes. When released into the atmosphere in this manner, it allows for the inhalation of vaporizing Hg (Center for Disease Control and Prevention 2009; Engel 2010).

Methylmercury (MeHg) is another naturally occurring form in the environment and is considered a relatively benign form of Hg when intake of it is monitored. However, MeHg can be absorbed into the body rapidly by penetrating the lung's membranes after which it is slowly secreted (Berlin 2015). Exposure to any form of Hg can cause adverse physiological effects such as damage to the immune and nervous systems and cause birth defects. It can also significantly hinder development in a growing fetus or child (Engel 2010). Molecularly, Hg reacts with sulfhydryl groups in proteins that inhibit enzyme activity and can alter cellular membranes. Hg can also increase oxidative stress genes and mitochondrial damage which has a plethora of negative consequences including the onset of neurodegenerative diseases (Center for Disease Control and Prevention 2015). There is considerable debate regarding the median reference value for high Hg levels in hair but one study found the median reference value in human hair to be 3.25ppm (Iyengar and Woittiaz 1988). There has been no reference median assessed for high hair Hg levels in nonhuman primates.

Arsenic (As)

Arsenic (As) can be found in the environment in both organic and inorganic forms. Inorganic trivalent and pentavalent As are the forms that are most toxic to living animals (Tobin 2005). When ingested, ~70-90% of As is easily absorbed in the gastrointestinal tract while almost 70% of that is excreted, primarily in urine. The most common way that As is ingested is through contaminated ground water or through a variety the compounds found in insecticides. Prolonged exposure to As can have physiological effects on the nervous and circulatory systems that result in ischemic heart disease, cerebral infarction, and abnormal electrocardiograms (Fowler et.al. 2015). At the molecular level, As binds with sulfhydryl groups, in ways similar to Hg, and can disrupt sulfhydryl containing enzyme functions. Additionally, As can replace phosphorus in a number of biochemical reactions leading to the rapid hydrolysis of energy bonds in compounds such as ATP which results in the loss of energy and can uncouple mitochondrial respiration. This prevents the effected cells from converting macronutrient energy into ATP (Center for Disease Control and Prevention 2011).

As is commonly detoxified in the liver through methylation. However, in cases of chronic exposure, the body's methylation capacity can be exceeded, resulting in the retention of As in the soft tissues (Drobna et al. 2009; Fowler et al. 2015). The median reference As value for human hair that is considered high is 0.26 ppm. There has been no reference median that is considered high for As in nonhuman primate hair.

Cadmium (Cd)

Cadmium (Cd) is frequently found in bottom sediments and suspended particles of natural ground water and are identified as low level (0.00007ppm) and not physiologically harmful. However, contaminated water systems, such as those near mine waste sites, have increased levels of Cd that are toxic to mammals utilizing it for drinking water (Rose 2013). Low intake of Iron, Zinc, Calcium, and dietary proteins can increase the rate of Cd absorption (Nordberg et al. 2015). Prolonged exposure to Cd can result in a disturbance to calcium metabolism, osteoporosis, and lung and other cancers (Nordberg et.al 2015). At the molecular level, Cd is known to increase oxidative stress by potentiating the formation of reactive oxygen species (ROS) resulting in increased inflammatory cytokine production (Center for Disease Control and Prevention 2011). The median reference value for high hair Cd levels in humans is 1.15ppm. There has been no reference median value assessed for high hair Cd levels in nonhuman primates.

Use of hair in heavy metals analysis

Currently, there are only three documented assessments of heavy metal concentrations using Old World monkey hair samples. These studies were conducted on rhesus macaques (*Macaca mulatta*; Engel et al 2010; Lee et al 2012) and long-tailed macaques (*Macaca fascicularis*; Schillaci et al 2011). Only two of these studies were conducted on free-ranging

Old World monkeys (Engel et al. 2010; Schillaci et al. 2011) and the third was conducted on macaques used for biomedical research (Lee et al. 2012). Since little research has investigated heavy metal concentrations on free-ranging nonhuman primate populations, there is no framework known to understand the true effect of heavy metals on nonhuman primate health, thus forcing one to provide reference human samples for degrees of toxicity for each element. In many aspects of nonhuman primate life history, this is less of a problem given as much as we know in terms of humans and nonhuman primates evolutionary histories, shared physiologies, and long-term field research on nonhuman primate health (Turner et al. 2015). However, information regarding high heavy metal concentrations in humans is classified by developmental stage at either adult, juvenile, or infant. This poses a problem when assessing high heavy metal concentrations in vervet monkeys, because although they may be adults, their body mass is closer to that of an infant human. Moreover, little if any research is currently focused on the differences between body mass and heavy metal concentrations. As a result, reference values for high heavy metal concentrations in vervets will be those of developmental stage in humans (adult, juvenile, infant).

In the early stages of using hair as a toxicological biomarker, scrutiny was given due to the apparent contamination of the hair samples during transportation from the field to the lab (Poon 2004). However, in recent years, new methods of sample collection and subsequent sample preparation have been developed that have reduced the potential for endogenous contamination.

Vervet monkey population description – by Anthropogenic Disturbance

For this study, anthropogenic disturbance, or human disturbance, refers to any influence or burden that humans impose on their surrounding environment. Examples include deforestation, agriculture, mining, predation, and disease transmission; all of which can have negative consequences on nonhuman primates (Garber and Estrada 2009; McKinney 2015). This thesis primarily focuses on the anthropogenic disturbance of mining and the processes associated with this practice (i.e. mine waste and acid mine drainage). However, special attention is also given to those vervet populations that live near large urban centers. Descriptions of which can be found in the preceding mining activities section of this thesis and in Figure 1. The criteria used to determine level of anthropogenic disturbance were based on informal interviews with local people and park/reserve rangers that were conducted by the field team who collected the hair samples. Additionally, I conducted an in-depth literature review of each location in regards to anthropogenic impacts, particularly as it relates to mining and industrial activity.

Dronefield/Kimberley population - high anthropogenic disturbance

Large scale diamond mining in South Africa started in the 1870s with the discovery of the 21¼ carat Eureka diamond and the 83½ carat "Star of South Africa" (Davenport 2013). The largest diamond mine to date, Kimberly (Figure 5), operated for 43 years between 1871 and 1914 and produced 14.5 million carats over its lifetime (Davenport 2013).

Figure 5. Dronefield/Kimberley Diamond Mine, commonly referred to as the Big Hole in Kimberly, South Africa, aerial view from public domain (pixabay)

Though the Kimberly diamond mine hasn’t been in operation for 103 years, the environmental perturbations that resulted from the mining process still linger in the surrounding areas. During its years of operation, the Kimberly mine was an open pit mine or surface mine. This type of mine is described as the cutting away of surface material to access deposits beneath the surface (Davenport 2013). Some of the environmental impacts of open pit mining include erosion, loss of biodiversity, and contamination of groundwater (Monjezi et al. 2009).

Since its closing, the Kimberly mine has accumulated water, filling approximately 41 meters of its original 215 meter depth (SouthAfrica.net). This water is in contact with a large surface area of exposed mine rock, which can contain heavy metals and other industrial mining chemical compouds. This vervet monkey population is categorized as high anthropogenic disturbance because over time, the contaminated water has leeched into underground water systems and exposed human, nonhuman primates, and other wildlife and livestock through contaminated surface water sources (Figure 3).

Parys population – high anthropogenic disturbance

Parys lies in the Free State Province of South Africa and is a city of approximately 50,000 people located on the banks of the Vaal River. The Vaal River flows from the greater Johannesburg region to the city of Parys. The Johannesburg area has a high concentration of abandoned mines (Figure 1), giving the Vaal River potential for significant contamination. Additionally, tributaries of the Vaal River include the Blesbok and Reit streams, and the Sulkerbosrand and Klip Rivers. All of which have been examined for levels of heavy metals, which were found to be present in both sediment and among the aquatic life (Pheiffer et al. 2014; Roychoudhury and Stake 2006). For these reasons, the vervet monkey population was categorized as high anthropogenic disturbance.

Polokwane Reserve population – high anthropogenic disturbance

The Polokwane Reserve vervet monkey population is directly on the edge of the large city of Polokwane in the Limpopo Province of South Africa. The approximate population of Polokwane is 130,000 people (South African census 2011). This population is categorized as high anthropogenic disturbance because the monkeys are located less than a kilometer from an operational aggregate quarry. Aggregate is used in the construction of road and concrete materials. The process of acquiring the aggregate is similar to open pit mining, including the use of waste rock piles for disposal of economically invaluable materials (LafargeHolcim 2017). These materials can include heavy metals and other potentially hazardous chemical compounds associated with this large urban area.

Soetdoring population – high anthropogenic disturbance

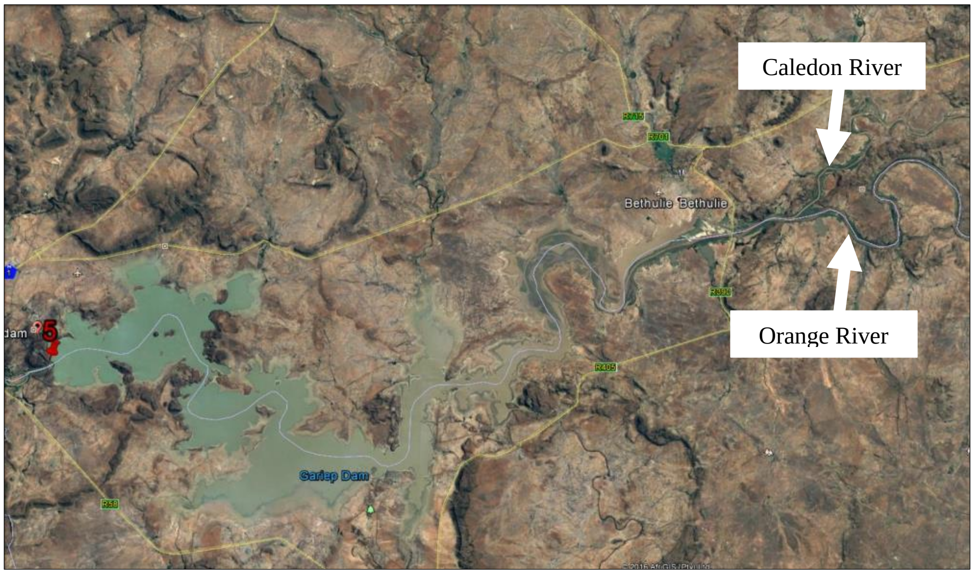
The Soetdoring population is near the city of Soutpan, a small salt mining village in the Free State Province. Salt has been mined in this area since 1908 by solution and solar evaporation mining. This type of mining consists of erecting wells over salt beds or domes and injecting them with water to dissolve the salt. The salt is then pumped into salt pans or holding ponds (Figure 6) where the water is then evaporated, leaving only the salt crystals (Delaurentis 2002 and Santhanakrishnan 2016). The potential contamination of these salt pans comes from the extraction of the salt solution from the Earth. As the solution passes through layers of sediment on its way to the surface, it can retain heavy metals found in the Earth. The salt solution, accompanied by the trace heavy metals is left to evaporate in a salt pan, however some of the water leeches into underground water sources. The heavy metals that were not exposed before, now have an opportunity to leech into the ground water system due to being in the water solution from salt mining (Santhanakrishnan 2016). For these reasons, the vervet monkey population at Soetdoring is placed in the high anthropogenic disturbance category.

Figure 6. Aerial view of saltpans at Soutpan, Free State, South Africa

Gariep Dam population – intermediate anthropogenic disturbance

The Gariep Dam population is located just outside the city of Norvalspont, which borders the Free State Province and the Eastern Cape Province. There are two large rivers, the Orange and the Caledon that flow directly into the Gariep Dam. (Gariep Dam 2017) (Figure 7). This population was categorized as intermediate anthropogenic disturbance because the source of each of these rivers begins at a different area in South Africa, therefore potentially bringing with them a variety of contaminants including heavy metals. The water in the Gariep basin behind the dam, is often used for the irrigation of thousands of hectares in the surrounding areas. Therefore, if heavy metals are being brought into the dam, they could be transported to surrounding environments as well (SA Places 2016).

Figure 7. Map of Orange and Caledon Rivers that flow directly into the Gariep Dam (red pin) area



Oribi Gorge population – intermediate anthropogenic disturbance

The Oribi Gorge vervet monkey population is located at the edge of the Oribi Gorge Nature Reserve and is surrounded by agricultural land. This site was categorized into the intermediate anthropogenic disturbance category because of the nearby Idwala Carbonates Mine (Figure 8). This mine has the potential to pollute the river that runs alongside it, and into the Oribi Gorge vervet population's habitat. In addition these river waters have the potential to pollute the shallow wells used by the farmers in the nearby agricultural areas. Carbonates are mined by quarrying, similar to open pit mining with mine waste sites (Idwala 2017; Gaiero 1998).

Figure 8. Aerial view of the Idwala Carbonates Mine, South Africa

St. Lucia population – intermediate anthropogenic disturbance

The St. Lucia vervet monkey population is located in the KwaZulu-Natal Province of South Africa which is an increasingly popular tourist and resort destination. A large portion of the St. Lucia area is considered a wetland reserve and has a staff of administrators and rangers who manage the park and landscape as a World Heritage Site (Nduru 2007). Additionally, in 1993, an environmental impact assessment deemed a proposed titanium mine in the area, "would cause unacceptable damage" (Mail & Guardian 1997). However there are still a rising number of commercial and residential developments in the area. The St. Lucia vervet monkey population is familiar but not completely habituated to humans but are frequently observed in areas with large numbers of people. This suggests that the potential hazards of construction and a changing landscape could be affecting this population, which is why they are categorized as intermediate anthropogenic disturbance.

Tsolwana population – low anthropogenic disturbance

The Tsolwana population is located in the Tsolwana Game Reserve in the Eastern Cape Province of South Africa. Although the vervet populations often share the same space as tourists visiting the reserve, they have the ability to avoid people at will and utilize other portions of the reserve (SA Venues 2016). Additionally, the reserve is not surrounded by agricultural land or any cities or towns (Google Earth 2017). For these reasons this monkey population is categorized as low anthropogenic disturbance.

Baviaanskloof Rooiport and Geelhousbout populations – low anthropogenic disturbance

The two Baviaanskloof vervet monkey populations (Rooiport and Geelhousbout) are part of the Baviaanskloof Nature Reserve in the Eastern Cape Province of South Africa. Both vervet monkey populations have the ability to avoid humans but sometimes interact with them in feeding contexts (Baviannskloof.net 2016). The Baviaanskloof populations are located on the edge of the Baviannskloof farming community, but because no record of the vervets consuming the crops has been recorded and the farmers don't appear to use harmful synthetic insecticides on their crops, these vervet monkey populations are classified as low anthropogenic disturbance (Loudon et al. 2014).

CHAPTER 3

METHODS AND MATERIALS

Location and materials

Vervet monkey (*Chlorocebus pygerythrus*) hair samples were collected during previous field seasons as part of an ongoing longitudinal study directed by Dr. Trudy Turner (University of Wisconsin-Milwaukee) and Dr. J Paul Grobler (University of the Free State, Bloemfontein). This long term project examines vervet monkey genetics (Turner 1981; Dracopoli et al. 1983; Grobler et al. 2006), morphometrics, and physical health (Turner et al 1994; Turner et al 1997) throughout Kenya, Ethiopia, and South Africa (Grobler and Turner 2010). The data analyzed here were all collected by Drs. Turner and Grobler, from 10 vervet monkey populations located throughout South Africa. Hair samples were initially sent to Dr. James E. Loudon for carbon and nitrogen stable isotope analyses.

Individual monkeys were baited with corn or oranges and captured in specialized traps that were designed to minimize physical and psychological stress to the monkeys (Grobler and Turner 2010). Once captured, the monkeys were administered a cocktail of Ketamine and Xylazine with a handheld syringe. In total, 60 hair samples were collected from 10 vervet groups ranging from locations with low anthropogenic disturbance to locations with high levels (see below). While sedated, blood samples and dental impressions were collected, and a clipping of hair from the upper shoulder. The hair samples were placed in individual envelopes identified with the group location, individual vervet monkey identification, and the age and sex of the vervet. All methods for trapping and sedation were approved by the Institution of Animal Care and Use Committee (IACUC) at the University of Wisconsin-Milwaukee and the Interfaculty Animal Ethics Committee at the University of the Free State.

The vervet monkey populations sampled consist of 10 groups with three levels of hypothesized anthropogenic disturbance (high, intermediate, low; see Table 1 and Figure 9). The populations were placed in these categories based on interviews with local people and park/reserve rangers, and an in-depth literature review of each location in regards to anthropogenic impacts, particularly as it relates to mining and industrial activity. A full description of the location of each vervet population site can be found in the preceding background chapter of this thesis.

Figure 9. Location of each vervet monkey study population (see Table 1 for numeric identification) shown on the map of South Africa

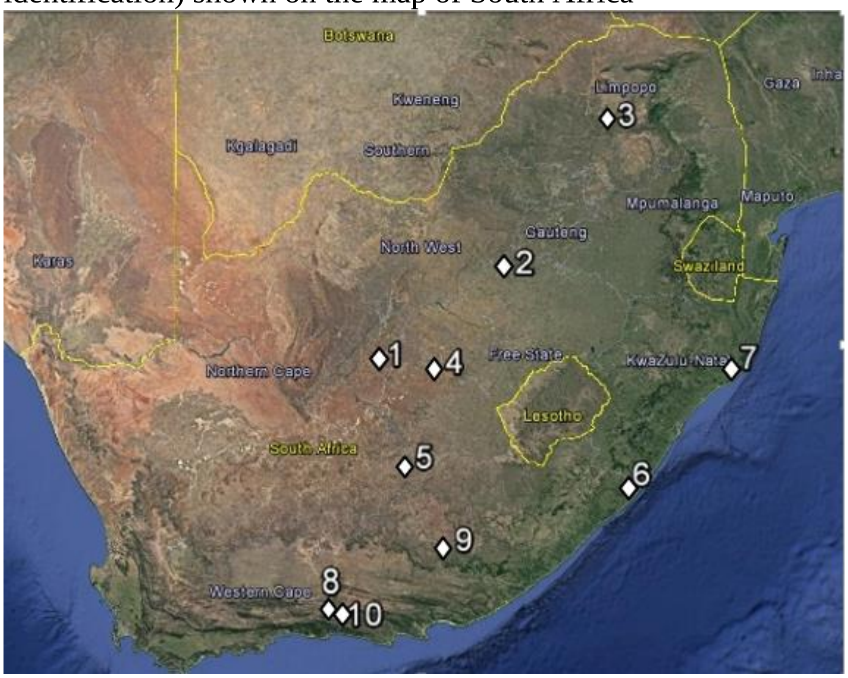


Table 1. South African vervet monkey populations placed in three categories based on the degree to which their habitats have been anthropogenically disturbed.

Vervet South Africa Locations (VSA) and Anthropogenic Disturbance (high, intermediate, low)		
High	**Intermediate**	**Low**
1 – Dronefield/Kimberly	5 – Gariep Dam	8 – Baviaanskloof Rooiport
2 – Parys	6 – Oribi Gorge	9 – Tsolwana
3 – Polokwane	7 – Saint Lucia	10 – Baviaanskloof Geelhousbout
4 – Soetdoring		

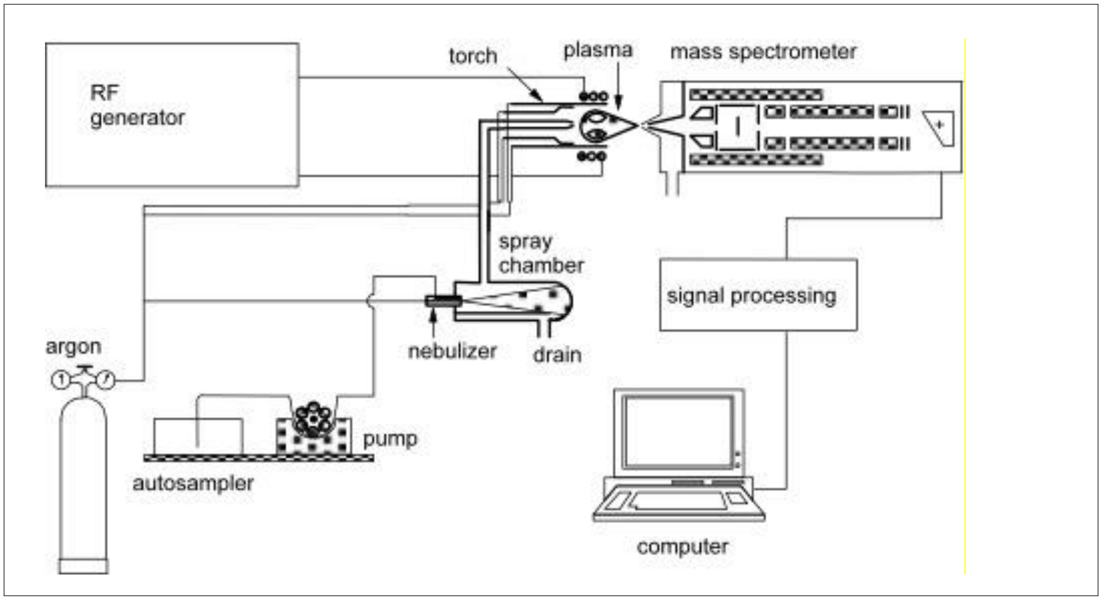
I analyzed six vervet monkey hair samples from each of the 10 locations (n = 60). The six individual samples were chosen based on which envelopes had enough hair to quantify their heavy metal concentrations. This approach also resulted in a random number of males and females from each group to be analyzed. To date, there have only been three published studies that have examined heavy metal concentrations in free-ranging Old World monkeys and each has focused on macaques (rhesus macaques: *Macaca mulatta,* and long-tailed macaques: *Macaca fascicularis*) (Engel et al 2010; Schillaci et al. 2010; Lee et al. 2012). For reference in my project, Old World monkey hair grows at approximately 0.5mm per day but this can be impacted by seasonality. Given the hair in this study were an average of ~50mm in length, each strand records nutritional and toxic biomarker data for approximately 100 days (Engel et.al 2010). This differs from blood toxicity analysis where the time frame is a matter of days, thus only recent acute exposure can be assessed. Though recent acute measurements might be more suited to pinpointing the source of exposure, prolonged measurements can assess the possibility of continued exposure. Using hair to assess heavy metal toxicity has received scrutiny due to its inaccuracy during early trials. The hair samples can become contaminated during transport to the labs where they were processed and further contaminated during analysis (Poon 2004). However, new methods of sample preparation have been developed and the contamination of samples has been greatly reduced.

One drawback for using hair for heavy metal toxicity analysis is the point at which levels become unsafe is unknown. Thus far, nonhuman primates have been scarcely the focus of such studies, even though these approaches are largely non-invasive. Those few studies that have utilized hair are most commonly focusing on captive nonhuman primates used in biomedical research. The reason for this centrality is that biomedical research is aimed to further human health; therefore understanding the levels of lethal heavy metal toxicity in nonhuman primates is crucial and is a basis for further study.

Heavy metal analysis

Heavy metal analysis for Arsenic (As), Cadmium (Cd), Lead (Pb), and Mercury (Hg) for each vervet monkey hair sample was conducted at the Department of Chemistry at East Carolina University by Dr. Jack E. Pender using an Agilent 7900X Inductively Coupled Plasma Mass Spectrometer (ICP-MS) (Figure 10). This instrument is a highly accurate mass spectrometer because it uses argon plasma. Samples are introduced to the argon plasma as aerosol droplets. The heat of the plasma quickly dries the aerosol and can easily remove an electron from the element. This process forms singly charged ions which are then directed into a filtering device, commonly known as the mass spectrometer. As the ions leave the mass spectrometer they hit a dynode of an electron multiplier. The impact of the ions on this multiplier release a cascade of electrons which are amplified until they form a measureable pulse. The ICP-MS software can compare the intensities of the pulses to those of commonly used standards. This process determines the concentration of the element being analyzed. The standards used in this study were natural human hair, European Reference Material (ERM) certified reference material from Sigma-Aldridge.

Figure 10. Diagram of Inductively Coupled Plasma Mass Spectrometer (ICP-MS)



To prepare the all hair samples for analysis using the ICP-MS, each individual hair sample was transferred to a digestion vessel and weighed. Once the hair weight had been recorded, 3.0 mL HNO3 and 7.0 mL internal stock 2 was added leaving a total volume of 10mL. From here the hair underwent a four stage digestion process within a controlled microwave digestion chamber (Mars 5 Microwave). Each stage was digested with 380w of power for five minutes. The pressure was increased in each stage, starting at 20psi, then 40psi, 85 psi, and finally 130psi. When the four stages of digestion were complete, the individual vessels were allowed to cool for a minimum of 10 minutes. Contents were then transferred to labeled 15mL centrifuge tubes. At this stage the samples have been digested and are in a liquid state. The tubes are then fed into the ICP-MS to quantify their heavy metal concentrations. The concentrations for each heavy metal is presented in parts per million (ppm).

Statistical Analysis

Assessing the heavy metal concentration of each hair sample at a specific location revealed the amount of exposure the individual vervet monkey accumulated over approximately 100 days. The statistical program JMP was used to analyze the heavy metal concentrations by location, estimated degree of anthropogenic disturbance, and sex. For these comparisons, I used Welch's ANOVA, which accounts for high levels of variation. I also used the Tukey's Honest Significant Difference (HSD) post hoc test for pairwise comparisons between each site and degree of anthropogenic disturbance.

CHAPTER 4

RESULTS

The concentrations of Cadmium (Cd) and Mercury (Hg) were below the level of quantification (LOQ) and therefore were not detected and will be excluded from further data analyses. Reasons for the low levels of Cd and Hg are discussed in the Conclusion and Discussion section below. Concentrations of Lead (Pb) and Arsenic (As) were found in all 60 samples. Mean Pb concentrations for all ten populations were higher than mean As concentrations across all ten populations (Figure 13). The Pb and As values for each vervet monkey by sex and corresponding site location is presented in Table 2. The mean and standard deviation for each vervet monkey population is presented in Table 3 and Figure 11 and the data for Pb and As values for each vervet monkey hair sample is shown in Figure 12. Mean Pb and As values for each population, categorized by anthropogenic disturbance is presented in Figure 13.

Table 2. Pb and As concentrations for individual vervet monkey, including their sex for each of the 10 populations

Group/Location and estimated degree of anthropogenic disturbance	Site ID	ID	Sex	Mass (mg)	Pb (ppm)	As (ppm)
Dronefield/Kimberly (high)	**K (1)**	173	M	34.8	1.338	0.289
		174	M	8	1.756	0.000
		175	M	28.6	4.107	0.330
		176	M	69.2	3.062	0.132
		177	M	21.3	1.982	0.000
		178	F	30.8	1.504	0.250
Parys (high)	**PA (2)**	413	F	68.7	0.588	0.128
		415	M	67.3	1.115	0.233
		416	M	42.6	0.949	0.151
		419	M	63.9	1.282	0.414
		420	M	49.3	0.796	0.149
		421	M	79.2	1.828	0.160
Polokwane Reserve (high)	**PK (3)**	152	M	27.3	0.597	0.223
		154	M	43.7	0.685	0.146

		155	M	22.5	0.753	0.000
		158	F	22.6	0.905	0.000
		161	F	11.3	1.602	0.000
		164	F	32.5	1.495	0.305
Soetdoring (high)	**SO (4)**	455	F	135.1	1.191	0.187
		456	F	80.3	0.484	0.073
		457	M	181.3	0.695	0.158
		462	M	63	0.658	0.261
		464	F	88.8	0.547	0.190
		466	F	93.3	1.070	0.062
Gariep Dam (intermediate)	**G (5)**	404	F	41.8	1.472	0.119
		406	F	43.5	1.427	0.000
		407	M	49.4	2.337	0.105
		408	F	46.2	1.771	0.000
		409	F	76.7	1.802	0.200
		410	F	67.5	4.118	0.128
Oribi Gorge (intermediate)	**O (6)**	131	F	37.8	1.076	0.165
		132	M	36.5	1.578	0.255
		133	M	16.2	0.901	0.000
		134	M	11.8	1.708	0.000
		136	F	77.9	1.220	0.180
		137	F	45.4	0.615	0.166
St. Lucia (intermediate)	**SL (7)**	503	F	101.3	1.104	0.102
		506	F	90.2	0.352	0.096
		507	F	146.9	0.630	0.099
		509	F	52.7	0.233	0.095
		516	F	101.1	0.778	0.084
		519	F	99.6	1.070	0.062
Baviaanskloof Rooiport (low)	**BR (8)**	490	M	131.1	1.289	0.214
		491	F	211	0.888	0.213
		492	M	243.3	1.022	0.112
		493	M	148.1	2.219	0.122
		494	F	133.4	0.585	0.084
		495	M	210.5	0.453	0.092
Tsolwana (low)	**T (9)**	471	M	112.1	1.389	0.179
		472	M	135.6	0.626	0.316
		473	M	M8	0.595	0.144
		479	F	F7	0.398	0.115
		480	F	F8	0.687	0.116
		482	F	F7	1.259	0.205
Baviaanskloof Geelhousbout (low)	**BG (10)**	485	F	138.1	1.044	0.446
		486	M	32.2	0.559	0.164
		488	F	39.8	0.658	0.167
		489	F	55.7	0.374	0.209
		497	F	53.1	0.653	0.236

		498	F	47.5	0.354	0.154

Table 3. Pb and As mean concentrations and standard deviations for each population

Population	Mead Lead (ppm)	SD	Mean Arsenic (ppm)	SD
Dronefield/Kimberly (high)	2.292	1.077	0.167	0.145
Parys (high)	1.093	0.434	0.206	0.108
Polokwane Reserve (high)	1.006	0.433	0.112	0.133
Soetdoring (high)	0.774	0.289	0.155	0.076
Gariep Dam (intermediate)	2.155	1.015	0.092	0.078
Oribi Gorge (intermediate)	1.183	0.411	0.128	0.104
St. Lucia (intermediate)	0.695	0.361	0.090	0.015
Baviaanskloof Rooiport (low)	1.076	0.635	0.140	0.059
Tsolwana (low)	0.826	0.400	0.179	0.076
Baviaanskloof Geelhousbout (low)	0.607	0.252	0.229	0.111

Figure 11. Mean Pb and As concentrations for each of the ten South African vervet populations

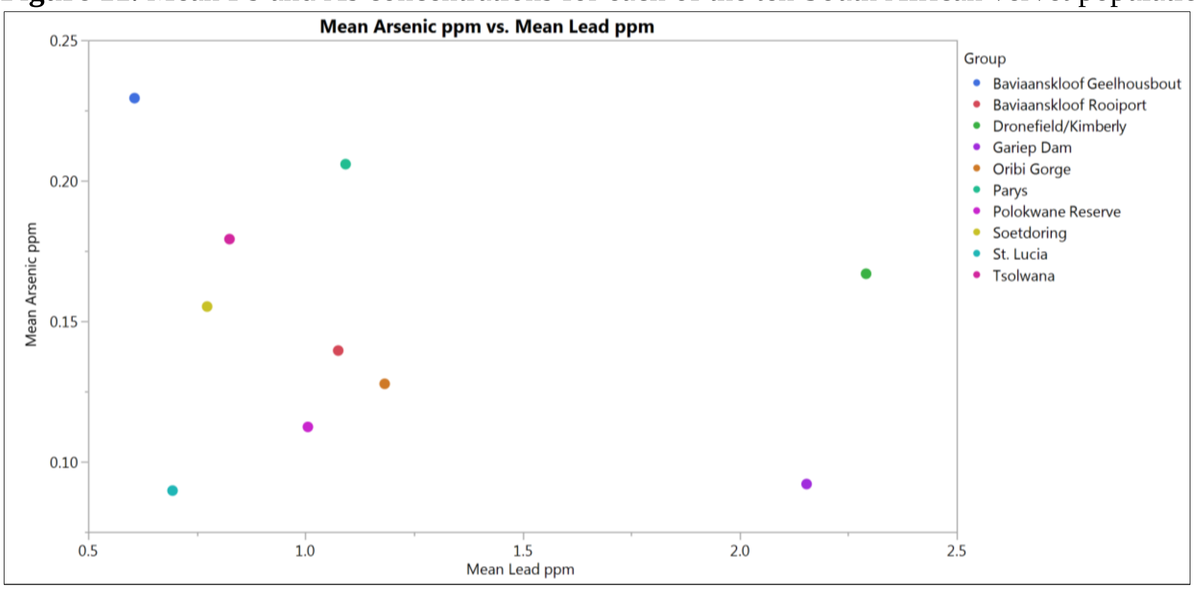


Figure 12. Pb and As concentrations for each vervet monkey, classified by site

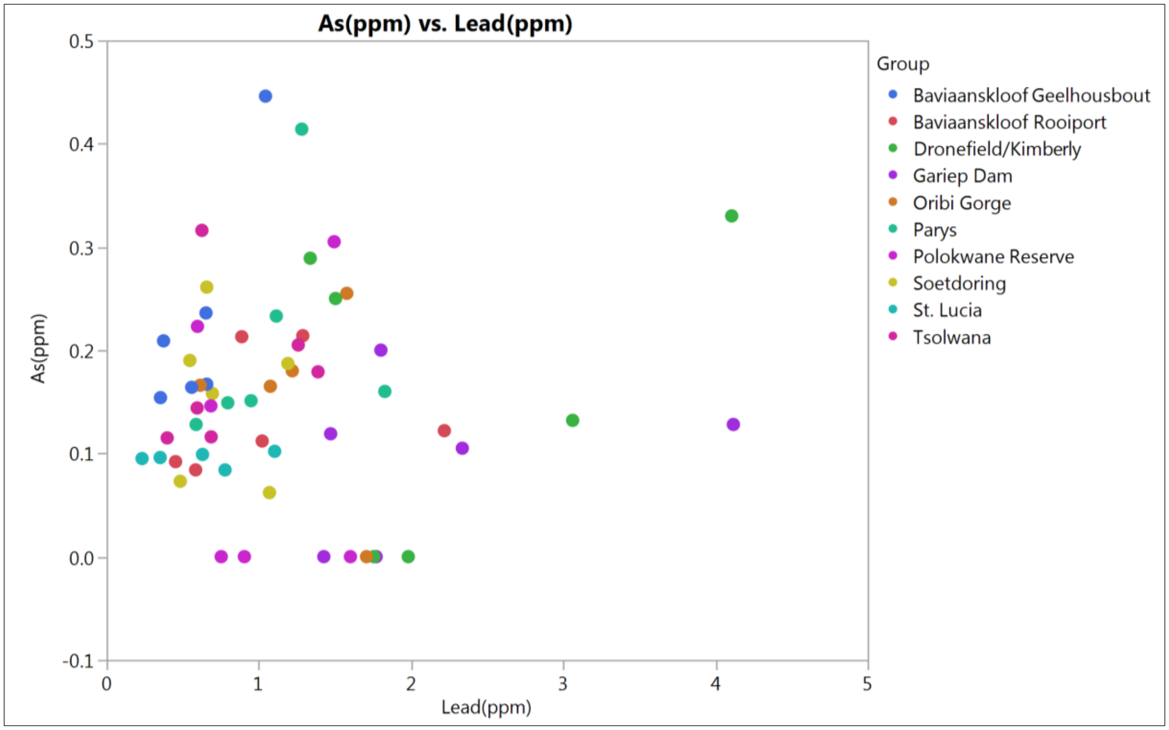
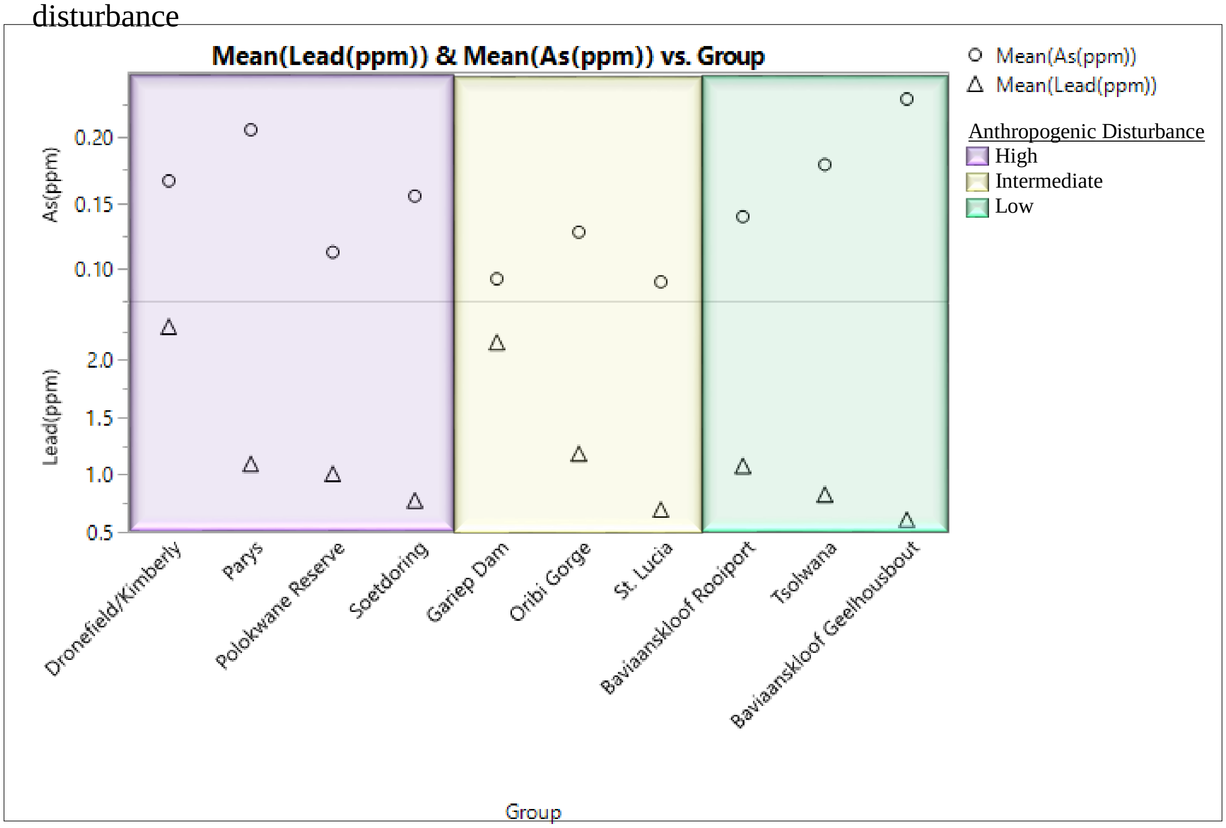


Figure 13. Mean Pb and As, categorized by population and estimated degree of anthropogenic disturbance



Among the ten populations there were significant differences in the Pb concentrations (Welch's ANOVA $F_{9, 20.2} = 3.08$; $P < 0.05$). Baviaanskloof/Geelhousbout exhibited the lowest Pb concentrations (mean = 0.607 ppm, s.d = 0.252), and Dronefield/Kimberly exhibited the highest (mean = 2.292 ppm, s.d = 1.077). Pairwise comparisons of the Pb concentrations between the populations using Tukey's HSD test are shown in Table 4.

Table 4. Pairwise comparisons of Pb concentrations between populations

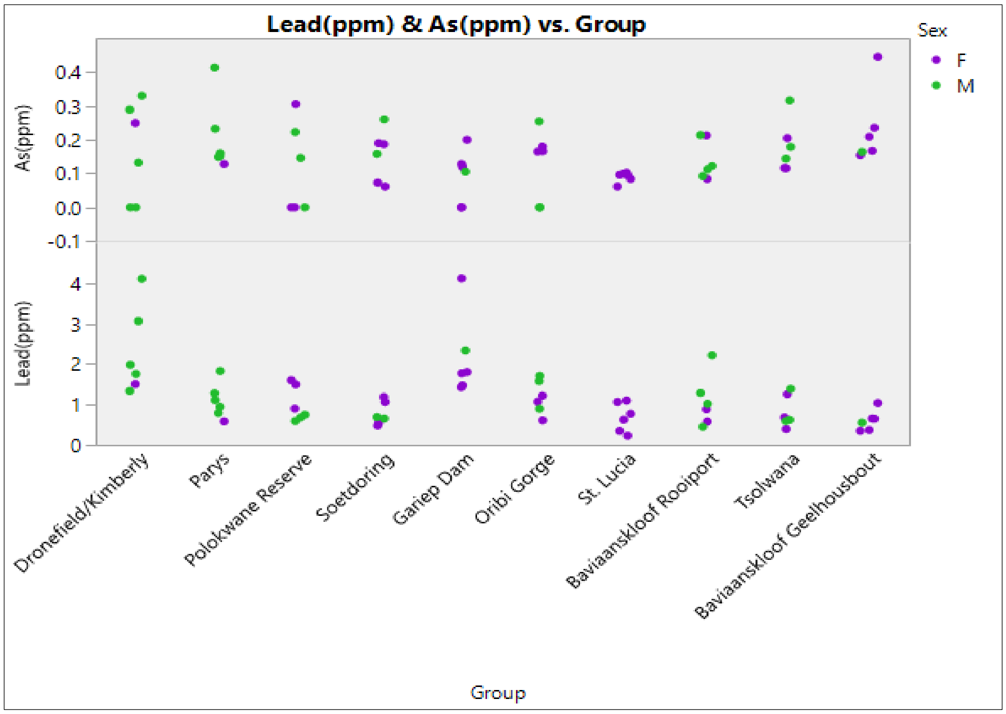
Higher concentration vs. Lower concentration	p-value
Dronefield/Kimberly vs. Baviaanskloof Geelhousbout	<0.001
Dronefield/Kimberly vs. Soetdoring	<0.01
Dronefield/Kimberly vs. Tsolwana	<0.01
Dronefield/Kimberly vs. St. Lucia	<0.01
Dronefield/Kimberly vs. Baviaanskloof Rooiport	<0.05
Dronefield/Kimberly vs. Parys	<0.05
Dronefield/Kimberly vs. Polokwane Reserve	<0.05
Gariep Dam vs. Baviaanskloof Geelhousbout	<0.01
Gariep Dam vs. Soetdoring	<0.01
Gariep Dam vs. St. Lucia	<0.01
Gariep Dam vs. Tsolwana	<0.05
Gariep Dam vs. Polokwane Reserve	<0.05

There were also significant differences in the As concentrations among the ten populations (Welch's ANOVA $F_{9, 19.1} = 2.68$; $P < 0.05$). St. Lucia exhibited the lowest As concentrations (mean = 0.089 ppm, s.d = 0.014) and Baviaanskloof/Geelhousbout exhibited the highest (mean = 2.292 ppm, s.d = 0.111). Tukey's HSD pairwise comparisons revealed no differences between the populations. Comparisons between male (n = 27) and female (n = 33) concentrations for Pb and As were not significant (Pb: Welch's ANOVA $F_{1, 50.6} = 2.35$; $P = 0.13$ and As: Welch's ANOVA $F_{1, 51.6} = 0.60$; $P = 0.44$). The mean and standard deviations for the Pb and As concentrations for males and females are presented in Table 5. The values of Pb and As for each monkey classified by sex and population are shown in Figure 14.

Table 5. Mean and standard deviations for Pb and As concentrations for female and male vervet monkeys

Sex	N	Mean Pb (ppm)	SD	Mean As (ppm)	SD
Female	33	1.029	0.709	0.140	0.093
Male	27	1.344	0.852	0.161	0.109

Figure 14. Pb and As concentrations for individual vervet monkeys classified by sex and population



I also compared the concentrations of Pb and As for the three categories of anthropogenic disturbance (see Table 6 and Figure 15). There were significant differences in Pb (Welch's ANOVA $F_{2, 35.3} = 3.72$; $P < 0.05$) and As (Welch's ANOVA F 2, 37.6 = 4.65; $P < 0.05$) among the three categories of disturbance. Collectively, the highest mean value for Pb was found among the groups in the intermediate category (mean = 1.344 ppm, s.d = 0.884). The highest mean values for As was exhibited by the groups which experienced the lowest degree of anthropogenic disturbance (mean = 0.183 ppm, s.d = 0.088). Tukey's PSD pairwise comparisons showed that As concentrations for the groups placed in the low category were significantly higher than the groups in the intermediate category (P < 0.05). There were no differences in the As concentrations between the groups in the low or high categories of anthropogenic disturbance (P = 0.73). Tukey's PSD pairwise comparisons revealed no differences in the levels of Pb between the three categories of anthropogenic disturbance.

Table 5. Pb and As mean and standard deviations concentrations in vervet monkey hair from populations placed in three categories of anthropogenic disturbance

Degree of anthropogenic disturbance	N	Mean Lead ppm	SD	Mean Arsenic ppm	SD
Low	18	0.836	0.473	0.183	0.088
Intermediate	18	1.344	0.884	0.103	0.073
High	24	1.291	0.845	0.160	0.116

CONCLUSION AND DISCUSSION

The purpose of this study was to identify the concentrations of heavy metals Arsenic (As), Cadmium (Cd), Lead (Pb), and Mercury (Hg) in 10 South African vervet monkey (*Chlorocebus pygerythrus*) populations categorized into three different levels of anthropogenic disturbance (high, intermediate, low). It was expected that the vervet populations with the highest level of anthropogenic disturbance would exhibit the highest levels of heavy metals concentration. Respectively, the vervet populations with the lowest levels of anthropogenic disturbance would exhibit the lowest levels of heavy metal concentration. These hypotheses were not supported by the data, and are discussed below. Concentrations of heavy metals Cd and Hg were above the limit of detection (LOD) but below the limit of quantitation (LOQ). This suggests that the 10 vervet populations were either not exposed to significant levels of Cd and Hg or levels were undetectable in hair because Cd and Hg have an affinity for tissues and lipids (Berlin at al. 2015) verses hair. In contrast, the heavy metals As and Pb were found above the LOQ in all 10 vervet populations and will be further discussed.

Arsenic (As)

To date, there has been one previous study conducted on heavy metal concentrations in Old World monkey hair that included the heavy metal, Arsenic (As). That study examined eight heavy metal concentrations in the 28 rhesus macaques (*Macaca mulatta*) from southwest China (Lee et al. 2012). Not much is known about the behavioral ecology or life history of these macaques as they were raised at the Guangxi Grand Forest Scientific Primate Company in Ping Nan County, China and were imported to South Korea for biomedical purposes. The mean As concentration for these macaques was 0.654 ppm (SD = 0.331; n = 28). This value is higher than the mean As concentrations found among the South African vervet monkeys sampled in this study, mean = 0.150 (SD = 0.1; n = 60) and exceeds the highest As value found among any of the vervets sampled in this study, 0.446 ppm. This vervet monkey was a female that lived in the Baviaanskloof Geelhousbout population which was placed in the low anthropogenic disturbance category. Mayo Medical Laboratories (2017) identifies toxic hair As concentration at >1.00 ppm in humans (all ages). However, no such reference has been evaluated for Old World monkeys, nor is there a reference for As concentrations in regards to body size. This lacuna encompasses Pb reference concentrations as well. Therefore, this study uses high human hair concentrations as a reference for heavy metal toxicity. The vervet monkey population that exhibited the highest concentration of As (0.229 ppm; SD = 0.111; n = 6) was found among the Baviaanskloof Geelhousbout population. The St. Lucia vervet monkey population, which was placed in the intermediate disturbance category, exhibited the lowest concentration of As (0.09 ppm; SD = 0.015; N = 6). These results do not support my expectations that those vervet monkey populations placed in the high category for anthropogenic disturbance would exhibit the highest concentrations of As.

Lead (Pb)

Two previous studies conducted on heavy metal concentrations in Old World monkey hair (Engel et al. 2010) examined the concentrations of Pb and Hg in three groups of rhesus macaques (*Macaca mulatta*) inhabiting the Buddhist temple of Swoyambhu located approximately 3 km from the densely populated urban center of Kathmandu in Nepal. The macaques at this site drink water from streams that are polluted with sewage and a gun powder factory (Engel et al. 2010) and are thus highly impacted by their human counterparts. The Pb concentrations exhibited by the three macaque groups at Swoyambhu vary greatly from 1.34 ppm to 10.2 ppm (n = 37) and the mean Pb values for each group was higher than the values found among the South African vervet monkey populations sampled in this study (vervet monkey range: 0.233 – 4.188 ppm; n = 60; see Table 3). This may be because the Swoyambhu macaques are drinking from water sources polluted by the gunpowder factory. However, given that these macaques live in close association with humans and raid the urban center of Kathmandu, it's difficult to determine the source(s) of their Pb exposure.

Schillaci et al. (2012) examined the Pb concentrations in the hair of long-tailed macaques (*M. fascicularis*) from eight populations throughout Singapore. The Pb concentrations ranged from 0.21 to 6.45 ppm (n = 17) (Schillaci et al. 2011). Six of the macaque populations were trapped in the Central Catchment Nature Reserve (CCNR), while the remaining two were trapped within the Bukit Timah Nature Reserve in Singapore. Macaque populations in all eight habitats had access to secondary and disturbed forest patches, city parks, roads and parking lots, and access to water. One specific macaque population located on Rifle Range Road, contains live firing ranges, and an ammunition manufacture and testing facility. Additionally, this area has a history of being an ammunition storage area. Due to the historically hazardous nature of this area, it is likely that the environment has been continually contaminated over a period of more than 50 years. The elevated levels of Pb in this particular macaque population (4.516 ppm; SD= 1.712; n = 5) could be the product of ingestion of contaminated plants and water and inhalation of dust from the ammunition manufacturing facilities (Schillaci et al. 2011).

Mayo Medical Laboratories (2017) suggests Pb hair concentrations <5.0 ppm are not clinically harmful to humans of any age. Using these values as a guideline I did not observe concentrations of Pb at level that would adversely affect the vervet monkeys' physical health.

Across all 10 vervet populations, Pb concentrations were found at higher levels than As, suggesting that each of the populations were exposed to higher concentrations of Pb and that Pb may be more readily available in their habitats (Figure 11). The Dronefield/Kimberly population, exposed to a high level of anthropogenic disturbance, exhibited the highest mean concentration of Pb (2.292 ppm) while the Baviaanskloof Geelhousbout population, exposed to a low level of anthropogenic disturbance, exhibited the lowest (0.607 ppm). This comparison follows the expectation that a high level of anthropogenic disturbance will result in a higher concentration of heavy metals. However, this comparison is expurgated and does not address high verses low anthropogenic disturbance in its entirety.

Heavy metal concentration by anthropogenic disturbance level

Concentrations of As and Pb were not linked to the levels of anthropogenic disturbance in the ways I expected. Vervet monkey populations originally categorized as low anthropogenic disturbance resulted in the highest mean As concentrations (0.183 ppm; SD = 0.088) and the lowest mean Pb concentrations (0.836 ppm; SD = 0.473). Whereas vervet monkey populations originally categorized as intermediate anthropogenic disturbance resulted in the highest mean Pb concentrations (1.344 ppm; SD = 0.884) and the lowest As concentrations (0.103 ppm; SD = 0.073). Vervet populations in the high anthropogenic disturbance category resulted in the intermediate mean for both As (0.160 ppm; SD = 0.116) and Pb (1.291 ppm; SD = 0.845). A few reasons could explain these discrepancies. First, there was a flaw in the original literature search conducted in order to place each population in an anthropogenic disturbance category. This flaw could be due to the amount of time between the data collection (2004-2008) and the data analysis (2017). Secondly, vervet monkey social hierarchy may be associated with heavy metal concentrations. When the hair samples were collected, the individual's social rank was not recorded. Therefore, the hair samples may have been collected from a low ranking individual or high ranking individual. This demographic is important because higher ranking vervets monkeys generally have more access to high quality food sources more readily than lower ranking individuals (Cheney and Seyfarth 1981). This could suggest that higher ranking individuals are eating more food that is potentially contaminated with heavy metals, or they are avoiding food that is contaminated, leaving it for the lower ranking individuals. Moreover, vervets live in a matriarchal society which allows me to assume that in a female to male comparison, females will exhibit higher concentrations of heavy metals because they are generally higher ranking. To test this hypothesis, a lengthy behavioral observational study paired with heavy metal concentration analysis of hair would need to be conducted.

The age of each individual vervet could have influenced the overall concentration within the hair samples but this information was not recorded for all individuals and was left out of the analysis. Vervets older in age could have been exposed to the heavy metals for a longer period than younger vervets. And although the 50mm hair samples used only accounts for 100 days of exposure, heavy metals can accumulate in other tissues of the body such as bones and lipids. Over time, these heavy metals can be re-released into the body and then accumulate in the hair.

Future directions

Although the results of this study did not support my expectations, it did reveal that vervet populations in South Africa are being exposed to heavy metals to some quantifiable degree. This find highlights the importance of understanding how we as humans are effecting our environment. Additionally, because these vervet populations are being exposed to heavy metal concentrations, it is easy to predict that humans utilizing the same areas are also being exposed. This study serves as a foundation for numerous future research projects. At present, there is a great need to identify how heavy metals are polluting environments at both a local and global scale. Because of our results regarding the Dronefield/Kimberley diamond mine had the highest levels of Pb, it can be assumed that mining to some degree is a cause of heavy metal exposure. However, identifying other sources is crucial to understanding the total human influence in heavy metal exposure. In addition to heavy metals, identifying what other hazardous compounds are being anthropogenically introduced to the environment (insecticides, herbicides, PCBs, etc.) and what effects they could potentially have on nonhuman primates and other wildlife. In vervet monkeys specifically, discovering if/how social hierarchy relates to heavy metal concentration could outline related behavioral consequences in the exposed individuals. And finally, comparing vervet monkey heavy metal concentrations to that of humans in the same areas can serve as a basis for consequential behavioral comparison studies in exposed individuals. It is often difficult to gain permission for studies that involve politically stressed human populations. Therefore, studying a mammal that is not only physiologically similar but utilizes the same spaces as those human populations can allow for a non-direct approach to the same desired information. This can lead to public health initiatives in those affected communities.

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APPENDIX

Vervet Monkey Demographics and Individual As and Pb Concentrations

Site Identification	Individual Identification	Sex	Weight (mg)	As (ppb)	As (ppm)	Cd (ppb)	Hg (ppb)	Pb (ppb)	Pb (ppm)	AVG Pb
SO	455	F	135.1	186.8925	0.187	Too Low	Too Low	1191.456	1.191	-
SO	456	F	80.3	73.13239	0.073	Too Low	Too Low	483.6696	0.484	-
SO	457	M	181.3	157.9389	0.158	Too Low	Too Low	694.6447	0.695	-
SO	462	M	63	260.8555	0.261	Too Low	Too Low	658.3766	0.658	-
SO	464	F	88.8	189.8735	0.190	Too Low	Too Low	547.1694	0.547	-
SO	466	F	93.3	61.95967	0.062	Too Low	Too Low	1069.764	1.070	-
PA	413	F	68.7	127.8305	0.128	Too Low	Too Low	588.1976	0.588	-
PA	415	M	67.3	233.3378	0.233	Too Low	Too Low	1115.119	1.115	-
PA	416	M	42.6	151	0.151	Too Low	Too Low	949	0.949	-
PA	419	M	63.9	414.2831	0.414	Too Low	Too Low	1282.014	1.282	-
PA	420	M	49.3	149	0.149	Too Low	Too Low	796	0.796	-
PA	421	M	79.2	160.1892	0.160	Too Low	Too Low	1828.076	1.828	-
SL	503	F	101.3	102.1084	0.102	Too Low	Too Low	1104.311	1.104	-
SL	506	F	90.2	96.31549	0.096	Too Low	Too Low	352.2383	0.352	-
SL	507	F	146.9	99.46442	0.099	Too Low	Too Low	629.8604	0.630	-
SL	509	F	52.7	95	0.095	Too Low	Too Low	233	0.233	-
SL	516	F	101.1	84.29003	0.084	Too Low	Too Low	778.0684	0.778	-
SL	519	F	99.6	61.95967	0.062	Too Low	Too Low	1069.764	1.070	-
K	173	M	34.8	289	0.289	Too Low	Too Low	1338	1.338	-
K	174	M	8	Too Low	0.000	Too Low	Too Low	1756	1.756	-
K	175	M	28.6	330	0.330	Too Low	Too Low	4107	4.107	-
K	176	M	69.2	132.0066	0.132	Too Low	Too Low	3061.913	3.062	-
K	177	M	21.3	Too Low	0.000	Too Low	Too Low	1982	1.982	-
K	178	F	30.8	250	0.250	Too Low	Too Low	1504	1.504	-
BR	490 A	M	120.4	178.3377	0.178	Too Low	Too Low	1111.499	1.111	AVG (490)
BR	490 B	M	141.7	248.522	0.249	Too Low	Too Low	1465.791	1.466	1.289
BR	491	F	211	213.1256	0.213	Too Low	Too Low	888.3191	0.888	-
BR	492	M	243.3	112.3981	0.112	Too Low	Too Low	1022.19	1.022	-

BR	493	M	148.1	122.4318	0.122	Too Low	Too Low	2218.584	2.219	-
BR	494	F	133.4	83.64403	0.084	Too Low	Too Low	584.8167	0.585	-
BR	495	M	210.5	91.80513	0.092	Too Low	Too Low	452.5256	0.453	-
BG	485	F	138.1	446.1514	0.446	Too Low	Too Low	1044.104	1.044	-
BG	486	M	32.2	164	0.164	Too Low	Too Low	559	0.559	-
BG	488	F	39.8	167	0.167	Too Low	Too Low	658	0.658	-
BG	489	F	55.7	209	0.209	Too Low	Too Low	374	0.374	-
BG	497	F	53.1	236	0.236	Too Low	Too Low	653	0.653	-
BG	498	F	47.5	154	0.154	Too Low	Too Low	354	0.354	-
G	404	F	41.8	119	0.119	Too Low	Too Low	1472	1.472	-
G	406	F	43.5	Too Low	0.000	Too Low	Too Low	1427	1.427	-
G	407	M	49.4	105	0.105	Too Low	Too Low	2337	2.337	-
G	408	F	46.2	Too Low	0.000	Too Low	Too Low	1771	1.771	-
G	409	F	76.7	199.7554	0.200	Too Low	Too Low	1802.009	1.802	-
G	410	F	67.5	128.0196	0.128	Too Low	Too Low	4118.132	4.118	-
T	471 A	M	122	143.1773	0.143	Too Low	Too Low	958.5146	0.959	AVG (471)
T	471 B	M	102.2	214.2497	0.214	Too Low	Too Low	1816.587	1.817	1.387551
T	472 A	M	111.9	292.0782	0.292	Too Low	Too Low	608.6704	0.609	AVG (472)
T	472 B	M	159.3	339.8255	0.340	Too Low	Too Low	643.0191	0.643	0.6258447
T	473	M	187.2	143.5112	0.144	Too Low	Too Low	595.4558	0.595	-
T	479	F	143.5	114.8187	0.115	Too Low	Too Low	398.468	0.398	-
T	480	F	204.8	115.5994	0.116	Too Low	Too Low	686.864	0.687	-
T	482	F	103.1	205.289	0.205	Too Low	Too Low	1259.09	1.259	-
PK	152	M	27.3	223	0.223	Too Low	Too Low	597	0.597	-
PK	154	M	43.7	146	0.146	Too Low	Too Low	685	0.685	-
PK	155	M	22.5	Too Low	0.000	Too Low	Too Low	753	0.753	-
PK	158	F	22.6	Too Low	0.000	Too Low	Too Low	905	0.905	-
PK	161	F	11.3	Too Low	0.000	Too Low	Too Low	1602	1.602	-
PK	164	F	32.5	305	0.305	Too Low	Too Low	1495	1.495	-
O	131	F	37.8	165	0.165	Too Low	Too Low	1076	1.076	-
O	132	M	36.5	255	0.255	Too Low	Too Low	1578	1.578	-
O	133	M	16.2	Too Low	0.000	Too Low	Too Low	901	0.901	-
O	134	M	11.8	Too Low	0.000	Too Low	Too Low	1708	1.708	-
O	136	F	77.9	179.8795	0.180	Too Low	Too Low	1219.547	1.220	-

O	137	F	45.4	166	0.166	Too Low	Too Low	615	0.615	-