



Assessing the potential risk of accumulation of selected freshwater contaminants in urban and peri- urban otters

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DECLARATION

I declare that this dissertation 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 other degree or examination at any other university.



Kaylee Clark

___1 June 2018_____

Submission date

ABSTRACT

As urban areas continue to expand rapidly and replace natural landscapes, the ability to conserve and manage native wildlife within urban environments is becoming increasingly important. As anthropogenic activities increase in these areas, there is a marked increase in levels of pollution which impacts both the terrestrial and aquatic ecosystems. Although some elements in the environment such as minerals and other compounds are necessary for animal and plant growth and metabolism, they can all be toxic at elevated levels, leading to severe health effects which could be fatal. Some aquatic organisms can bioaccumulate many heavy metals and transfer them to consumers higher in the food chain, which exposes them to significant health risks. The present study aimed to use non-invasive sampling techniques in order to assess the potential risk of the bioaccumulation of heavy metals in the freshwater rivers of Gauteng through the food chain of otters. This study was conducted along the Hennops River and the Klip River, focusing on the two otter species in Gauteng, the *Aonyx capensis* and *Lutra maculicallis*. The nature and concentrations of contamination were assessed at four different levels of the otters' food chain - water (ingested by prey species), sediment (contaminants may accumulate here and be released into the water), crab (ingested by the otter) and scat samples (eliminated by the otter). The heavy metals which were analysed throughout this study included lead, mercury, titanium, uranium and arsenic. Heavy metal analysis showed that there may be potential for arsenic and mercury to bioaccumulate through the otter's food chain as these heavy metals were detected at lower concentrations in the otter's scat than in its food source, suggesting a high risk for the otter as these contaminants are not being eliminated from the otter. In addition to the accumulation of these metals, titanium and uranium also showed potential for bioaccumulation as the physical variables of the water quality indicated favourable conditions for potential bioaccumulation. The present study demonstrates the concentrations of the contaminants present in the Gauteng rivers and to what extent they may accumulate through the food chain of otters and pose serious health risks to the species. The potential risks to the otter species occurring throughout Gauteng are not fully understood. It is therefore suggested that future studies continue to focus on determining the potential risk of accumulation and health effects of freshwater contaminants for all rivers in Gauteng in order to further encourage the conservation of the otter species.

KEYWORDS:

Aonyx capensis, Bioaccumulation, Contaminants, Heavy Metals, *Lutra maculicollis*, Peri-Urban, Urban.

DEDICATION

To

My loving family, Steven, Pauline and Megan.

For your continuous love and support!

With all my love!

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CHAPTER 1: INTRODUCTION

More than 50% of the world's population is living in urban centres (Goulart and Teixeira 2010). Urbanisation is forming an important part of global and land transformation (Pickett *et al.* 2008), and disturbances from these cities have resulted in the decline of natural habitat availability for many animal species. The increased levels of human activity resulting in habitat destruction degradation pose huge threats to amphibian, reptile and mammal populations (Price and Dorcas 2011). Urban wildlife is also increasingly exposed to a number of different pollutants associated with urban development (Ditchkoff *et al.* 2006). These increased pollutant levels place further stress on wildlife in these regions in addition to an already considerable level of physiological stress, as further stress occurs through impaired reproduction and immune system health which in turn negatively affects the survival of these species (Ditchkoff *et al.* 2006).

As a result of anthropogenic activities in and around cities, a number of different pollutants have entered aquatic ecosystems; many of these are known to be harmful to animals at high concentrations. Heavy metal contamination for example is one of the main environmental concerns (Zhang and Shao 2013), ruining biological communities, destroying the landscape ecology and the environmental functions within affected ecosystems. The introduction of these contaminants into aquatic ecosystems may result in increased accumulation of harmful substances through the food chains of apex predators (Hamilton 2004). In essence the contaminants which settle on the sediments of the rivers are taken up by smaller organisms. Larger animals then feed on these contaminated organisms, and so the toxins are taken up into their biomass; this process thus continues further up the food chain resulting in an increase in the concentration of the toxins which is a process known as biomagnification (Khayatzaheh and Abbasi 2010). Due to the exposure to and bioaccumulation of toxic pollutants, top predators mainly dependant on the aquatic ecosystems have suffered population declines and even local extinction (Khayatzaheh and Abbasi 2010).

The integrity of an ecosystem is often measured by the health of its carnivore populations. Due to their trophic position as well as their sensitivity to certain toxic environmental contaminants (Wren *et al.* 1987), some species are considered to be indicator or flagship species. Indicator species reflect the quality of and changes in conditions of biological, physical and chemical attributes of the environment. Changes in distribution, abundance and demographic characteristics of these species may indicate adverse changes to the ecosystem as a whole (Leader-Williams and Dublin 2000). Flagship species are a symbol for a specific habitat or environmental cause and are therefore often used to assist in conservation studies, focusing on protecting habitats in human-inhabited areas (Gangadharan *et al.* 2016).

Urban conservation is challenging due to the difficulty of creating corridors to further the success of urban wildlife, allowing them to occupy and move through to their preferred habitats (Longcore 2010). The development of greenways within the open public spaces in urban areas is becoming increasingly popular. There are many reasons for this development, including recreation, buffering of adjacent land uses, beautification, but most importantly wildlife conservation. The goals for these greenways are usually stated in a city's development plans. However, the actual contributions of these greenways for the wildlife present within these areas are often unclear, especially for species which are more sensitive to human disturbances as well as those with larger area requirements (Schiller and Horn 1997). Urban development is limiting conservation measures by limiting the habitats or space available in order to create adequate corridors and refuges for wildlife.

The aquatic ecosystems present in urban areas are also greatly threatened by the pollution from the development within the urban areas (Schiller and Horn 1997). Understanding the importance of water contamination is becoming a vital focus for scientists within the conservation management field as it allows for identification of more feasible and successful conservation efforts. The main issue regarding water pollution is that most of the contaminants are soluble in water and therefore the contamination is not visible immediately once introduced into the water body. This means that the contamination impacts to the aquatic ecosystem could only be noticed once the damage has already been done to the environment as well as the health of aquatic organisms. At this point it is impossible to reverse the impact. It is therefore essential that water quality

management strategies are put in place in order to prevent or limit these impacts to the health and quality of these ecosystems. The need to understand past species populations is important in order to inform the current management about the significance of quantifying the impacts of urbanisation and pollution on the species to encourage conservation action to take place (Longcore 2010).

1.1. South African otters

In southern Africa there are two endemic otter species, the African clawless otter (*Aonyx capensis*) and the spotted-necked otter (*Lutra maculicallis*) (Nel and Somers 2007). These two otter species are both semi-aquatic species, occupying a number of different habitats associated with freshwater and coastal systems (Kruuk *et al.* 1993). The African clawless otter is the most widely distributed otter species throughout South Africa, with the spotted-necked otter being limited mainly to inland water sources across the eastern half of the country (Rowe-Rowe 1990). The spotted-necked otter is known to be highly adapted to feeding on fish, whereas the African clawless otter relies primarily on crabs, however when these prey populations are low, both species will resort to feeding on fish and other food sources such as invertebrates, small mammals and birds (Rowe-Rowe and Somers 1998).

The current knowledge on the African otters within South Africa is to some extent limited (Nel and Somers 2007). However, even though otters appear to be very rare in Gauteng (the focus area for this study), recent studies have shown that these species do occur throughout the urban and peri-urban areas of Gauteng province (Ponsonby submitted). Both the spotted-necked otter and the African clawless otter are recorded as having a 'near-threatened' conservation status according to the IUCN Red Data List. As they are widespread, the decline in numbers across the range is not believed to be sufficient to warrant a 'threatened' status (IUCN 2016). The population trend for the African clawless otter is currently stable. However, the spotted-necked otter's population is continually decreasing. In areas with suitable habitats, including highland streams and man-made lakes in South Africa, it is estimated that there is one spotted-necked otter per 1-2km of stream (Perrin and Carugati 2000), while in less suitable habitat, Rowe-Rowe (1992) estimated one otter per 6-11km. The African clawless otter population densities are estimated that there is

one otter per 2 km in the more pristine areas and in the less suitable areas it is estimated one otter per 3-4 km (Rowe-Rowe 1992).

There are a number of studies that have been conducted on these two species, however the majority of these studies have focused on the otters occurring in Kwa-Zulu Natal and the Cape regions of southern Africa (Somers and Nel 2004). To date it appears that none have focused on the impacts of urbanisation on these species. Gauteng is known to be the industrial and commercial centre of South Africa, thus contributing to greater disturbances for wildlife found in this region. The human industrial disturbances as well as mining and residences are the primary contributors of the high levels of contaminants found within the urban and peri-urban ecosystems in Gauteng, more specifically the aquatic ecosystems (Mason 1989).

The deteriorating conditions of the otters' habitat may pose a great risk to otters as they are more likely to take up contaminants from the water. Because they are a higher level predator and due to the increasing rates at which their population numbers are declining, the Gauteng Department of Agriculture and Rural Development (GDARD) is particularly concerned about the conservation of these species (Seegers, *pers. comm.*)¹. It is thus important that an action plan is put in place, not only for the survival of the otter population but also for the survival of the remaining waterways and wetlands which they inhabit.

1.2. Importance of this study

Habitat degradation and water pollution from increasing urbanisation are two of the most significant threats to the aquatic ecosystems as well as the animals depending on them for their survival (McKinney 2002; Romanowski *et al.* 2013). In urban areas sewage, dumping and run-off waste present in the water ways are of great concern. Most of this waste contains a variety of harmful substances, the most concerning of these being heavy metals and toxic chemicals, as they are known to accumulate to top predators and lead to poor health (Romanowski *et al.* 2013).

¹ Christina Seegers, Biodiversity Officer: Biodiversity Stewardship, Gauteng Department of Agriculture and Rural Development.

Studies have shown that otters tend to avoid highly polluted water bodies, and rather stay in areas with low anthropogenic activities, as these activities have been found to have a negative effect on the abundance of the otter populations (Nel and Somers 2007; Baltrūnaitė *et al.* 2009; Romanowski *et al.* 2013). However, several studies have also shown the presence of otter populations in urban areas (Ditchkoff *et al.* 2006; Ponsonby submitted; Martinez-Abraín and Jimenez 2015).

In the aquatic ecosystems of Gauteng, the two otter species occupy the role of top predator (Peterson and Schulte 2016). These species are also important flagship species for the freshwater ecosystems as well as potential indicator species for water quality throughout Gauteng and the rest of South Africa (Peterson and Schulte 2016). The Gauteng province presents several threats to the otters' survival as their natural habitat is being transformed due to urban and agricultural land changes, as well as altering pollution events and threatening their health (Romanowski *et al.* 2013). Presently there are very limited data on the distribution and conservation status of the otters in Gauteng. Therefore, there is no reference point to assess the combined effects of pollution on the abundance and health of otters within the aquatic ecosystems. To my knowledge no studies have focused on the impacts of urbanisation on otters in southern Africa, and the corresponding effects that urban contaminants have on these animals in aquatic ecosystems.

Otters are at risk of exposure to pollutants due to their dependence on freshwater habitats. They are very cryptic animals and are mostly active at dusk and dawn, making it difficult to obtain direct samples. However, otters scat can easily be found, thus making it easier to study the species by indirect sampling using non-invasive sampling techniques. Indirect sampling has been tested and applied by Mason and MacDonald (1987); Elliott and Wilson (2003) and Zwiernik *et al.* (2008) suggesting that the non-invasive sampling through scat analysis provides an efficient approach for estimating the accumulation of contaminants and the threats posed to the otter. In fact, the use of otter scat has been a successful method in determining physiological variables such as the reproductive hormones (Kalz *et al.* 2006), as a source of genomic DNA for the identification of individuals (Hung *et al.* 2004), to monitor and evaluate the occurrence of disease (Gaydos *et al.* 2007), as well as to describe food habits (Ben-David *et al.* 2005). Most importantly scat sample analysis has been successfully used to monitor the heavy metal exposure as well as other

contaminants to the otter species (Elliot *et al.* 2008). Through the non-invasive assessment of the accumulation of the environmental contaminants in the food chain, I asked what the likelihood is that chemical contaminants from the environment bioaccumulate in the animals' food chain to the extent that they are at toxic concentrations when they enter the diet of a top predator.

1.3. Aim and objectives

The aim of this study was to assess the risk of bio-accumulation of five common urban contaminants in southern African otters inhabiting the surrounding areas of Johannesburg in Gauteng, South Africa. This was achieved through the following objectives:

1. determining the most common contaminants found in two major rivers of Gauteng;
2. identifying the contaminants which pose potential threats to mammals;
3. determining the contaminant concentration levels through the otter's food chain by sampling tissue from their primary food source (crabs);
4. collecting scat samples to determine the concentration of the contaminants eliminated through the otters' digestive process, to assess whether or not there may be some level of contaminant accumulation within the otters;
5. interpreting the possible impacts this bio-accumulation may have on otters;
6. identifying possible trends in the concentration levels of several aquatic contaminants in Gauteng rivers based on water quality data from the Department of Water and Sanitation to assess possible future risks to aquatic predators.

Specifically, I asked what the likelihood of bio-accumulation of chemical contaminants from the environment might be through the animals' food chain to the extent that they are at toxic concentrations when they enter the diet of a top predator. What are the potential risks to otters exposed to high concentration levels of contaminants in the urban and peri-urban rivers of Gauteng? My hypothesis for this study is based on the extensive literature review and the increase in contamination of contaminants in the rivers of Gauteng. I expect the otters to accumulate some level of contaminants through their food chain.

1.4. *Layout of the dissertation*

This Masters thesis was done by dissertation. It begins with chapter one being the introduction of the thesis and presenting the aims and objectives of the research. Chapter two gives a literature review covering the impacts of pollution on wildlife with specific focus on the risks of accumulation and biomagnification of heavy metals through a top predator's food chain. Chapter three is the description of the methods employed in order to conduct this project. Chapter four presents the results of the study, and chapter five provides a risk assessment, where the results of this study are assessed against academic literature and other studies to discuss the potential risks of heavy metal accumulation in otters in Gauteng. Lastly, chapter six finalises this thesis with the discussion and conclusion in which the main arguments are presented. A list of the literature cited follows chapter six, with the thesis ending with the appendices.

CHAPTER 2: LITERATURE REVIEW

More than 50% of the world's human population is living in urban centres (Goulart and Teixeira 2010). As the human population continues to grow, natural resources are being exploited through agriculture, industrialisation, mining and urbanisation, resulting in ecological disturbances. In addition to this rapid growth in human population, various other anthropogenic factors add to the immense pressure on and detrimental impacts to the environment (Price and Dorcus 2011) such as overexploitation, habitat loss, climate change, invasive species and pollution (McKinney 2006; Goulart and Teixeira 2010). These impacts do not only affect the terrestrial ecosystem but also aquatic ecosystems. Urbanisation is the most rapid transformation with a significant impact on wildlife (McKinney 2002).

2.1. Urbanisation pressures on the natural environment

The most prominent feature of the earth's ecosystems is that they are becoming increasingly human-dominated (Vitousek *et al.* 1997). Since the 1950's, landscapes have evolved in quality and in spatial structure, endangering a number of species through the influences of natural and human-induced disturbances (Pichancourt *et al.* 2006). This not only exerts immense pressure on the natural environment but also poses a threat to the survival of wildlife within their natural habitat (McKinney 2006). As urban development spreads rapidly across the world, it sets challenges for conservationists to understand the effects it has on biodiversity (McKinney 2002 and 2008) and ecosystem services such as air and water purification as well as amenity value for enjoyment and recreation (McKinney 2008).

Although the impacts of urbanisation and other forms of land transformation on the natural environment are mostly negative, urban systems may result in a more complex interaction between the local biodiversity and the surrounding environment. In most cases urbanisation causes a drastic decline in species diversity, as urban development reduces the area of natural habitat available, resulting in only fragments of land left for species to live. Species endangerment and extinction are often a direct result of fragmentation and habitat destruction resulting from urbanisation (Magel *et al.* 2010). Most species are able to find

refuge in patches of habitat within or adjacent to urban areas, however there are a number of species that are unable to adapt to this changing environment (Bonier, Martin and Wingfield 2007; Magel *et al.* 2010). However, urbanisation may also have a positive effect on biodiversity due to high spatial habitat scales of heterogeneity resulting from different land uses in these urban areas, which may lead to increased species richness as well as diversity, however this is often due to the introduction of non-native species that replace the native species at a faster rate than the rate at which these are lost (McKinney 2006).

There are a number of different ways in which fauna and flora are affected by urbanisation around the world. Habitat fragmentation poses a significant threat to biodiversity (Solé *et al.* 2004). In most cases fragmentation reduces a species' habitat in such a way that the area is too small for the species to maintain a viable population size or home range (Tigas *et al.* 2002). Habitat destruction resulting from urbanisation is another significant contributor to rapid decline in species richness and diversity (McKinney 2002). Habitat destruction has driven species populations into isolation (Prugh *et al.* 2008). This results in fragmented populations with reduced numbers and varying species occurrence depending on the patch size. Improving the quality of these patchy and isolated areas in conservation planning may encourage the species which persist in the aftermath of habitat destruction (Prugh *et al.* 2008). There are many examples of the negative impacts of fragmentation and habitat destruction, such as in southern California with the two most threatened species, the bobcat (*Felis rufius*) and the coyote (*Canis latrans*), being most sensitive to fragmentation due to their trophic needs and low numbers. As a result, there were signs of behavioural adjustments of these species persisting in fragmented areas (Tigas *et al.* 2002). These individuals restricted their home ranges to small fragments of habitat within or adjacent to the urban development. In terms of their movements, these animals mostly crossed through the urban area, including crossing over roads which led to high mortality rates due to vehicle collisions, reducing the size of their population in these areas (Tigas *et al.* 2002). In southern California, a similar study was conducted by Riley *et al.* (2003) which focused on the ecology and behaviour of bobcats (*Lynx rufus*) and coyotes (*Canis latrans*) in landscapes compromised by human activity. Home range size was negatively correlated with urban associations for these carnivores, suggesting that a human dominated area is less suitable than natural areas. It was seen that, in order for the bobcat

population to remain in the area, preserving open spaces of sufficient quantity and quality is necessary for the population's viability (Riley *et al.* 2003).

However, physical habitat impacts are not limited to destruction and fragmentation, but degradation can also occur through waste and pollution. Pollution has widespread effects on both terrestrial as well as aquatic environments. One of the reasons why pollution is so devastating is because its effects are not noticed until the impact has occurred, i.e. the effects of pollution are not as obvious as those brought about by fragmentation and habitat destruction. This makes it difficult to stop or limit the pollution impact as soon as the challenge arises before the impact has caused detrimental effects to the wildlife as well as their environment. Aquatic ecosystems may be jeopardised by pollution, more specifically pollution of heavy metals (Govind and Madhuri 2014).

2.2. Pollution in urban environments

Urban wildlife is exposed to a number of different pollutants associated with urban development, which have detrimental effects on the survival of many species (Ditchkoff *et al.* 2006). The wildlife in these areas is coping with a considerable level of physiological stress due to pollution levels present in their environment, which includes impaired reproduction, immune system and health (Schell and Denham 2003; Ditchkoff *et al.* 2006). Several studies have shown that there is an increased level of lead and organochlorine pollutants accumulated in the tissues of some species (Dip *et al.* 2003). For example, in the case of the urban red fox (*Vulpes vulpes*), significantly higher mortality rates were seen among adult males compared to adult females due to females being able to reduce the levels of pollutants by passing these to offspring during lactation, whereas males have no way of eliminating these pollutants from their systems (Ditchkoff *et al.* 2006).

Many pollutants such as organochlorines and heavy metals are ingested by mammals via their food supply. If these contaminants are persistent, as many of them are, accumulation within living tissues will occur. This is a concern not only for terrestrial habitats but especially for freshwater ecosystems as there are a number of different input sources flowing into the rivers, causing an increase in the contamination levels. The main sources of

these pollutants include rainfall bringing in atmospheric pollutants, agriculture (fertilisers and pesticides washed into rivers), industries, sewage works, mining and domestic waste (Foster-Turley *et al.* 1990).

Pollution effects resulting of human activities and are driven by urbanisation result in impacts at not only the local scale but also extending to regional and global scales. More specifically, local impacts alter the ecosystems within, surrounding and at greater distances from urban centres. Pollution from the urban and surrounding agricultural areas has been found to influence ecosystems in profound ways (McKinney 2002). Emissions which are commonly associated with these areas include nitrogen oxide, volatile organic compounds, greenhouse gases, hydrocarbons as well as heavy metals and other reactive gases from combustion sources (e.g. vehicles and power plants) (Grimm *et al.* 2008). An example of the effects this has is seen by the excess emission of ammonium and nitrate from combustion in the Midwest counties of the United States. These emissions elevate reactive nitrogen loading in sensitive ecosystems such as high-altitude forests. Nitrogen loading changes the plant chemistry, photosynthesis and ecosystem carbon balance within these sensitive areas (Aber *et al.* 1991; Grimm *et al.* 2008). These pollutant effects may result in a shift in dominant plant species, further impacting the entire ecosystem and the animals which rely on these plant species (Grimm *et al.* 2008).

One of the greatest sources of pollution which causes significant damage within urban areas is sewage. Urban sewage has had drastic impacts on aquatic ecosystems and water biogeochemistry around the world (see Singh *et al.* 1999; Lampman *et al.* 1999; Daniel *et al.* 2002; Chigor, Sibanda and Okoh 2013), and is a major contributing source of heavy metals (Sanudo-Wilhelmy and Gill 1999), pathogens (Donnison and Ross 1999), and inorganic compounds (Ono *et al.* 2000; Daniel *et al.* 2002) to aquatic systems, resulting in water not only being severely ecologically degraded but also posing serious health risks to humans and other mammals using these rivers (Walsh 2000). Faecal pollution can have detrimental effects on the health of mammals as well as the ecological balance and functioning of ecosystems (Chigor *et al.* 2013). Some of the health risks which are associated with faecal pollution are posed by and antibiotic-resistant strains of bacterial pathogens and microbes. EDCs (Endocrine Disrupting Compounds) which are also found in sewage include a number of natural and synthetic chemicals and compounds, but most importantly steroidal

oestrogens and heavy metals (Rodriguez *et al.* 2007; Faul *et al.* 2013). Hormonal systems of various animals may be directly affected by these compounds as they alter hormone synthesis and metabolism and modify hormone receptor levels (Faul *et al.* 2013). There have been numerous studies indicating that aquatic systems and their fauna are often very sensitive to the effects of EDCs (Porte *et al.* 2006; Faul *et al.* 2013). The effects on the endocrine and neural systems in mammals are manifested as adverse effects on the growth, development and reproductive success of individuals, thus eventually having an impact on the whole ecosystem (Faul *et al.* 2013).

Most forms of pollution contain traces of heavy metals. The most common heavy metals which are of main concern in environmental science as they are most commonly found in water waste include lead, mercury, cadmium, chromium, copper, zinc, nickel, manganese and silver (Govind and Madhuri 2014; Jaishankar *et al.* 2014). Even though many metals are essential to maintain the body's metabolism and health, at higher concentrations heavy metals can be toxic (Jaishankar *et al.* 2014). Heavy metals can enter an individual's body via food, drinking water and air and can be harmful if taken up in excess quantities as they can bioaccumulate in plants and animals. This will lead to toxic effects on individuals and eventually lead to death (Govind and Madhuri 2014). There are numerous health impacts heavy metals can have on wildlife. Iron for example is an essential metal, however in the presence of toxic metals iron can change form, resulting in toxicity by interfering with the metabolism process (Govind and Mudhuri 2014). Carcinogenic effects can be seen when metals have abnormal oxidation phases such as Cr (VI), whereas Cr (III) is an essential trace metal. All heavy metals in high concentrations have toxic effects on individuals via metabolic interferences and mutagenesis (Govind and Mudhuri 2014). These toxic effects include the reduction in fitness of the individual, reproduction interference leading to carcinoma and finally death (Govind and Madhuri 2014; Jaishankar *et al.* 2014). Chronic toxicity of heavy metals is seen in the bioaccumulation of the metals through the body as well as the food chain. For example, metals such as radium which is radioactive, can imitate calcium and be incorporated into bone matter; similar hazards can be due to effects from lead and mercury (Govind and Madhuri 2014). The increase in heavy metals has become a serious threat and has brought hazards to not only the growing populations but the environment as well.

2.3. Water pollution

Despite its importance, water is the most poorly managed resource worldwide (Phiri *et al.* 2005). Rivers located in the urban areas of most African countries are found at the end points of the disposal of the effluents discharged from industrial areas. The impacts of industrial effluents and other wastes on the water quality of the rivers in Nigeria for example showed that the chemical parameters studied tend to accumulate downstream (Fakayoda 2005). The careless disposal of these effluents may be the main contribution to the poor water quality of river water found in these urban areas (Olayinka 2004; Phiri *et al.* 2005). Rapid industrial growth may increase this careless disposal of effluents, creating a difficult task in environmental conservation for the implementation of the laws and policies set out by the governments with regards to the protection of the water quality of the rivers within the urban areas of the African countries (Kadongola 1997; Phiri *et al.* 2005).

Industrial effluent is however not the only source of contamination for aquatic systems. Mining industries as well as other secondary industries and domestic wastes contribute a significant amount of leaching metals into the streams and rivers in Gauteng (Schoonbee *et al.* 1995; Haywood *et al.* 2004). Acid mine drainage is often caused by high concentrations of pyrite from the gold ore on the Witwatersrand. As a result, the acidification causes heavy metal speciation, increasing their bioavailability and toxicity to the wildlife in these areas. Most heavy metals in the aquatic ecosystems originate from mining activities, increasing their concentrations to lethal levels due to their accumulation abilities in organisms (Haywood *et al.* 2004).

Mining does not only pose direct threats to aquatic mammals through ingestion and accumulation of toxins, it also presents indirect threats by affecting the animals' habitat quality and food availability. Mining can place immense pressure on the surrounding environment as it creates a large amount of dust as well as runoff from tailings into the surrounding rivers. Therefore, mining does not only affect the surrounding natural vegetation, but also increases the silt levels of the water which blocks the gills of fish (Mason and Macdonald 1988). Mining can also increase the acidity as well as the heavy metal content of the water through arsenic, titanium, cadmium and mercury, which are toxic to fish, making the otherwise ideal river habitats unsuitable for mammal species that

rely on fish as their main food source, such as the Eurasian otter (Mason and Macdonald 1988; Mason 1989; Foster-Turley *et al.* 1990).

Another example of how pollution from mining and industrial development affects surrounding environments is shown in a study conducted by Durand (2012), discussing the impacts of gold mining in the Witwatersrand on the river systems of Gauteng, South Africa. Gauteng's rivers have evidently deteriorated over the past decade due to the constant release of mine effluent. Mining activities have further exposed the aquatic environments to the threat of Acid Mine Drainage (AMD) pollution. AMD is a long term threat to the environment as it leaches from abandoned mine waste materials. This directly affects the rivers, drinking and ground water, and thus affects the wildlife and their habitat (Durand 2012; Ayokunle 2014). AMD changes the acidity of the water from the oxidation of pyrite, causing dissolution of the surrounding rocks within parts of the mines, tailing dams or dumps and therefore releasing the metals present in the rock. Metals being released into the environment include aluminium, radium, uranium, manganese, iron, zinc, nickel, lead, cobalt, arsenic, copper and thorium (Durand 2012; Ayokunle 2014). Overall this produces toxic and acidic water affecting the bioavailability of the heavy metals and resulting in detrimental effects on the ecosystems (Ayokunle 2014), such as soil quality degradation, reduction of crop yields, poor quality primary producers, posing a health risk to animals higher along the food chain and thus affecting the whole ecosystem health (Singh *et al.* 2011).

It must be noted though that not all heavy metals in the river water come from pollution but also from the underlying geology in Gauteng (Durand 2012). The most important geological event for the Gauteng province occurred about 3 billion years ago with the formation of the Witwatersrand Supergroup (Durand 2012; Environomics 2014). This sedimentary rock was made up of numerous layers of black chert or white quartz pebbles, sand and silt. The Witwatersrand Supergroup is known to consist of a number of different rock types such as quartzites, banded ironstones, mudstones, tillites, conglomerates and some marine lava (Durand 2012). In aquatic systems within Gauteng the primary source of trace metals is the underlying rock types. These trace metals enter the river systems by rock weathering, atmospheric dry and wet deposition and by anthropogenic activities (Gaillardet *et al.* 2003). In an aquatic ecosystem the behaviour of the trace metals does not

solely depend on the source but is also controlled by the soil and in-stream processes and reactions between elements (Gaillardet *et al.* 2003). Quantifying the proportions of contaminants which are derived from either natural or anthropogenic activities is complex and is conducted using isotope ratios of the elements (Gaillardet *et al.* 2003). Nriagu and Pacyna (1988) suggested that the estimated heavy metal contamination from anthropogenic activities in aquatic ecosystems far exceeds any natural process to contaminate these ecosystems. The main heavy metals recorded in the natural environment that far exceed the levels that could be attributed to the underlying bedrock include arsenic, cadmium, copper, mercury, molybdenum, nickel, lead, antimony, selenium, vanadium and zinc (Gaillardet *et al.* 2003; Durand 2012). It was reported that for arsenic, lead, cadmium, mercury and selenium the enrichment factors due to the human activities were several times higher than what is currently being detected in the Gauteng rivers, with respect to the natural levels of these heavy metals (Nriagu and Pacyna 1988), suggesting that the concentration measured in the aquatic ecosystems are far higher than what is being released from the underlying bedrock.

2.4. Risk associated with the accumulation of contaminants through the food chain

Heavy metals and other contaminants are released into the environment by natural as well as anthropogenic sources. These heavy metals can be highly toxic to human and ecosystem health, even if they are found at low concentration levels in the environment (Gall *et al.* 2015). They are found to enter the soils and groundwater, bioaccumulate through food webs, and adversely affect biota. Once these contaminants enter the environment, it is possible that they will remain present for years, even after the point source may have been removed. However, their presence poses a long-term risk to biota living within these areas in form of behavioural, dietary and physiological impacts to these individuals (Gall *et al.* 2015). Not only is the individual directly affected by the presence of contaminants in the environment but is also indirectly affected. These main indirect effects are seen as their habitats are affected by the accumulation of heavy metals within the aquatic ecosystem. The impact of the accumulation of toxic contaminants is seen at various levels of the food chain, posing risks to both aquatic and terrestrial fauna (Hamilton 2004).

Figure 1 provides a clear illustration of the full complexity of the interaction between the aquatic and terrestrial food chains (taken from Clarkson 1995, p 683).

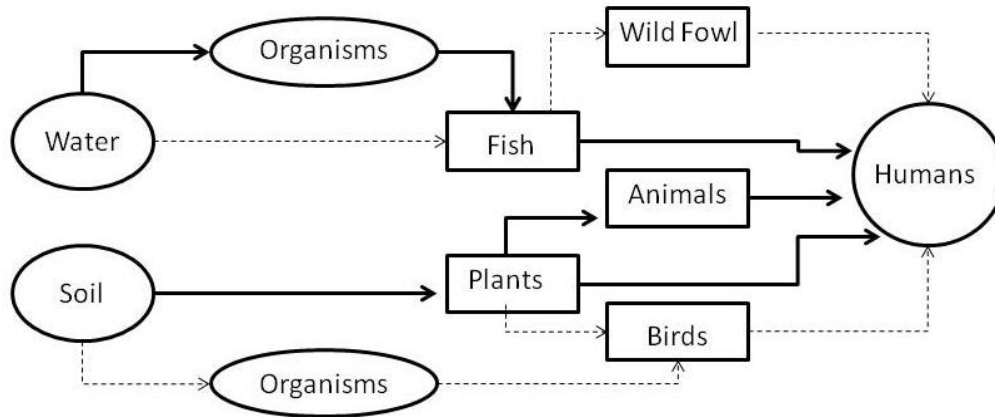


Figure 1. A representation of both an aquatic and terrestrial food chain, where both chains may share common pathways. Both chains start with the water and soil where the contaminants are present. The solid lines represent the most direct and common food chain and the dotted line represents additional transfer pathways (source: Clarkson 1995, p 683).

It is well established that environmental contaminants present in the soil, air and water are transferred to aquatic and terrestrial organisms such as invertebrates, fish and mammals, by means of feeding, respiration and through skin contact, resulting in the transfer from one organism to another through the food chain (Sharpe and MacKay 2000; Figure 1). This often results in higher concentration levels occurring at higher trophic levels, posing a health risk to the individuals that consume them. In a study conducted by Walters *et al.* (2015) it was found that mercury and selenium biomagnify in aquatic food chains and are toxic to fish and other wildlife. Similar results can be seen in a study conducted by Krishna *et al.* (2014), where fish, considered to be an ecologically significant link between the aquatic and terrestrial habitats, as they are an important prey source for a diverse number of terrestrial consumers, may continue to the accumulation of mercury or other toxic contaminants in their tissue through the food chain and bioaccumulate, directly affecting predator health (Krishna *et al.* 2014).

Bioaccumulation refers to the net accumulation of heavy metals as well as organic compounds in the tissues of an organism due to the uptake from all dietary or water sources (Luoma and Rainbow 2008). The measure of the accumulation of metals is of particular value because metals do not completely break down as they pass through the food chain. Metal bioaccumulation is more complex and there are additional influences such as metal-specific influences, species-specific influences, environmental influences and the exposure route of the metal (Luoma and Rainbow 2008). The balance between the uptake and the loss of the metals in an organism directs the process of accumulation. The interaction between the different metals present may arise from the disturbance of the physiochemical balance controlling the metals' speciation, or the interaction between the protein carrier and membrane channels which affects the uptake rates of the metals (Luoma and Rainbow 2008). The uptake of a metal is determined by how much metal has crossed the cell membrane (e.g. the cells of the gut epithelium in mammals). Absorbed metals that have yet to cross this membrane have not been taken up by the organism, and therefore do not play a role in that metal's toxicity to the organism (Luoma and Rainbow 2008). These factors which affect the metals uptake rate also affect the toxicity of the metals to the individual. Toxicity is ensured once the metal availability threshold has been past, at which point the uptake rate will exceed the rate of excretion (Figure 2). This metal accumulation results in the disruption of important molecular functioning in the individual (Luoma and Rainbow, 2008, p 207).

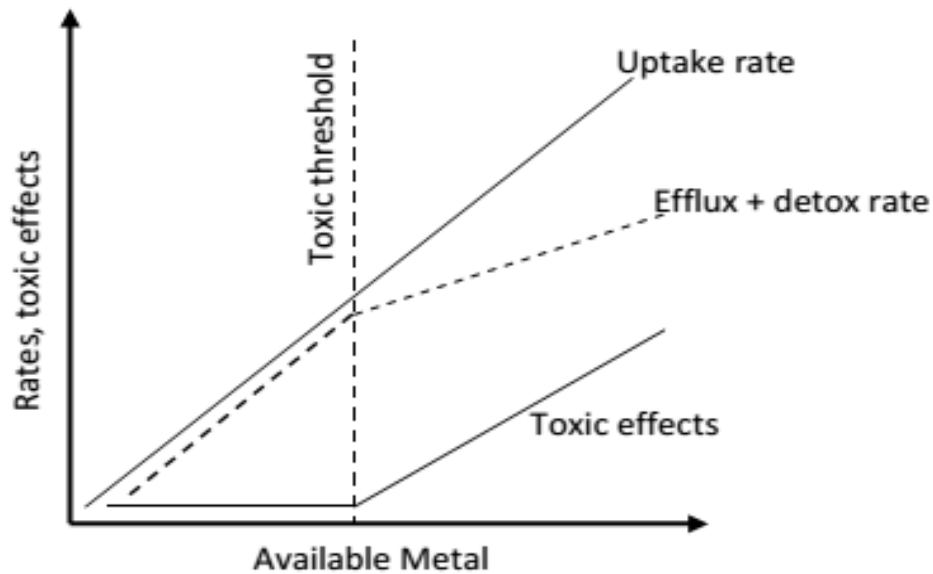


Figure 2. The uptake rate of trace metals and the toxic effects after threshold will increase with the availability of the metals within the individual (source: Luoma and Rainbow 2008, p 207).

The health effects of these contaminants depend on the amount consumed and their bioavailability, i.e. the amount of a substance which becomes available for bioactivity in an organism's body when introduced through ingestion or skin contact (Manach *et al.* 2004). Clarkson (1995) suggests that there are three requirements that a substance must meet in order for that substance to bio-accumulate from water. Firstly, the substances must have a high octanol-water partition coefficient, which is defined as the ratio of a contaminant's concentration in the octanol phase to its concentration in the aqueous phase in a two-phase octanol-water system. Secondly, they need to be stable in water as well as other compartments of the aquatic system, suggesting that these substances are metabolically stable in species involved in the food chains (i.e. fish and mammals), and lastly, their toxicity should be low enough that they do not eliminate any of the intermediate species, resulting in the continual accumulation of harmful contaminants (Clarkson 1995).

The risk of bioaccumulation affects a number of factors within the aquatic environment and not just the health of the individual. These areas include prey availability, reproduction, health and most importantly the physiological effects on the individual being exposed to high levels of contaminants. Food-web complexity and stability of relationships are the

fundamentals of ecology. Aquatic ecosystem pollution is gaining more interest and is a cause for concern worldwide. Besides the health risks posed to the mammals, pollutants alter important chemoreceptive abilities of many aquatic species, thus negatively impacting the predator-prey relationships in these ecosystems (Lahman *et al.* 2015). In some species the ability to respond to sensory information can be affected before contaminant concentrations reach levels far above the threshold, often resulting in fatal outcomes for individuals. Many organisms use chemical cues to not only find food and partners, but also to sense their natural enemies and to avoid predation (Lurling and Scheffer 2007). Lurling and Scheffer (2007) found that chemicals, both heavy metals as well as pesticides ranging from high to low concentrations, can disrupt the transfer of chemical information, manipulating responses in both the signaller and the receiver. These results were also confirmed in a study conducted by Lahman *et al.* (2015), where the exposure for crayfish to toxic levels of copper affected their ability to detect and respond appropriately to chemosensory signals within their environment to successfully locate food and other necessary resources. These 'information disruptors' are similar to endocrine disruptors, forming a new class of chemical threats to aquatic organisms. Therefore, the increasing contamination levels disrupting these signals is an additional factor lowering the survival rate of the prey species within these aquatic ecosystems. These effects could have far-reaching implications for not only ecosystem functioning but conservation management as well (Lurling and Scheffer 2007).

Predator-prey relationships are greatly affected by increased heavy metal concentrations in the environment (Petchey 2000). Once prey exposure to heavy metals increases, it will not only result in bioaccumulation through the food chain as discussed, but can have lethal effects on the prey populations, thus affecting the survival of the predator populations. The presence of toxic contaminants can cause changes in predator-prey interactions by altering the prey capture and predator avoidance behaviour in individuals, thus resulting in a change in both predator and prey populations (Weis *et al.* 2001). Overall this may change the community structure within an ecosystem. The interactions between predators and their prey is an important component in the structuring of a community and serve as an important link between the toxic contaminant and the induced effect to the individual as well as the effects at higher trophic levels within the same community (Weis *et*

al. 2001). The presence of the toxic contaminants can change predation rates and patterns and may lead to changes in the predator species population, these changes include reduced growth, survival and reproduction (Weis *et al.* 2001).

With increasing industrial and urban development worldwide, there is a growing concern as metals continue to increase and accumulate in the environment, resulting in detrimental effects to many biological aspects within a community including mammalian reproduction (Henson *et al.* 2016). The biological effects of exposure to these metal compounds and elements are of concern due to their pervasive occurrence and increasing rates of production within the aquatic environments (Clarkson 1995; Chedrese *et al.* 2006; Henson *et al.* 2016). The chemicals within these polluted environments contain a number of reproductive toxins, including metal compounds which act as reproductive as well as development toxins within humans, mammals and other wildlife (Henson *et al.* 2016).

In mammals the most common exposure to and intake of metals and other toxic contaminants, namely iron, zinc, copper, selenium, manganese, lead, cadmium, arsenic, mercury, uranium and nickel, is through their food sources. The metals which cause the most detrimental effects to the reproduction processes of a mammal are those which directly impact the reproductive organs, adult sexual functions or maternal-offspring interactions (Henson *et al.* 2016). The latter consists of impacts to the fertilized ovum, the embryo or the neonate (Henson *et al.* 2016). A number of studies have focused on the harmful effects that pollutants may have on wildlife, these studies range from bees (Moron *et al.* 2013), and mammals (Jensen *et al.* 2015), to humans (Skinner 2014; Rzymiski *et al.* 2015). Studies have concluded that exposure to toxic pollutants affects reproduction, resulting in abnormalities in offspring, diseases being passed on to the offspring or increasing the proportion of dead offspring, as well as increasing the mortality of the individuals itself.

The most important risk affecting mammals inhabiting a contaminated aquatic environment is the risk posed to an individual's health, both physically as well as to their DNA. Health effects can result from short term and long term exposure to the toxic contaminants present. These impacts can also occur when present in low concentrations as well as having fatal impacts when exceeding threshold levels (Jaishankar *et al.* 2014). The toxic

effects are due not only to the degree of contaminants present within the environment, but also due to the biochemical role in the metabolic process and to what extent the contaminants are absorbed or excreted from the animal (Jakimska *et al.* 2011). Heavy metal toxicity in aquatic wildlife can impact life processes differently in different parts of the trophic pyramid (Hylland 2006; Jakimska *et al.* 2011). When toxic metals are present in high concentrations, the endocrine system, reproduction and growth may be affected. In addition to these impacts, toxic metals which have the ability to bioaccumulate and biomagnify in the tissues and organs of an animal may have negative effects to their cellular functioning due to their interactions with the system's enzymes. Therefore, there is a further disturbance to the individual's growth, reproduction, immune system and metabolism (Jakimska *et al.* 2011). The health risk to an individual will vary depending on the different contaminants it has been exposed to. Health risks for each heavy metal analysed in this study is discussed in detail within the section below.

2.5. Characteristics and health impacts of heavy metals

Numerous heavy metals are found in varying concentrations in aquatic ecosystems. Once heavy metal concentrations reach their risk threshold levels, they have varying effects on the health of the organisms exposed to them (Wilson 1978). Contaminant concentrations are greatly influenced by the physical parameters of the surrounding environment, these parameters being the pH, electrical conductivity (EC) and temperature (Meador 1991). It is thus important to include the analysis of these physical variables in metal monitoring protocols. In South Africa some of the most common heavy metals found at high levels include arsenic, mercury, lead, titanium and uranium (Lenntech 2016; Cukrowska, *pers. comm.*)². Other contaminants which are also assessed regularly by the Department of Water and Sanitation include aluminium, chlorine, calcium, copper, sodium and magnesium (Department of Water Affairs and Forestry 1996) all the above contaminants were selected for study.

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2.5.1. Arsenic

Arsenic is ranked as the 20th most abundant element found in the Earth's crust (Eisler 1988). It occurs in soils and minerals and can enter air, water and land through water run-off and wind-blown dust. However, human activities such as the burning of fossil fuels are responsible for a rapid increase in arsenic levels in our environment.

The degree of toxicity of arsenic to mammals depends on the form and oxidation state of the arsenic as it is taken up by the mammal. It has been found that both the inorganic and organic forms may cause a number of effects on mammals tested in the laboratory (Gomez-Caminero *et al.* 2001; Lenntech 2016). Inorganic arsenic compounds are known to be more toxic than the organic form, especially when found in high doses. These effects can range from chronic impacts such as cancer through to death (Gomez-Caminero *et al.* 2001). However, the organic form does not cause cancer nor damage an individual's DNA, but high doses will still affect the health of the individual, causing nerve injury and stomach aches (Lenntech 2016).

2.5.2. Mercury

Mercury can be found in a variety of different forms. It can be found as a metal, as mercury salts, or as organic mercury compounds. Mercury can occur naturally in the environment through the breakdown of minerals in the soils and rocks which are exposed to water and wind. These natural processes remain fairly stable. However, mercury concentrations in the environment are increasing, most likely due to human activities such as fossil fuel combustion, mining, smelting and solid waste combustion which release high levels of mercury. Applications such as agricultural fertilisers and industrial wastewater disposal are directly released into the soil and water, however all mercury deposits released into the environment will eventually enter the soil and water of the nearby rivers within the areas near to these sources (Lenntech 2016).

The bioaccumulation of mercury has significant consequences to not only individuals but to animal populations as a whole. Mercury negatively affects the survival of adults and juveniles of a number of mammal species, having adverse effects on reproduction in both

males and females, the higher the concentrations are in the diet of the mammal (Aulrich *et al.* 1974; Ben-David *et al.* 2001). High concentrations of mercury are also known to affect the mammals' central nervous system which eventually results in the death of the animal (Ben-David *et al.* 2001). Mercury is also able to penetrate the body tissue, accumulate in the brain and nervous tissue, and the intestinal and salivary glands (Aulrich *et al.* 1974).

2.5.3. Lead

Lead occurs naturally in the environment and is usually found in ore along with zinc, silver and copper, however most of the lead concentrations measured in the environment are a result of human activities. The industrial lead cycle is more extensive than the natural lead cycle, causing lead pollution to extend worldwide. Lead salts are produced when the lead is burned in car engines, these salts then enter the environment. The larger particles immediately drop to the ground and pollute the soils or water sources. The smaller particles are the particles which enter this vicious cycle, by travelling longer distances through the air and remaining in the atmosphere. The lead is then returned back to the earth's surface as it rains (Lenntech 2016)

Lead is known to accumulate in organisms and can be taken up by mammals from the intake of food (Lenntech 2016). The most common pathway of intake for mammals is through their food chain. Constant exposure to lead is more concerning than acute poisoning, due to the irreversible effects of environmental contamination. The chemical effects of lead intoxication in mammals are nonspecific, resulting in weakness, fatigue, changes to the nervous system and gastrointestinal tract functions. The overall predicted risks of lead effects in mammals correlate with the toxicity endpoints of neurological, renal, hematopoietic or musculoskeletal nature (Ma 1996). Younger individuals in the population are at greater risk of complications with brain functioning, developmental stages including during foetus development (Ma 1996).

2.5.4. Titanium

Titanium is one of the most abundant elements on Earth. In the environment titanium is often present in its oxidation state in common mineral forms. Titanium is known to be an immobile element for mass-balance weathering rate studies. However, it has been seen to be mobile in soils. This mobility is enabled by the dissolution of minerals by both organic and inorganic acids. Concentrations of titanium in rivers and near coastal waters vary between 1pM and > 100 nM. Concentrations of dissolved titanium tend to decrease with an increase in salinity until it reaches the open sea waters. Titanium solubility is increased in some natural waters with oxalic acid and citric acid mainly increasing its solubility from natural mineral sources (Buettner and Valentine 2011).

Titanium has been found to accumulate in some organs. In studies where titanium was injected into hamsters, it accumulated in the kidney, spleen, liver and blood (Buettner and Valentine 2011). When present in high concentrations, it has the potential to accumulate in bones. This accumulation can have negative impacts on the animal, as chemical changes in the blood and urine could cause liver damage. Titanium at high levels can be toxic, affecting embryos, lowering the number of foetuses, and decreasing mean foetal body weight development (Buettner and Valentine 2011).

Titanium dioxide (TiO₂) nanoparticles easily cross the blood-brain barrier and enter the central nervous system, causing neurodegeneration in exposed individuals (Saraswat *et al.* 2016). Other impacts caused by TiO₂ include carcinogenic effects, effects to the nervous system, dermal and mucosal systems, the cardiovascular system, the liver, hematopoietic and immunological systems, renal system, musculoskeletal systems, reproductive systems, and genotoxicity (Iavicoli *et al.* 2012).

2.5.5. Uranium

Uranium is found naturally in very small amounts in air, soil, rocks and water (Lenntech 2016). Additional sources of uranium enter the environment due to human activities, mainly during mining and milling processes. The main concern around uranium is that it is radioactive and is very difficult to avoid in the environment (Lenntech 2016). In water most

of the uranium present is dissolved from the underlying rocks and soils. Large amounts of uranium are found in the environment once they have been released through the erosion of mine and mill tailings (Lenntech 2016). The natural levels of uranium have been shown to have no harmful radiation effects on individuals who are exposed to it. However, when exposed to higher levels of uranium, the chemical effects after the uptake of higher levels can cause health effects such as kidney disease (Cooley and Klaverkamp 2000).

As with most toxic environmental compounds, mammals exposed to uranium experience some health effects. The target organ for uranium toxicity in mammals is the kidney (Domingo *et al.* 1989). Additional complications caused by this exposure included reduce foetal body weights, foetotoxicity, internal and external malfunctions and developmental variations. Given the nature of the radiation damage to the DNA of individuals, any radioactive substance (depending on the radiation dose) in the body will increase the probability of cancer (Wren 1985).

2.5.6. Physical variable analysis

In metal monitoring protocols, pH is one of the most important parameters to determine, as chemical speciation and thus the bioavailability of many metals is greatly affected by this parameter (Meador 1991), where lower pH levels promote the solubility and mobility of the metals. This increase in mobility of the metal particles results in increased toxicity of these metals to the aquatic species (Sanders 1997). High pH values could alter the toxicity of pollutants in the rivers, however a decrease in the pH values will also cause a decrease in the solubility of the element (Morrison *et al.* 2001). Low pH also increases the solubility of other elements such as aluminium, boron, copper, cadmium, mercury, manganese and iron (Morrison *et al.* 2001).

Temperature is another important parameter in the assessment of metal bioavailability in an aquatic system. It is known that an increase in temperature will result in an increase in metal uptake by the organisms. As a result, it is suggested that certain metal concentrations may be more toxic to organisms during the summer seasons (Sanders 1997). The

temperature of the water greatly affects the salinity of the water, where the EC values can increase from up to 3% per 1°C (Oyem *et al.* 2014; Lenntech 2016).

Lastly, the electrical conductivity of a water body provides a good indication of the total dissolved salts in the water (Lenntech 2016). The total dissolved salts can influence the organisms either directly due to the sudden changes in the salt levels, or indirectly by influencing the metal toxicity in the water (Sanders 1997). The EC of the water may increase due to a number of different factors including waste waters, urban runoff, agricultural runoff and acid mine drainage. The increased salt levels affect how some of the metal compounds bond in water, either with the water molecules or with other metal compounds. Lower conductivity levels where there is less salt available for the metals to bind, result in an increase in the toxicity levels in the water (Sanders 1997). This then determines the concentrations of the heavy metals as well as whether or not they will be accumulated through the food chain of aquatic organisms reaching the top consumers.

2.5.7. Heavy metals studied by the Department of Water and Sanitation

Other substances known to occur in Gauteng rivers include aluminium, chlorine, calcium, copper, sodium and magnesium. These substances are known to occur naturally in the earth's crust (Lenntech 2016). However, these heavy metals enter the environment through sewage waste, agricultural runoff as well as industrial wastes (Department of Water Affairs and Forestry 1996; Lenntech 2016). These sources increase the concentrations of contaminants in the aquatic ecosystems, having direct impacts to the organisms inhabiting them. These contaminants are accumulated through food, inhalation and by skin contact (Lenntech 2016). The health effects of the heavy metals studied by the Department of Water and Sanitation depend largely on their concentration as well as an animal's length of exposure to these metals. They cause a variety of symptoms to the organisms exposed to them, these range from less intense to more concerning impacts and include gastrointestinal pains, respiratory distress, nausea, dehydration, loss of memory, kidney, liver, lung and brain damage, damage to the central nervous system (CNS) and suppression of heart functioning (Department of Water Affairs and Forestry 1996; Agency for Toxic Substances and Disease Registry 2010; Lenntech 2016). The release of these heavy metals to

the environment allows them to be available for accumulation in plants and animals, thus affecting animals further up the food chain.

2.6. Common urban pollutant thresholds

While urban environments have been significantly impacted by a number of different pollutants, not all pose a health risk to humans or animals. Most pollutants are relatively harmless as long as they do not exceed a biological threshold above which their concentrations are high enough to have a negative effect on mammal health and reproduction (Wilson 1978). The heavy metals which are found naturally in the environment are commonly found in animal tissue (Manson and Macdonald 1986). Heavy metals are not directly involved in the decrease of animal populations, but result in rather subtle, more sub-lethal effects (Olsson *et al.* 1979; Foster-Turley, Macdonald and Mason 1990). In a study conducted by Brueske and Barrett (1991), the least shrew (*Cryptotis parva*) was used to evaluate the accumulation of heavy metals from their diet of earthworms collected from a long-term sludge-treated municipal field in Oxford, Ohio. It was found that cadmium (Cd), copper (Cu), lead (Pb), and zinc (Zn) accumulated in significantly higher levels in the livers of the shrews than in the earthworms, indicating the potential health hazard to the higher trophic level organisms (Brueske and Barrett 1991). Weight loss seen in the shrews may have been an additional symptom of metal toxicity in these mammals (Ma 1989; Brueske and Barrett 1991). In an additional study conducted by Halbrook *et al.* (1994), hair, muscle tissue and livers were used in order to determine the mercury accumulation in river otters (*Lutra Canadensis*). The results of the study indicated that mercury was accumulating in otters with concentration levels which are much higher than the concentrations in their prey source, fish. These mercury concentrations were found to be near those causing death in a closely related species, the mink (Halbrook *et al.* 1994). Otters displayed clinical signs of mercury intoxication, with major concerns being evident in the physical and behavioural effects of sub-lethal mercury exposure to otters. These signs are thought to affect the reproduction rates of the otter, thus potentially causing population level effects (Halbrook *et al.* 1994). It is seen through a number of similar studies that the accumulation of heavy metals within higher trophic levels results in potential health impacts to these organisms, as

these substances interfere with their normal metabolic processes, resulting in potentially lethal outcomes (Goyer *et al.* 1970; Brueske and Barrett 1991).

The thresholds set by the Agency for Toxic Substances and Disease Registry (2016) for the heavy metals and other selected contaminants of interest in this study are represented in Table 1 below. These concentration levels indicate the intake risk level of each contaminant where, if exceeded, the contaminant may have harmful effects on mammal individuals.

Table 1. The thresholds concentration levels for mammals for the selected contaminants.

Contaminant Name	Minimum Risk Level
Arsenic*	0.005 mg/kg
Cadmium*	3x10 ⁻⁸ mg/l
Chlorine**	200mg/l
Magnesium**	70 mg/l
Mercury*	2x10 ⁻⁷ mg/l
Titanium*	0.00001 mg/l
Uranium*	0.02mg/kg
Lead**	1x10 ⁻¹¹ mg/l
Aluminium**	5 mg/l
Copper *	0.01 mg/kg
Sodium**	600 mg/l

* Agency for Toxic Substances and Disease Registry (2016).

** Department of Water Affairs and Forestry (1996).

*** mg/l is equivalent to mg/kg (Taylor and Thompson 2008).

2.7. Research gap

In recent years, a number of environmental issues concerning contaminants in the rivers of South Africa have arisen. The continual discharge of pollution from a variety of sources into the river ecosystem makes the food and drinking water harmful to humans and the wildlife dependant on these water sources for survival (Chopra *et al.* 2011).

There have been several studies which have been conducted in South Africa regarding the effects of the bioaccumulation of contaminants on wildlife. The most researched field on contaminants has mainly been focused on aquatic organisms. Some examples include amphibian species, mainly frogs and tadpoles (see Gutleb *et al.* 2000 and Haywood *et al.*

2004), fish species (see Retief *et al.* 2006), the Nile crocodile (see Ashton 2010), and the South African river crabs (see Sanders *et al.* 1999; Schuwerack *et al.* 2001). All these examples have focused mainly on heavy metals in the water systems in South Africa and have concluded that these contaminants at high concentrations have been hazardous to the survival rates of these species.

Very few studies have been conducted on the impacts of aquatic pollution on the mammal wildlife of South Africa. The few studies which have been conducted have been focused on small mammals such as bank voles and shrews (see Reinecke *et al.* 2000 and Milton *et al.* 2003). All of the above mentioned studies have used invasive sampling methods, whereby blood samples, muscle tissue, bone, skull, hair or organs were used in order to determine the concentration levels of contaminants which have bio-accumulated in the study species. Previous studies have indicated that concentration levels of contaminants in scat can be used to predict the concentration levels in the mammal's liver and adipose tissue (Guertin *et al.* 2010; Gupta 2012). Elliott and Wilson (2003) and Zwiernik *et al.* (2008) assessed the accumulation of polychlorinated dioxins (PCDDs), furans (PCDFs), PCBs, mercury and cadmium in scat and carcass samples of both mink and otter species. Results showed that the scat samples can be used as a useful tool to assess contaminant concentrations in the otter and mink species, as it was found that concentrations of heavy metals detected in scat were directly correlated with those determined in the liver and adipose tissue analysed ($r^2 = 0.94 - 0.97$, $p < 0.01$). Thus, scat for both species appears to be a useful tool to assess the contaminant level accumulated in the individuals, allowing for a prediction on the effects these contaminants may have on the reproduction of these species (Elliott and Wilson 2003; Zwiernik *et al.* 2008).

To determine the contaminant accumulation in an animal is often a difficult assignment, as many species are endangered, rare or protected, especially within the urban areas. Due to the conservation concerns and the difficulty in capturing and handling the animal, invasive techniques are not ideal for the tissue analysis of pollutants, thus limiting the ability to determine the potential threats to their populations in South Africa. It is for this reason that an alternative technique is essential to be employed to provide clues to their rare and possible decreasing populations (Foster-Turley *et al.* 1990). In order to achieve this, the most successful non-intrusive technique would be to analyse their prey, followed by an

assessment of their scat for the presence of environmental pollutants (Mason and Macdonald 1994). This study set out to determine the risk of bioaccumulation of the pollutants in otters from the rivers of Gauteng using a non-invasive sampling technique.

CHAPTER 3: METHODS AND MATERIALS

3.1. Study area

This study was conducted in Gauteng Province of South Africa between June 2014 and May 2015. Study sites were located along the Hennops River in the north and the Klip River in the south (Figure 3). Gauteng is the smallest of the nine provinces in South Africa, covering an area of 16191km² (Grobler *et al.* 2002), and consisting of both urban and peri-urban areas. The urban areas are considered as those areas close to the city centre of Johannesburg, whereas peri-urban areas are located further away from the city centre and consist primarily of open fields and agricultural areas. The province is dominated by urban areas with an approximate population of 12.9 million people, making it the most populated province in South Africa (Statistics South Africa 2015).

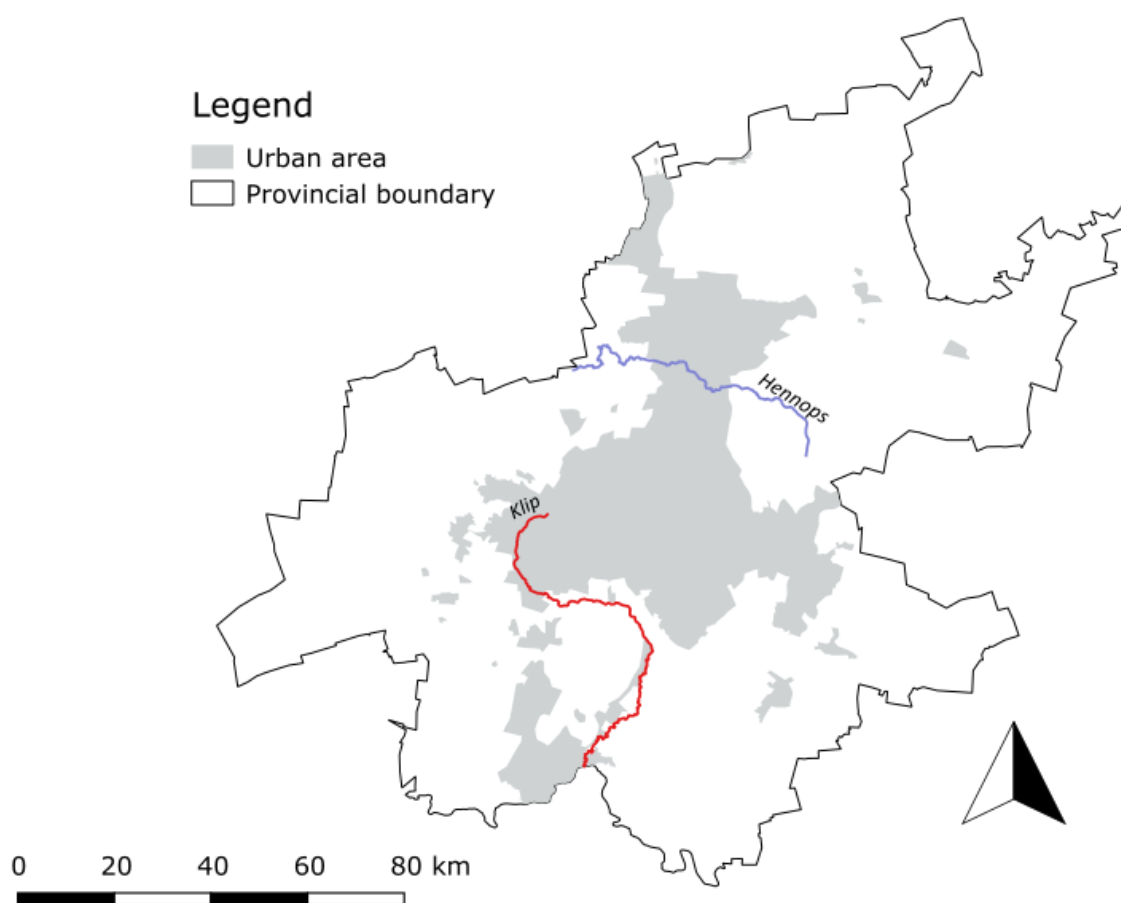


Figure 3. The two rivers being studied (Hennops River and the Klip River), including the urban edge which is represented by the grey area (GDARD, 2013, CPLAN 2).

Gauteng experiences hot summers, most commonly associated with hail and rain, whereas the winters are cold, dry and often experience frost (Grobler *et al.* 2002). Gauteng experiences a mean summer temperature ranging between 25 - 26 °C with a mean winter temperature between 16 - 19 °C (SAWS 2016). The annual rainfall in Gauteng is 544 - 608 mm per year (SAWS 2016). Gauteng vegetation is predominantly part of the Grassland biome (Rutherford and Westfall 1986), however it also partly includes the Magaliesberg, which is located in the Savannah biome (Grobler 2000). There are three additional vegetation types in this area which have been recognised by Bredenkamp and van Rooyen (1996), these include Mixed Bushveld, Moist Cool Highveld Grassland and Rocky Highveld Grasslands.

The two rivers studied were selected based on the fact that they both flow through the peri-urban as well as the urban areas of Gauteng (Figure 4). Previous studies (Ponsonby submitted) have confirmed the presence of otters along both of these rivers. The Hennops River is one of the smallest rivers in Gauteng and is located southeast of Pretoria. The Hennops River has become heavily polluted due to the different surrounding land uses and activities within the area, such as informal settlements and agriculture, and has therefore lost most of its aesthetic appeal (Singh and Todd 2015). The pollution has also affected the natural environment as well as social and economic aspects as it has become less useful for recreational activities as well as agriculture, but more importantly the Hennops River has become less suitable for sustaining aquatic life (Nawn 2004; Singh and Todd 2015) as a result of its poor ecological condition (Singh and Todd 2015). The Klip River drains into the southern Witwatersrand region of Gauteng and is surrounded by agricultural fields (McCarthy *et al.* 2007). This river is one of the most heavily impacted river systems in South Africa as it is subjected to a number of different pollution sources as well as various anthropogenic activities between the river's source and its confluence with the Vaal River (Kotze 2005; McCarthy and Venter 2006).

Several suitable sampling sites were identified along each river, set at approximately 5 km from each other. In total 32 sites were sampled and analysed along the Hennops and Klip River, however only 12 sites (six per river; Figure 4) yielded positive samples for all four sources required (water, sediment, crab and scat). Previous studies on the presence of

otters along a river have indicated that 5km is a large enough distance between sites to avoid collecting scat from the same individual otter at several sites (Ponsonby submitted). When selecting these sites, it was ensured that at least three of the six sampling sites along the rivers were located in the urban regions closer to the built up areas whilst the remaining sites were located further away from these built up areas, in the peri-urban regions transected by the river. This was done in order to determine if there was a difference in risk effects posed to mammals in the urban and peri-urban areas along the selected rivers.

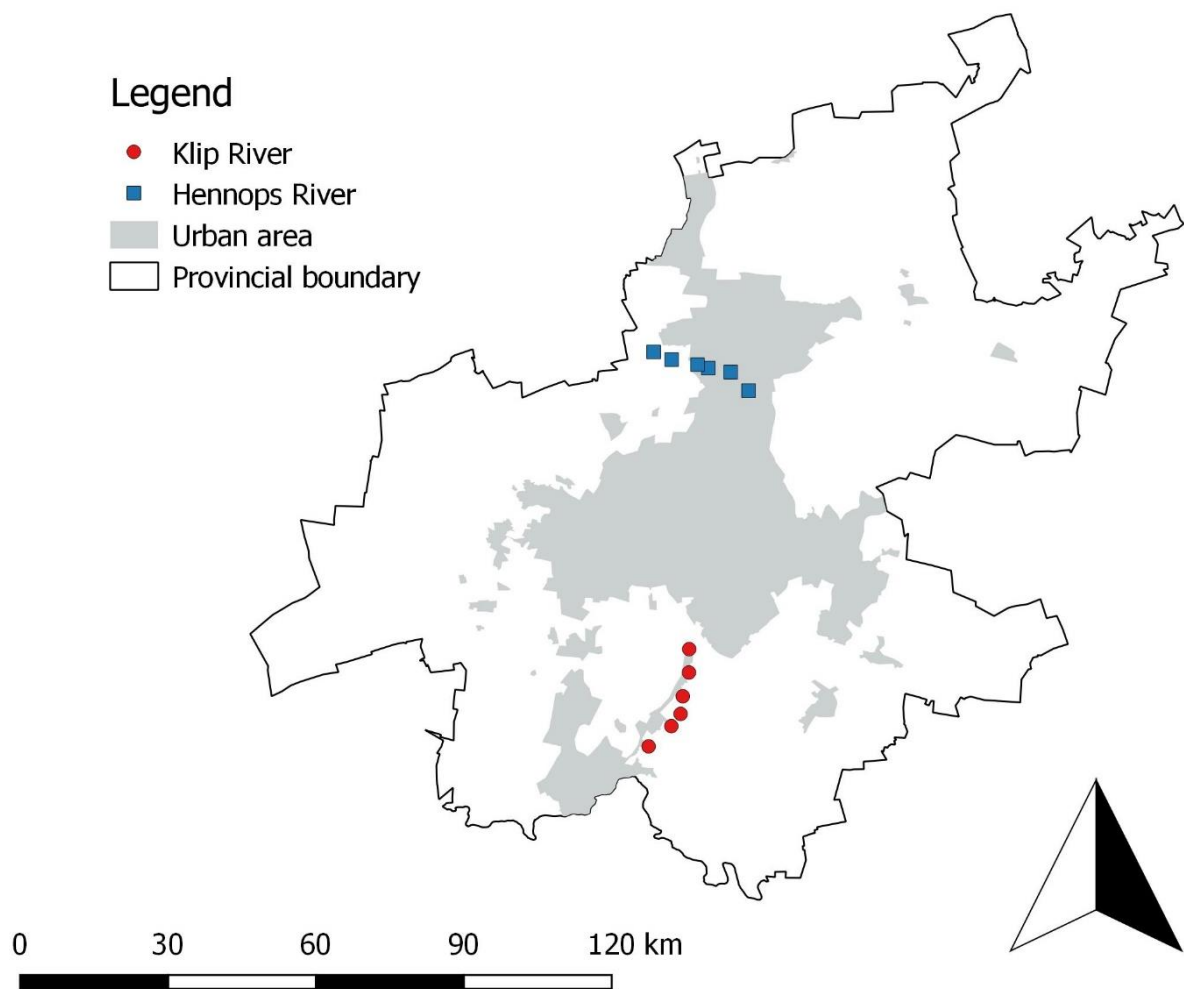


Figure 4. The six sampling sites occurring within the urban and peri-urban regions for each river being studied in Gauteng (GDARD, 2013; CPLAN 2).

3.2. Experimental design and protocol

Indirect sampling techniques were used, as per Mason and MacDonald (1987), Elliot and Wilson (2003) and Zwiernik *et al.* (2008). Data were obtained from the University of the Witwatersrand's Environmental Chemistry laboratory regarding the most common contaminants found in Gauteng's river waters at significant concentrations (Cukrowska, *pers. comm.*)³. This narrowed down my choice for further testing based on the list which was supplied. The contaminants which were of main interest for this study were selected based on whether or not they were potentially harmful to mammals.

The five common urban heavy metals which were selected based on literature as well as the data available from the University of the Witwatersrand's Environmental Chemistry laboratory were uranium, titanium, lead, arsenic and mercury.

3.2.1. Sample collection

At each of the 12 study sites otter scat, crabs, water and sediment samples were collected for contaminant testing. A study conducted by Ponsonby (submitted) indicated that the majority of both otter species' diet consisted of crabs. Due to very limited sightings of fish in the rivers and little to no success in capturing them, fish were not included in the study. All samples were sent to the School of Chemistry's Environmental Analytical Chemistry laboratory at the University of Witwatersrand for all chemical analysis.

Sampling collection was done as follows: at each site, fresh otter scat (scat that was still intact with no sign of disintegration yet) was collected for testing by using latex gloves or inverted ziplock bags. The ziplock bags were then labelled with the GPS coordinates taken at that site as well as the date, name of the river and the site number. The labelled ziplock bags were then taken back to the University of the Witwatersrand and stored in a refrigerator at 2-4°C until preparation for analysis was conducted. The preparation for the chemical analysis was conducted within 1-2 days of collection. In order to prepare the samples, the scat from each site was broken up into smaller pieces and placed in a plastic bowl in order to composite the scat from each site (see Patil 2002). One subsample, for

³ Prof E. Cukrowska is a professor at the School of Chemistry, University of Witwatersrand.

chemical analysis, was taken from the plastic bowl, placed in labelled CARBI disposable petri dishes and covered with parafilm. The petri dishes were placed back in the freezer in the research laboratory until they were sent to the chemistry laboratory for analysis. The remaining scat left in the bowl was placed into a 50ml polypropylene container and labelled with the site details and placed in the freezer as a backup sample of that site, in case chemical analysis had to be repeated.

Crabs were caught using crab nets which were baited with canned sardines and placed on the sediment of the river (as per Dellinger *et al.* 2016). At least four crab nets were placed at each site along the river, these were left overnight before they were removed from the river. At each site at least two crabs were caught, however there were four sites where no crabs were caught, due to low crab number in the rivers. Once a crab was caught, it was stabbed through its centre to kill the crabs instantly to ensure no suffering or a slow death occurred. Crab sizes were not taken into account as crab numbers were low in the rivers making it very difficult to collect, therefore I used any crabs I was able to collect. The crabs collected at the same site were placed in a labelled ziplock bag and kept in a cooler box packed with icepacks for transport to the University, where they were stored in a freezer at -18°C until the samples were ready to be blended. Blending occurred within 1-2 days of collection using a blender with a stainless steel blade. In order to obtain the tissue samples, all crab individuals caught at each site were blended from frozen. All the blended crab samples from each site were placed in a plastic bowl and composited to ensure the maximum amount of contaminants taken up by the individuals which may have accumulated in different parts within each individual at the corresponding site (see Jarvis 2007). One subsample, for chemical analysis, was then taken from the bowl and placed in a CARBI disposable petri dish until the dish was full. Parafilm was used to close up the samples. The petri dish was labelled and placed back in the freezer in the research laboratory until it was sent off to the chemistry laboratory for analysis. The remaining crab tissue left in the bowl was placed into a 50ml polypropylene container and labelled with the site details and placed in the freezer as a backup sample of that site, in case chemical analysis had to be repeated. The equipment was then thoroughly washed between each sample preparation in order to prevent cross-contamination. This was repeated for each of

the 12 sites sampled. Animal ethics clearance was given by the University's Animal Ethics Screening Committee (Ethics clearance no. 2014/41/O).

At each site the pH, temperature and conductivity of the water were measured and recorded (using a HANNA HI 99300 EC/ TDS/Temperature meter and a HANNA HI 9210N ATC pH meter). After testing the probes were rinsed using de-ionized water in order to prevent contamination between sites. Water and sediment samples were also collected in 1l polypropylene containers which were labelled and placed in the cooler box with ice packs to keep the samples cool during transport back to the University. The samples were then divided into two 500ml polypropylene containers. One sample was acidified by adding 5ml of nitric acid. This would ensure that none of the heavy metal traces present in the water sample denatured before chemical analysis took place (see Tshumah-Mutingwende 2014). The samples were placed in the research laboratory fridge at 3°C, until they were sent to the chemistry laboratory at the University.

Once all the scat, crabs, and sediment samples were collected and prepared, they were sent to the School of Chemistry, where they were freeze-dried at -40°C to prepare for further analysis (Elliott *et al.* 2008); 10g of each sample were extracted in order to obtain a representative sample for testing.

Chemical analysis was conducted by the Environmental Analytical Chemistry Lab at the School of Chemistry, University of the Witwatersrand, Johannesburg. The following methodology was applied by the School of Chemistry, University of the Witwatersrand: Firstly, all the equipment used for the chemical analysis of all the samples went through a cleaning procedure. This procedure minimises the risk of any contamination of the samples. The samples were first wet digested in the microwave digestion systems, after which an Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP-OES) was used in order to determine the total metal concentration. Lastly an Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) instrument was used for trace metal determination (see Appendix A for a detailed description of methods).

3.3. Data from the Department of Water and Sanitation

Additional water quality data were obtained from the Department of Water and Sanitation to determine any additional risks to otters in Gauteng. I used the data to identify any trends in contaminant levels to see if any additional contaminants which had been assessed over the past 10 years could suggest any further potential risks to the mammals along the rivers. The data included trace metal concentrations which the Department of Water and Sanitation had tested for, these being aluminium (Al), cadmium (Cd), chlorine (Cl), copper (Cu), sodium (Na), magnesium (Mg), as well as the electric conductivity (EC) and pH levels of the water. The above water quality data were obtained for four rivers in Gauteng, the Hennops River, the Klip River, Blesbokspruit River and the Jukskei River (Figure 5). The two additional rivers were selected as otter occurrence was confirmed in a previous study (Ponsonby submitted). The data obtained were recorded hourly on the days when analysis was conducted. These records date back to 1976. For the purpose of this study I only looked at the last three years of records (2013-2015) to align with my study period.

The five main contaminants (lead, arsenic, mercury, titanium and uranium) were selected as they are regarded the most common contaminants found in Gauteng's river waters at significant concentrations (Cukrowska, *pers. comm.*)⁴. Data for the other contaminants analysed were provided by the Department of Water and Sanitation and were also regarded as common occurring contaminants in the rivers of Gauteng. All of the above common contaminants are thought to have a high impact to the health of an individual when being exposed at levels higher than the suggested minimum threshold.

3.4. Data analysis

Statistica vs 12 (Statsoft, 2014) was used to perform a two sampled t-test in order to determine the difference between the two rivers for each water quality variable (temperature, EC and pH). A general linear model (GLM) was used to see whether river, site or source was a predictor for heavy metal concentrations. A Fisher post-hoc test was then conducted to see which metals and which sources specifically were significant within the

⁴ Prof E. Cukrowska is a professor at the School of Chemistry, University of Witwatersrand.

study. The significance level was set at $p < 0.05$ for all statistical analysis performed. Lastly, simple regression models were used in order to determine any possible relationships between the four different sources analysed. Due to the small sample size it is understood that the statistics obtained are not solid but rather indicate possible trends in the accumulation of contaminants through the food chain of Gauteng otters.

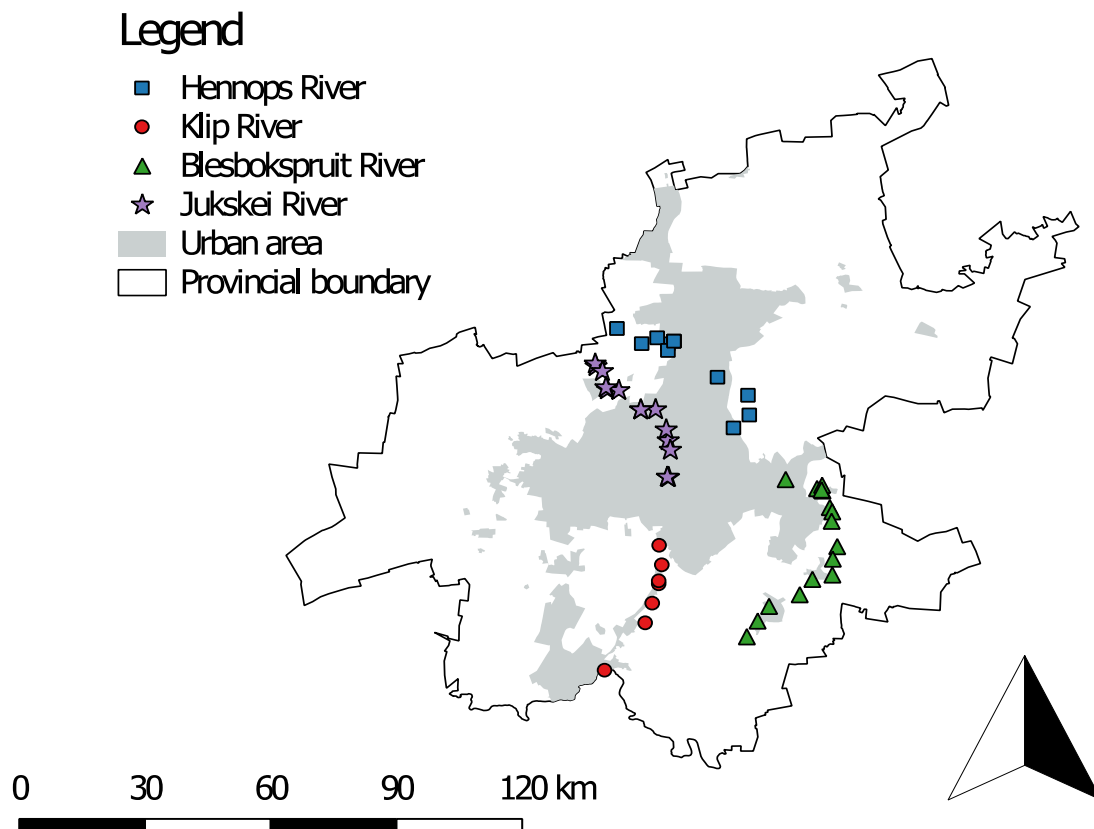


Figure 5. Sampling sites for data obtained from the Department of Water and Sanitation for the Hennops River, Klip River, Blesbokspruit River and the Jukskei River within Gauteng (Arc GIS Mapping 10.2).

CHAPTER 4: RESULTS

Overall, I had a very small sample size. In total 32 sites were sampled and analysed along the Hennops and Klip River, however only at 12 sample sites was I able to gather data. Of the 12 sample sites, six sites were assessed along both the Hennops and Klip River. From these 12 sites a total of 12 water samples, 12 sediment samples, 7 crab samples and 10 scat samples were collected (see Appendix B). Fish could not be included in the analysis as a food source as there was no presence of fish at the sites sampled.

No significant differences were found for the concentration values for the metals analysed for all four sampling sources between the two rivers studied ($F_1 = 0.028$, $p > 0.05$), nor was there a significant difference between the urban and peri-urban areas ($F_{10} = 0.532$, $p > 0.05$). Due to the rivers not being significantly different, the data for both rivers were combined for further analysis, thus making the overall sample size 12. The small sample size allowed for simple statistics to be conducted which highlighted trends in contaminant accumulation through the otter's food chain in rivers across Gauteng. Furthermore, the study's aim was to assess the risk of accumulation of heavy metals in a food chain and not the difference between rivers or spatial variation, which therefore allowed for the data to be combined.

When analysing the heavy metals, it is important to acknowledge that the metal concentration is affected by the temperature, EC and pH of the water (see Baba *et al.* 2008) as these physical properties of water affect the form and therefore the bioavailability of the heavy metals. Overall the pH was seen to be neutral for both rivers analysed (Table 2). EC for both rivers also fell within the normal range which is 20 mS/m to 100 mS/m. Statistical analysis was conducted, where a two sampled t-test was performed in order to determine if there was a significant difference between the two rivers for each parameter. Temperature was the only parameter to show a significant difference between the two rivers ($p < 0.05$), where for EC and pH there was no statistically significant difference between the two rivers.

Table 2. The total average (min/max) temperature, EC and pH levels for the water quality the Klip River and the Hennops River.

River	Temperature (°C)	EC (mS/m)	pH
Klip River	16.5 (13.7/19.0)	63.35 (49.3/69.9)	7.4 (7.2/7.8)
Hennops River	20.1 (19.4/20.8)	55.25 (18.2/74.2)	7.1 (5.6/7.8)

4.1. Water sample analysis

Both mercury and titanium concentration levels were higher than the suggested minimum risk thresholds reported by the Agency for Toxic Substances and Disease Registry (2014) (Table 3). In particular it was found that titanium had the highest concentration in water (0.0195 mg/l) reported across the board in all water samples. Mercury was found to be the second heavy metal to exceed the suggested minimal risk threshold.

Table 3. The averaged contaminant concentrations in the water samples analysed compared to the suggested minimal risk thresholds.

Element	Sample Concentration (mg/l)	*Suggested Minimal Risk Threshold (mg/l)	Higher/lower than Suggested
As	0.0017	0.005	3x Lower
Hg	0.0029	0.2x10⁻⁷	15x Higher
Ti	0.0195	0.1x10⁻⁵	>100x Higher
Pb	0.0118	10	>100x Lower
U	0.0019	0.02	11x Lower

* Source: Agency for Toxic Substances and Disease Registry (2016).

The average concentrations for the heavy metals found in the water samples taken from both rivers were not statistically significant ($F_5 = 0.982$, $p > 0.05$). A Fishers post-hoc test further revealed that there was no significant difference between any of the heavy metals in the water samples ($p > 0.05$). Although the concentrations were not statistically different, it was evident that the Hennops River had a higher concentration of both lead and titanium (Figure 6), where the maximum titanium concentration recorded was close to double the levels seen in the Klip River. Mercury however was not recorded in the Hennops River.

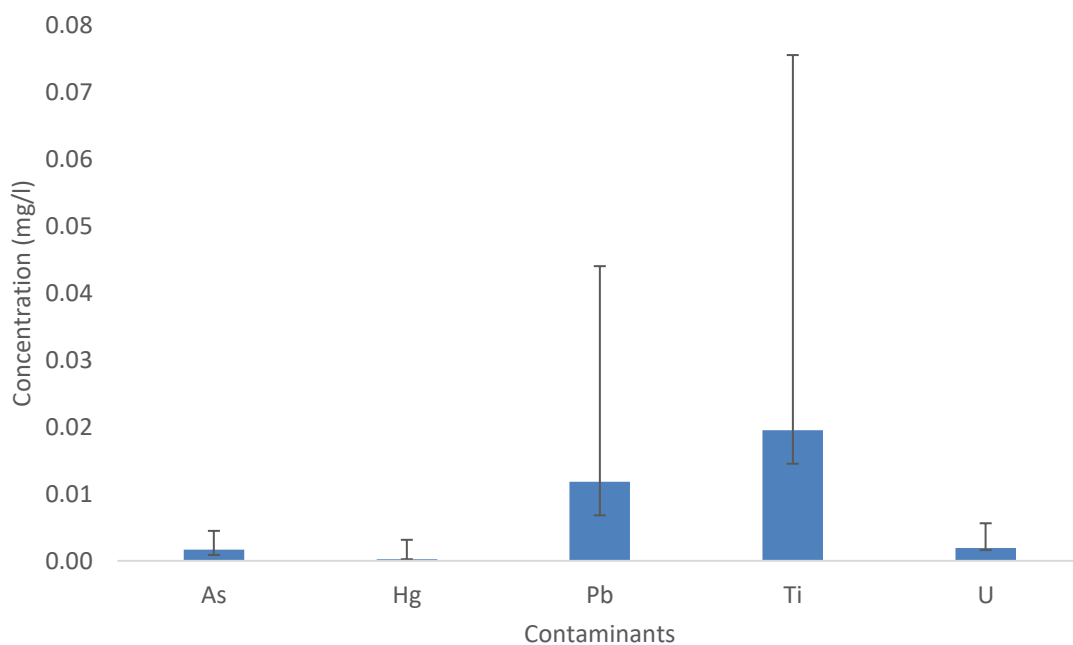


Figure 6. The average (min/max) concentration of the five heavy metal contaminants found in the water samples from the two rivers.

It was clear that titanium and lead concentrations were the highest at each study site sampled. There was little to no mercury found in the water samples. Titanium concentrations were substantially higher at two sites along the Hennops River and at three sites along the Klip River (Appendix C).

Within both the urban and peri-urban areas, lead and titanium had by far the highest concentrations (Figure 7) with a large variation in the readings for both heavy metals recorded in the water samples. Overall, the urban area had the highest average concentration for these two contaminants. All metals were found at higher concentrations in the urban areas compared to the peri-urban area, however this was not statistically significant ($F_1 = 0.202$, $p > 0.05$). A Fishers post-hoc was performed for the difference in elements Pb and Ti and showed no significant difference between the two areas ($p > 0.05$). Overall, there was no significant difference between the urban and peri-urban areas.

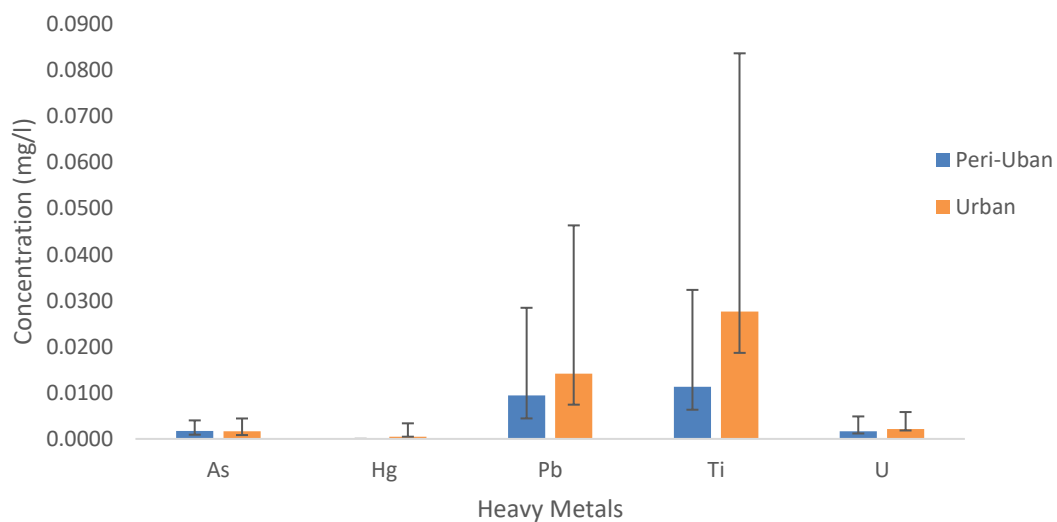


Figure 7. The average (min/max) concentration of all heavy metals in the water samples between the urban and peri-urban sites.

4.2. Sediment sample analysis

A GLM showed that there was a significant difference between the metals analysed in the sediment samples ($F_3 = 9.408$, $p < 0.05$). A Fisher post hoc analysis showed that the titanium concentration was significantly higher compared to the other metals analysed ($p < 0.05$; Figure 8). In contrast to titanium's high concentration levels, lead and mercury had the lowest concentration levels. There were only two recorded sites where lead was present, one along the Klip River and the other along the Hennops River, with only 7 mg/kg and 3 mg/kg respectively (Appendix D). However, statistically the Fishers post-hoc test showed that there were no significant differences between any of the other metals analysed ($p > 0.05$).

Titanium occurred at the highest concentration in the urban as well as the peri-urban area (Figure 9). Mercury on the other hand was found to have the lowest concentration in both the urban and peri-urban area. Statistically, once again there was no significant difference found between the urban and peri-urban areas for the sediment analysis report values ($p > 0.05$).

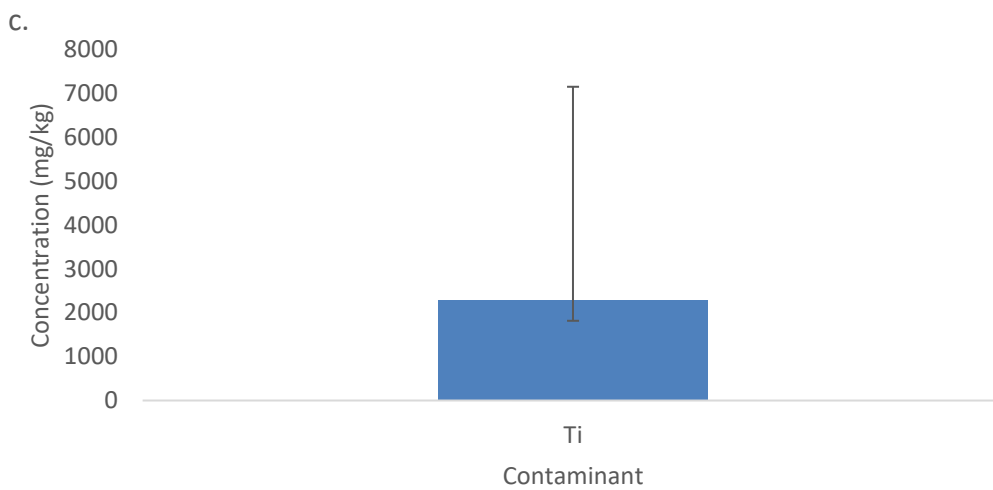
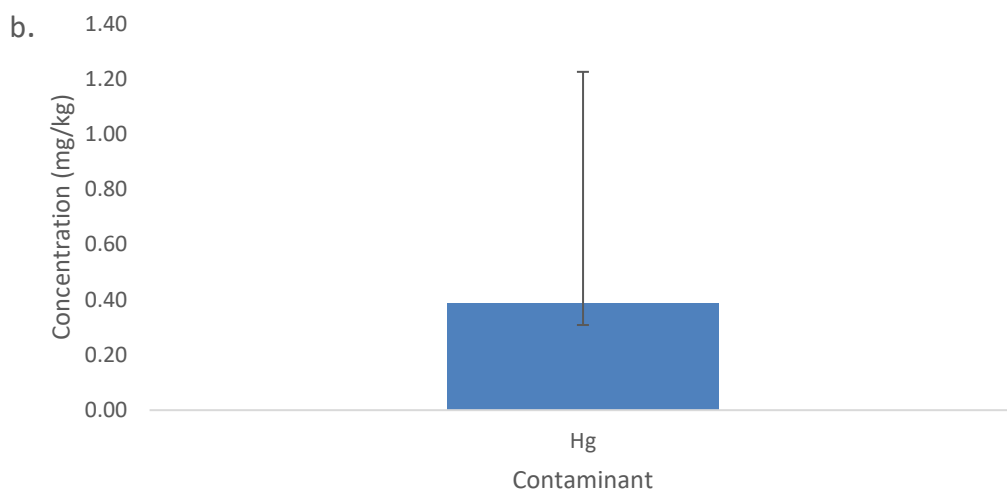
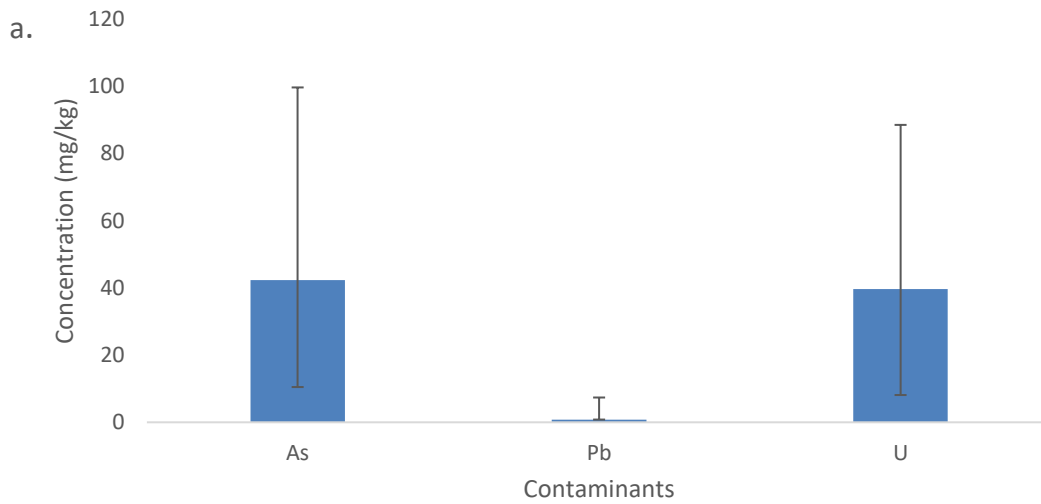
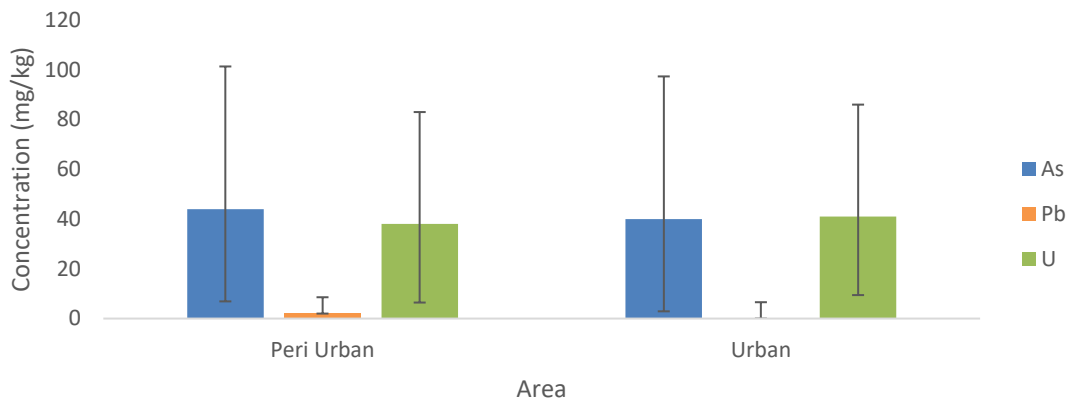
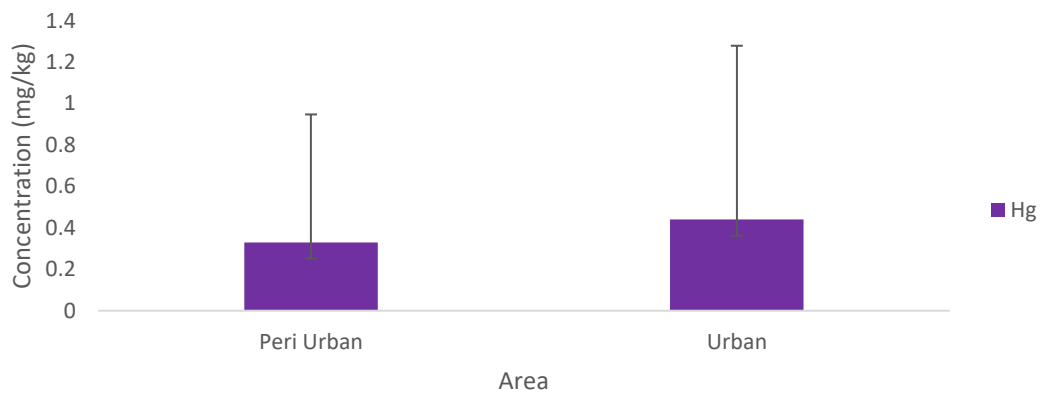


Figure 8. Average (min/max) contaminant concentrations for the sediment samples (a. Arsenic, Lead and Uranium. b. Mercury and c. Titanium).

a.



b.



c.

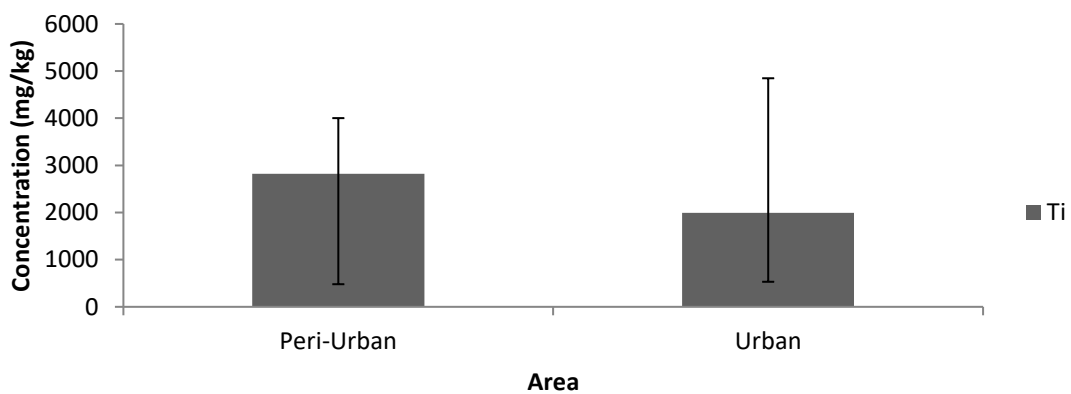


Figure 9. The average (min/max) contaminant concentrations for the sediment samples in urban and peri-urban areas (a. Arsenic, Lead and Uranium, b. Mercury and c. Titanium).

4.3. Crab and scat analysis

The GLM for scat and crab samples revealed no statistical differences between rivers ($F_3 = 9.408$, $p < 0.05$), thus data were pooled. The crab samples for mercury, arsenic and titanium had concentration levels which exceeded the minimum risk threshold. All three metals were recorded to be over 100 times higher than the threshold. Lead was the only metal which had a concentration level lower than the threshold, this being recorded at 22 times lower than the minimum risk threshold. Uranium was not detected in any of the scat samples along either of the rivers sampled (Figure 10). The scat collected along the Klip River had higher concentrations of arsenic and titanium than the Hennops River samples, while the scat collected along the Hennops River had higher concentrations of mercury and lead compared to what was recorded for the Klip River. There was no detection of uranium in the crab samples along either river studied (Figure 11).

Overall, arsenic concentrations were highest with 35.636 mg/kg in the crab samples. The maximum level recorded for arsenic was very high (237.154 mg/kg) compared to the average concentration of arsenic. Mercury concentrations were the lowest (Figure 10). However, in the scat samples titanium had the highest concentration, while mercury had the lowest concentration (Figure 11).

Figure 12 shows that arsenic and titanium were found in higher concentrations in crab samples from the peri-urban areas, however the opposite trend is seen for the scat samples, where the majority of the contaminants detected were found in the urban areas (Figure 13). Once again, as seen in Figures 12 and 13, when comparing the urban and peri-urban areas along the Gauteng rivers, there is no statistically significant difference ($F_{35} = 0.186$, $p > 0.05$). Statistically there was no difference found between the scat and crab samples for any of the heavy metals tested ($p > 0.05$).

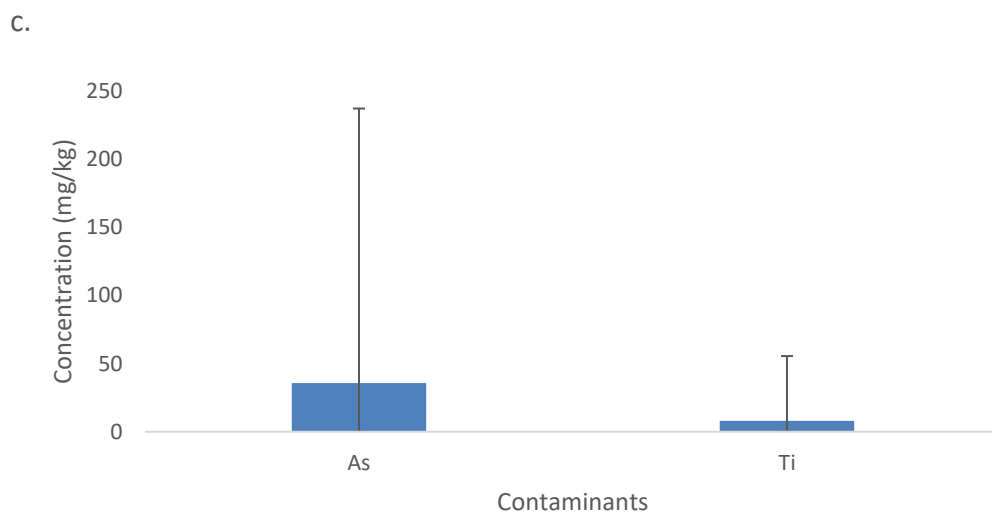
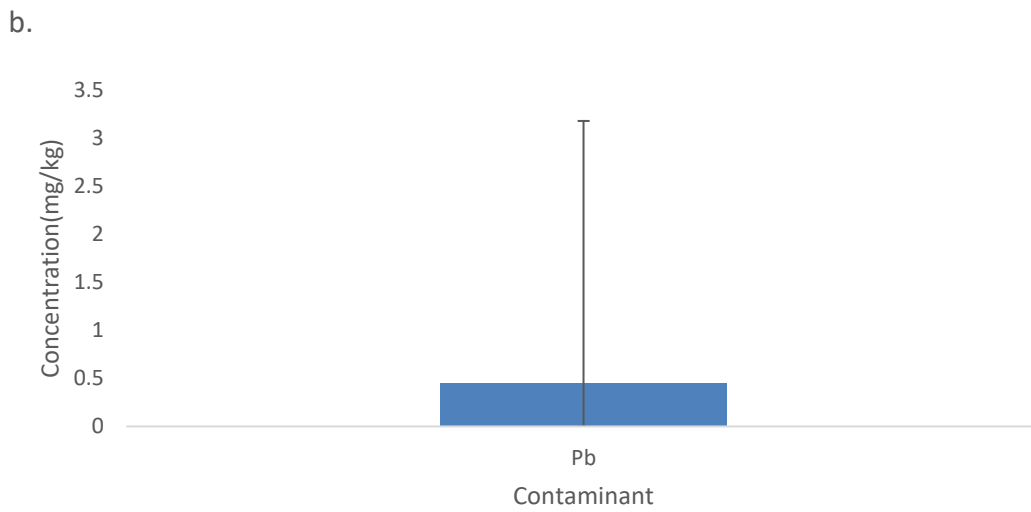
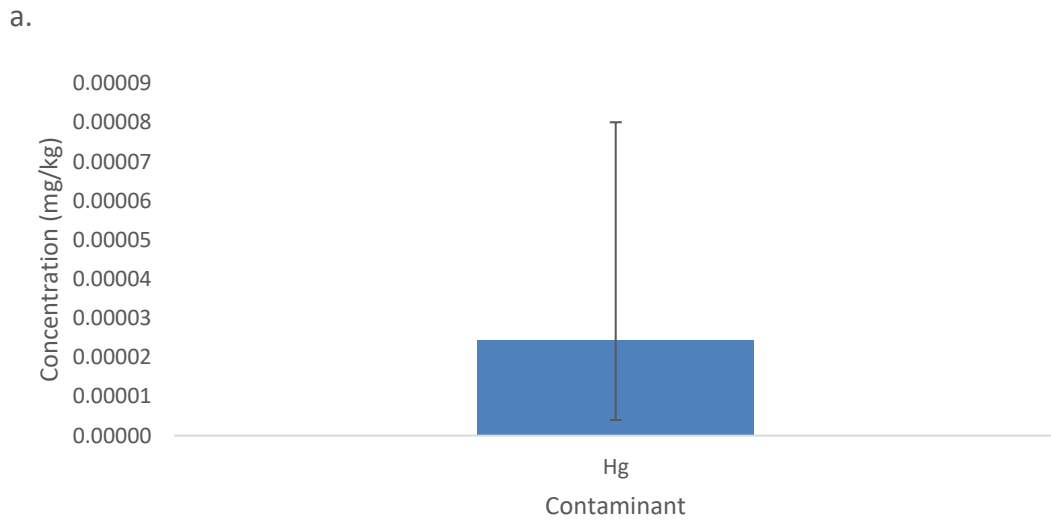


Figure 10. The average (min/max) concentration of the metals analysed in the crab samples (a. Mercury, b. Lead, c. Arsenic and Titanium).

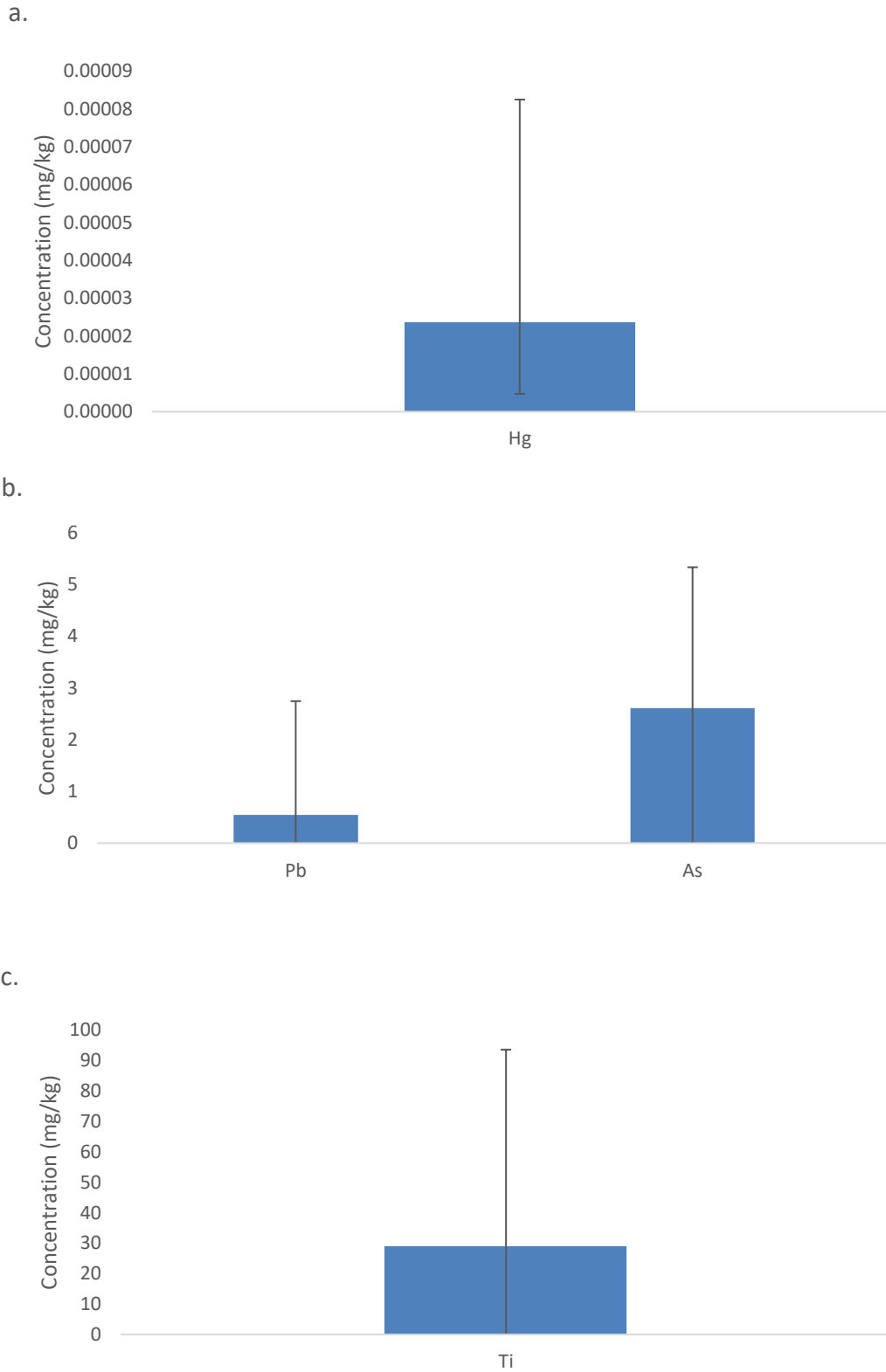


Figure 11. The average (min/max) concentration of the metals analysed in the scat samples (a. Mercury, b. Lead and Arsenic and c. Titanium).

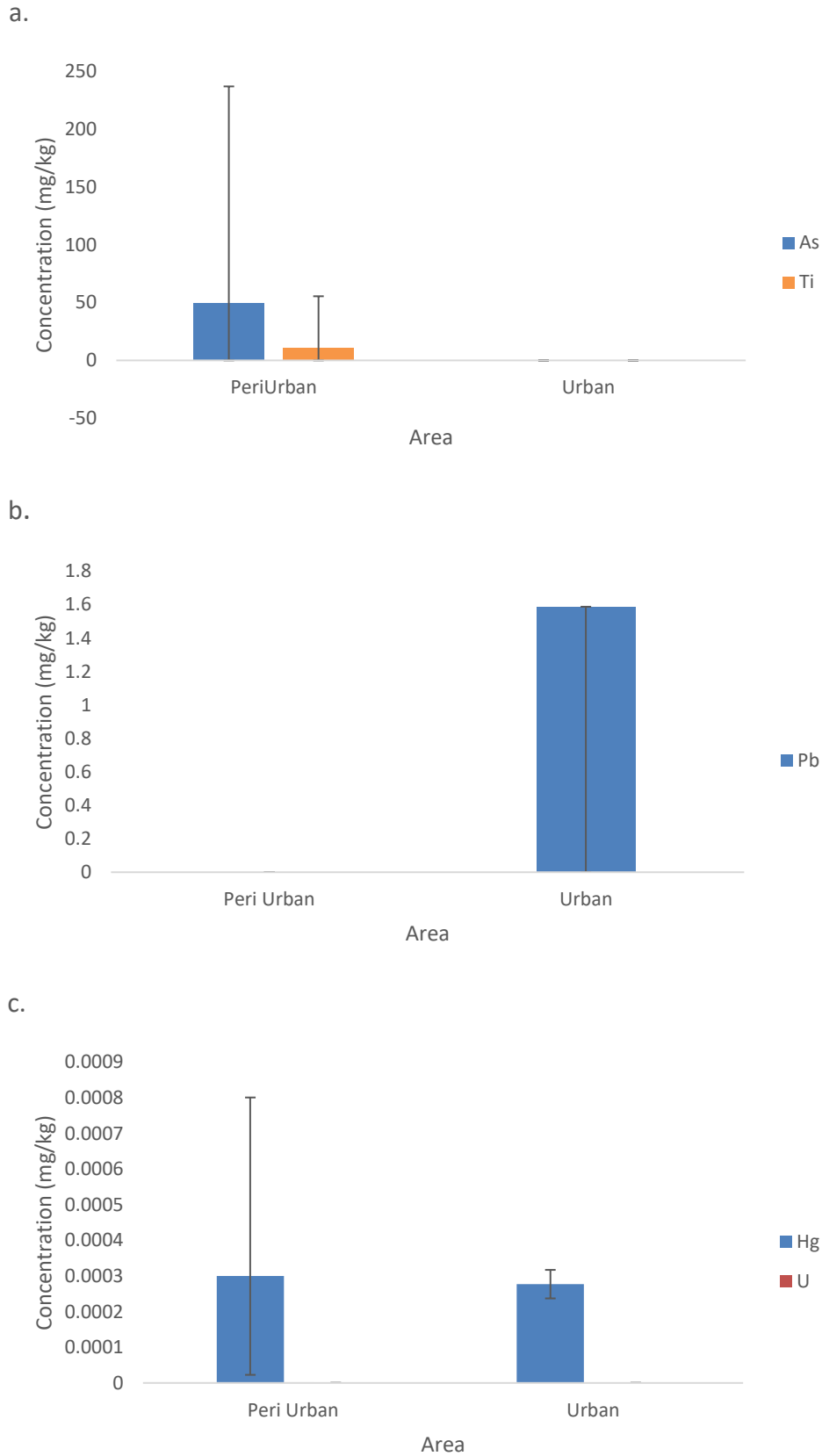


Figure 12. The average (min/max) concentrations comparing the urban and peri urban areas for the five heavy metals in the crab samples (a. Arsenic and Titanium, b. Lead and c. Mercury and Uranium).

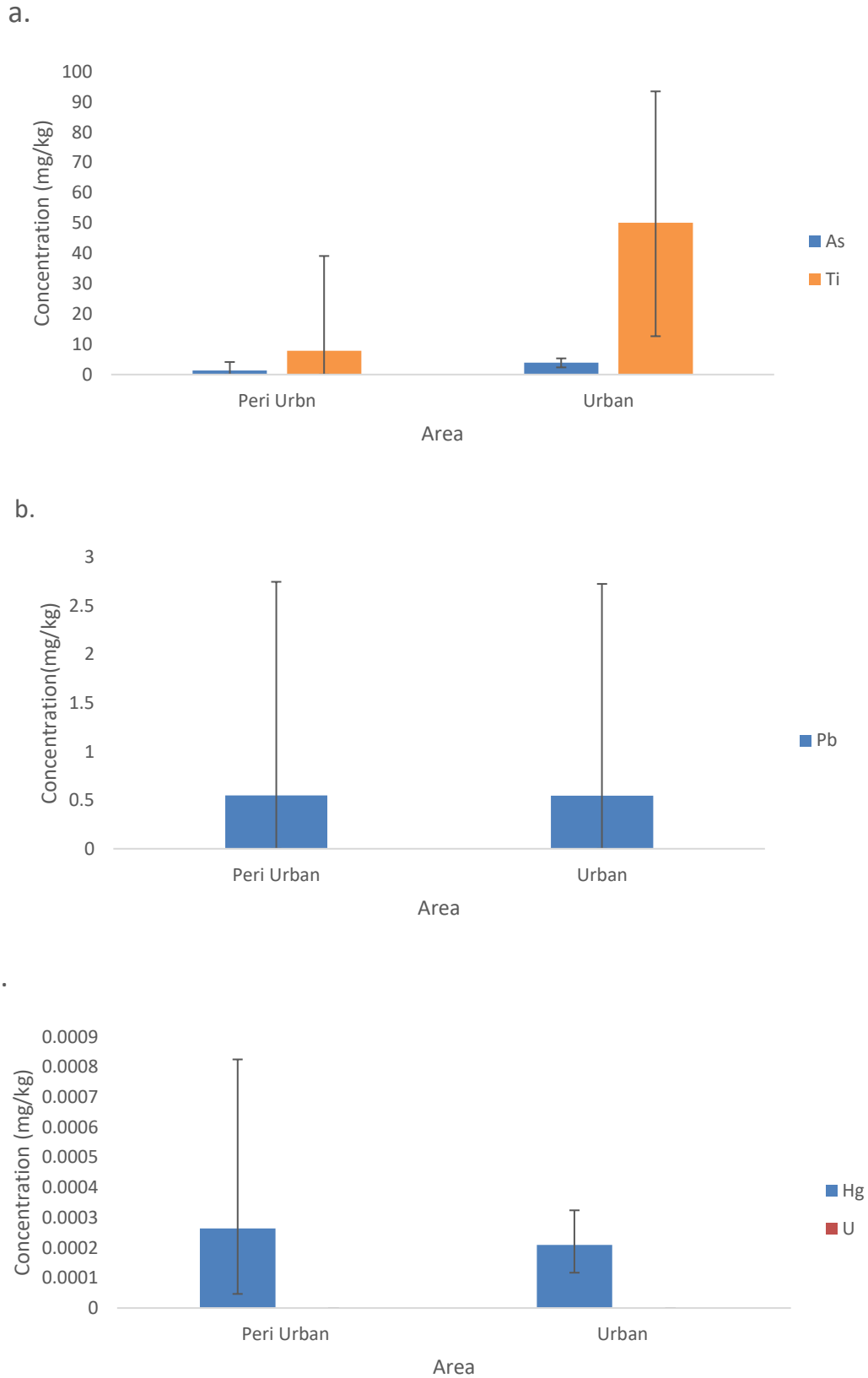


Figure 13. The average (min/max) concentrations comparing the urban and peri urban areas for the five heavy metals analysed in the scat (a. Arsenic and Titanium, b. Lead and c. Mercury and Uranium).

4.4. Heavy metal analysis through the food chain

The concentrations of arsenic and mercury shown in Figure 14 revealed that there was a higher concentration for both heavy metals in the otters' food source than was being eliminated from the otter through their scat. Significant accumulation of all contaminants analysed occurred within the sediments of both rivers (Figures 14 and 15). There was no trace of uranium in the crab and scat samples (Figure 15c).

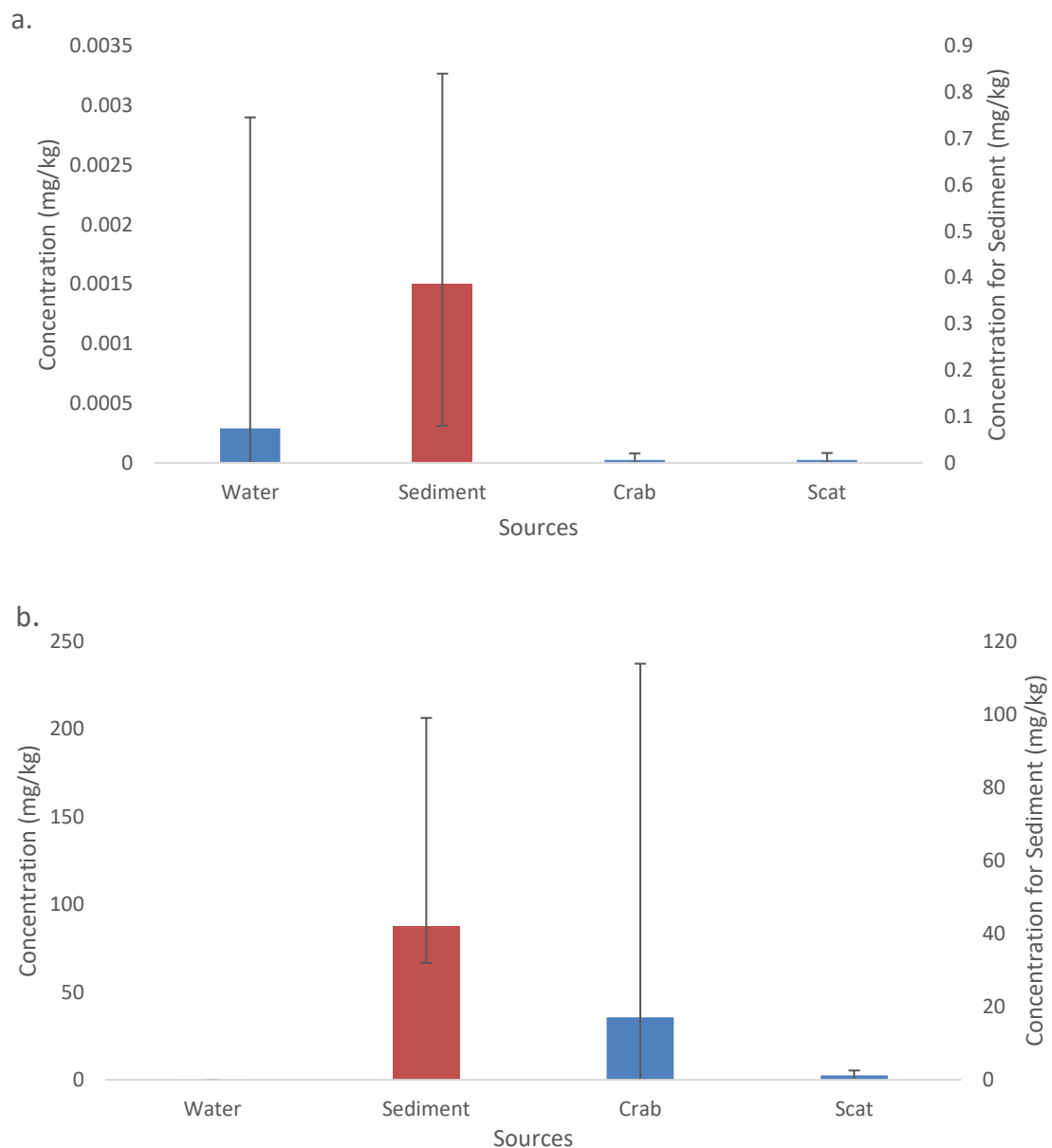


Figure 14. The overall average (max/min) concentrations of the two contaminants analysed through the otter's food chain along the Hennops and Klip River in Gauteng (a. Mercury and b. Arsenic). *mg/l is equivalent to mg/kg (Taylor and Thompson 2008).

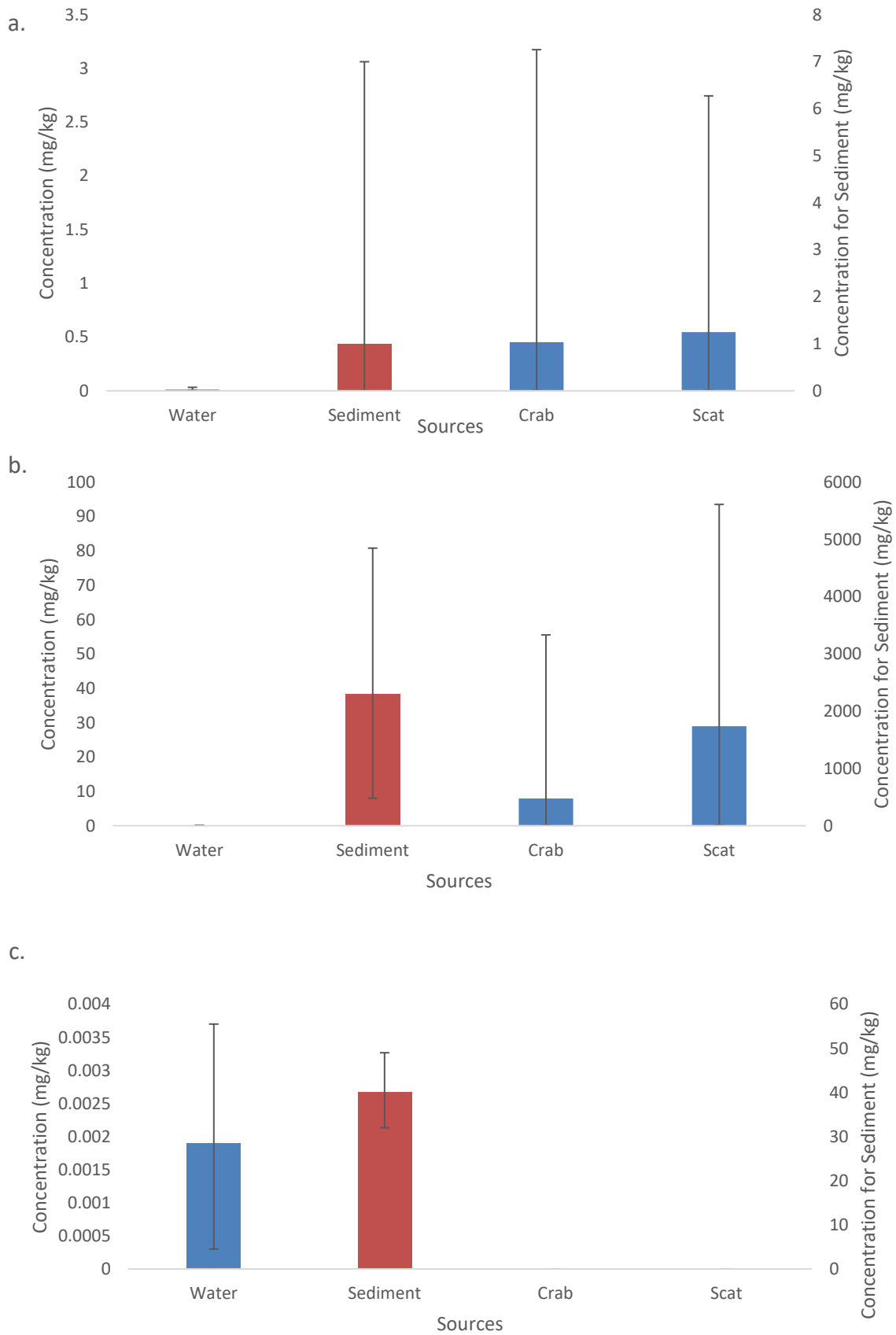


Figure 15. The overall average (max/min) concentrations of the two contaminants analysed through the otter’s food chain along the Hennops and Klip River in Gauteng (a. Lead b. Titanium c. Uranium). *mg/l is equivalent to mg/kg (Taylor and Thompson 2008).

When comparing the four different sources of samples which were collected (water, sediment, crab and scat) the GLM showed that there was a significant difference between the sources ($F_3 = 8.956$, $p < 0.05$). Furthermore, a Fisher post-hoc test showed a significant difference in metal concentrations between sediment and the other three sources ($p < 0.05$).

A regression was done in order to see whether there was a relationship between the different sources for all five of the heavy metals. For mercury two strong regressions were seen, the first (Figure 16) was a strong negative relationship between sediment and scat ($r^2 = 0.512$; $p < 0.05$), and the second (Figure 17) was a strong positive relationship for mercury between the crab and the scat samples ($r^2 = 0.557$; $p < 0.05$). Concentrations for mercury however were found to be marginally higher in the crab than in the scat, suggesting that mercury is most likely being accumulated in the otter.

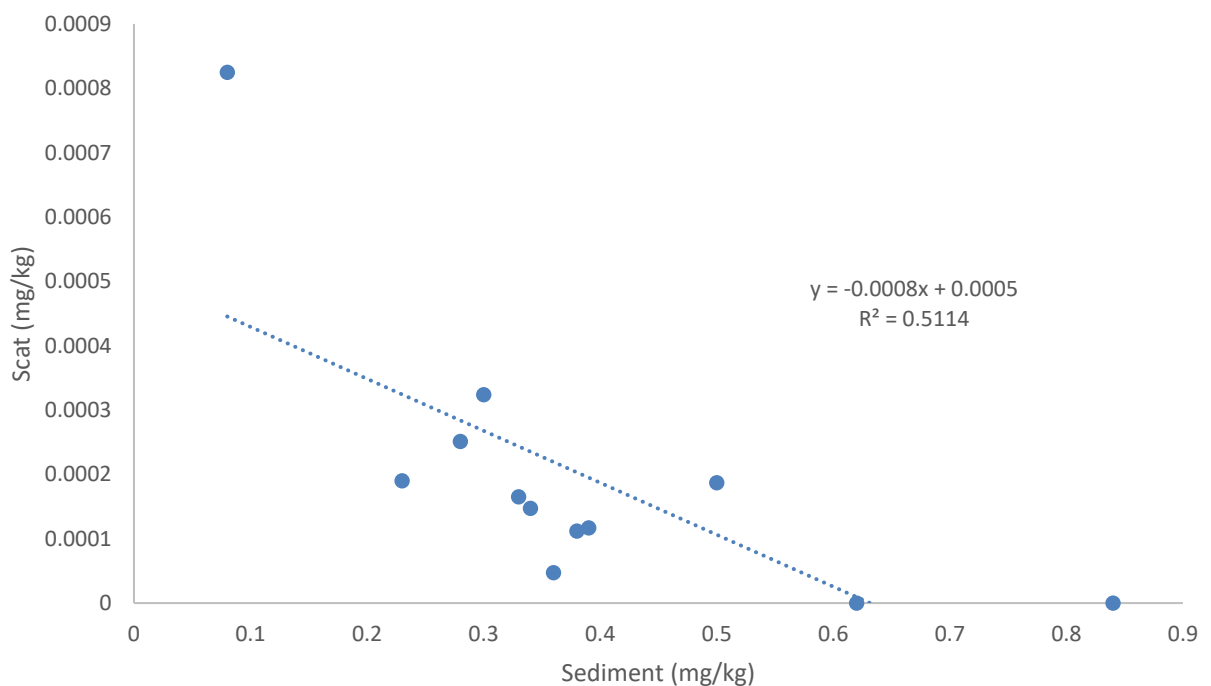


Figure 16. A strong negative regression between sediment and scat samples for Mercury.

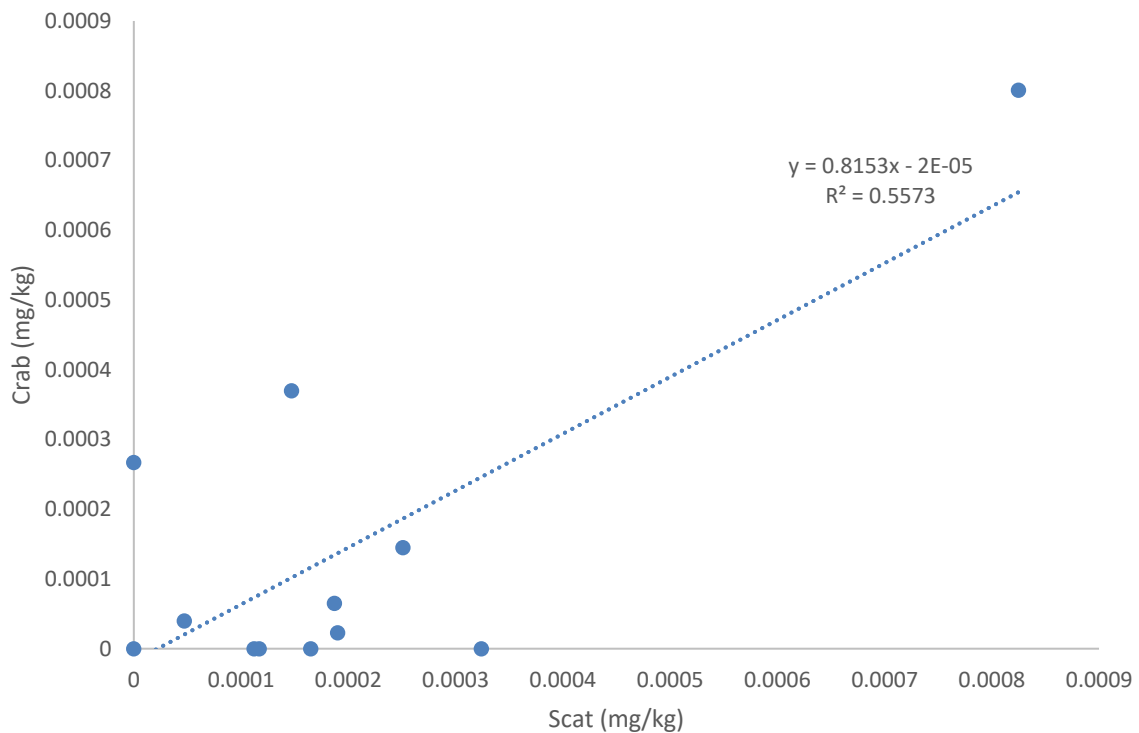


Figure 17. A strong positive regression between scat and crab samples for Mercury.

Arsenic showed no strong regression between the sources. However, there were three tests (sediment vs. scat; sediment vs. water and crab vs. scat) which had a negative regression. Lead also had no strong regressions, however all tests displayed a negative trend. Uranium only had one weak regression between sediment and water. Lastly, titanium had a strong negative regression between sediment and water sources ($R^2 = 0.414$, $p < 0.05$). As the sediment concentration increased, the concentration of titanium in the water decreased (Figure 18).

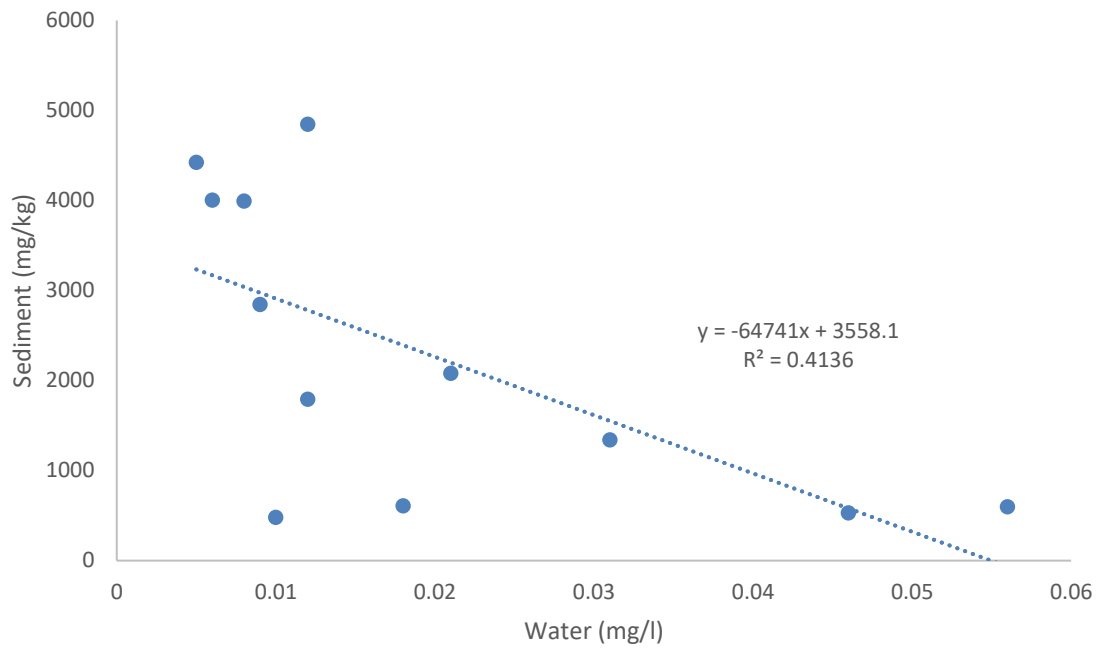


Figure 18. A General Regression model between water and sediment samples for Titanium (Statistica 2016). *mg/l is equivalent to mg/kg (Taylor and Thompson 2008).

4.5. Water quality data from the Department of Water and Sanitation

Over the past 10 years, there were no obvious trends observed for the water quality data for the Gauteng Rivers. Heavy metal concentrations fluctuated continuously throughout this period (Figure 19). Calcium, chlorine and sodium had the highest concentrations (Figure 19a), whereas aluminium and copper had the lowest concentrations (Figure 19b) for the averaged heavy metal concentration for all four rivers analysed. These trends were also seen for each river individually (Appendix E).



Figure 19. The averaged water quality data for all four rivers (Klip River, Hennops River, Blesbokspruit River and the Jukskei River) analysed by the Department of Water and Sanitation over the past 10 years (a. Calcium, Chlorine, Sodium and Magnesium; b. Aluminium and copper).

The average EC ranged between 53-80 mS/m over the 10 year period (Figure 20a). This was the same for each river individually with the exception of the Blesbokspruit River. The Blesbokspruit was the only river to experience higher EC values over a 4 year period (2008-2011). These levels were recorded between 100-140 mS/m. There were no obvious trends for conductivity, as conductivity continued to fluctuate around the same values over the 10 year period.

Since 2009, there has been a steady increase in pH levels within the rivers of Gauteng, reaching pH levels around 8 (Figure 20b). This trend was also seen in the Klip River as well as the Hennops River where increases in pH levels were evident from 2012 onwards (Appendix E). The Blesbokspruit however had the highest pH levels since 2005, with a slight drop during 2008 and 2009. However, those levels have again drastically increased (Appendix E).

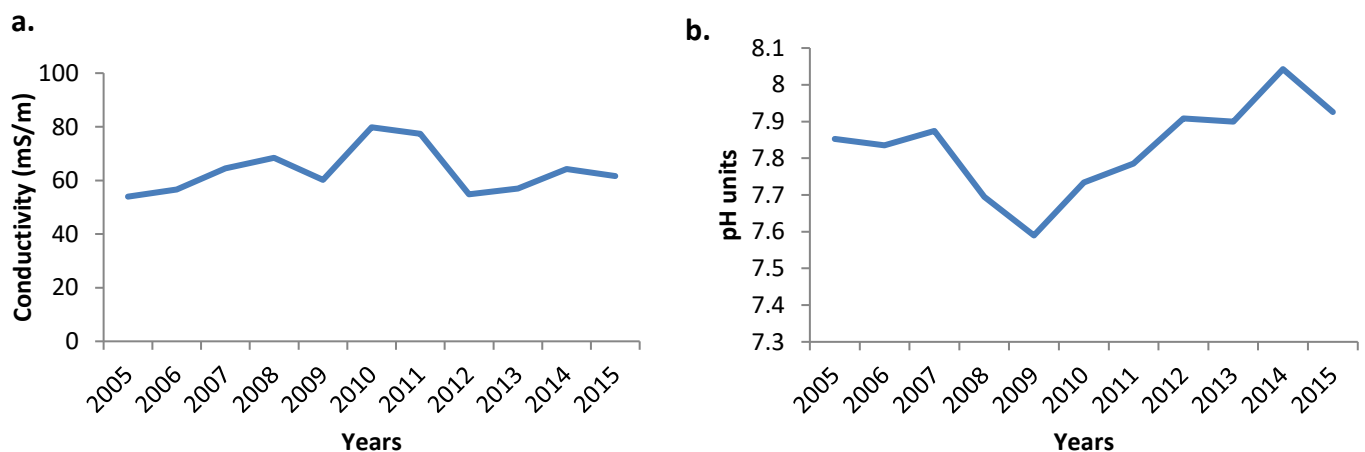


Figure 20. The averaged water quality data for all four rivers (Klip River, Hennops River, Blesbokspruit River and the Jukskei River) analysed by the Department of Water and Sanitation over the past 10 years (a. EC readings and b. pH levels).

Over the past three years the water quality data for the total averaged contaminants from the four rivers analysed show that only two heavy metals, calcium and chlorine, had much higher concentrations than the suggested minimal risk threshold (Table 4). Therefore, it appears that there is a possibility for these two contaminants to bioaccumulate in the otter. Fortunately, there was no increase in these contaminants in the rivers over the past years. However further studies need to be conducted by the Department of Water and Sanitation in order to determine a definite bioaccumulation of these contaminants.

Table 4. The averaged contaminant concentration per year (2013-2015) for the six heavy metals analysed for the Klip River, Hennops River, Blesbokspruit River and the Jukskei River, by the Department of Water and Sanitation, compared to the suggested minimal risk thresholds.

Element	Year	Averaged analysed concentration (mg/l)	Suggested minimal risk threshold (mg/l)	Higher/Lower than suggested
*Al	2013	0.155	1.0	6 x Lower
	2014	0.0045		>100 x Lower
	2015	-		-
*Ca	2013	32.688	3×10^{-8}	>100 x Higher
	2014	40.24		>100 x Higher
	2015	38.89		>100 x Higher
*Cl	2013	44.33	4.0	11 x Higher
	2014	47.19		12 x Higher
	2015	44.61		11 x Higher
*Cu	2013	0.009	0.01	1 x Lower
	2014	0.0013		7 x Lower
	2015	-		-
**Na	2013	44.86	600	13 x Lower
	2014	21.01		29 x Lower
	2015	55.55		11 x Lower
**Mg	2013	16.15	70	4 x Lower
	2014	19.85		4 x Lower
	2015	17.67		4 x Lower

* Agency for Toxic Substances and Disease Registry (2016).

**Department of Water Affairs and Forestry (1996).

In this study we are only able to state whether or not a heavy metal appears to be accumulating within the otters' organs, based on the relationship between the concentration levels taken up to that which is expelled from the otter. In order to determine an estimated level of the heavy metals being accumulated, future studies need to develop statistical predictive relationships (log-log relationships) which will accurately predict the concentrations in the organ using the concentrations found in the scat samples as seen in a study conducted by Zwiernik *et al.* (2008).

When comparing the mean contaminant concentrations of each river, no clear trends were seen. There was an increase in the calcium levels in the Klip River (Figure 21), with a 12 mg/l increase from 2013-2015. In the Hennops River (Figure 22), there were no records obtained for aluminium and copper. During 2014, there was a spike for all contaminants analysed, however concentrations decreased back to their stable levels in 2015. The Blesbokspruit River concentration levels for chlorine and sodium decreased by 18 mg/l and 29 mg/l respectively, whereas calcium and magnesium increased by 17 mg/l and 9 mg/l respectively (Figure 23). Overall, the Jukskei River had the lowest contaminant concentrations compared to the other rivers in Gauteng (Figure 24).

The EC values of all four rivers were below the World Health Organisation (WHO 2016) salinity limits for water quality which is recorded at 156 mS/m. Figure 24a shows a gradual increase of 12 ms/m in the EC readings in the Klip River between 2013 and 2015. The EC values were constant in the Hennops River over the three year period, as it reached its highest level at 69.60 ms/m in the Hennops River during 2014 (Table 5). Overall, the EC in the Blesbokspruit showed a slight decrease of 4 mS/m over the three years (Table 5). Lastly, the Jukskei River was the only river to have the EC readings within the specified range (Table 5).

In all four rivers the pH levels remained between 7 and 8 throughout the three years. However, records showed that the readings were closer to pH 8 (Table 5).

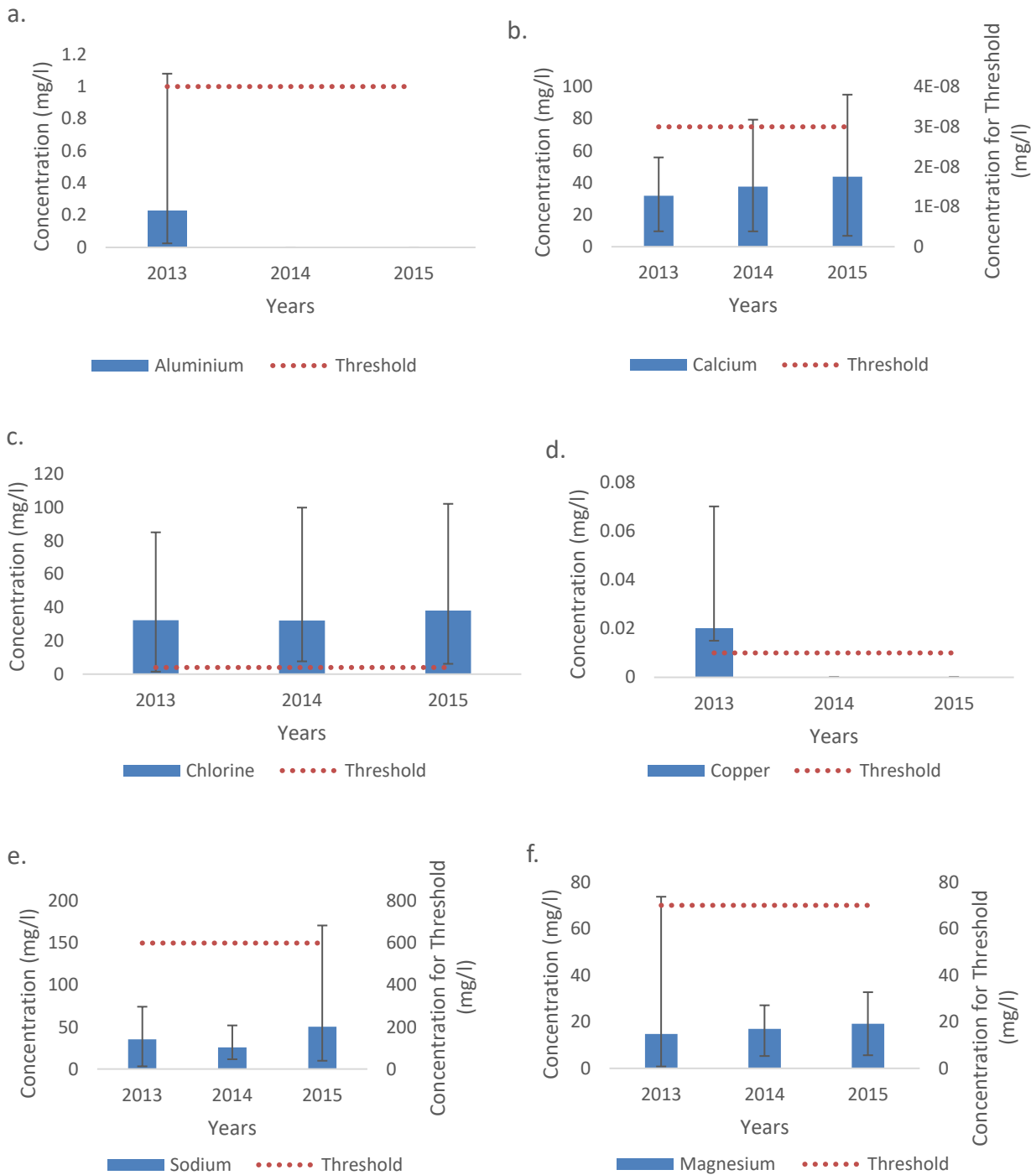


Figure 21. The mean (min/max) concentrations for the six heavy metals analysed in the Klip River, obtained from the Department of Water and Sanitation. Where the dotted line represents the suggested minimum risk threshold suggested by WHO (a. aluminium, b. calcium, c. chlorine, d. copper, e. sodium, and f. magnesium).

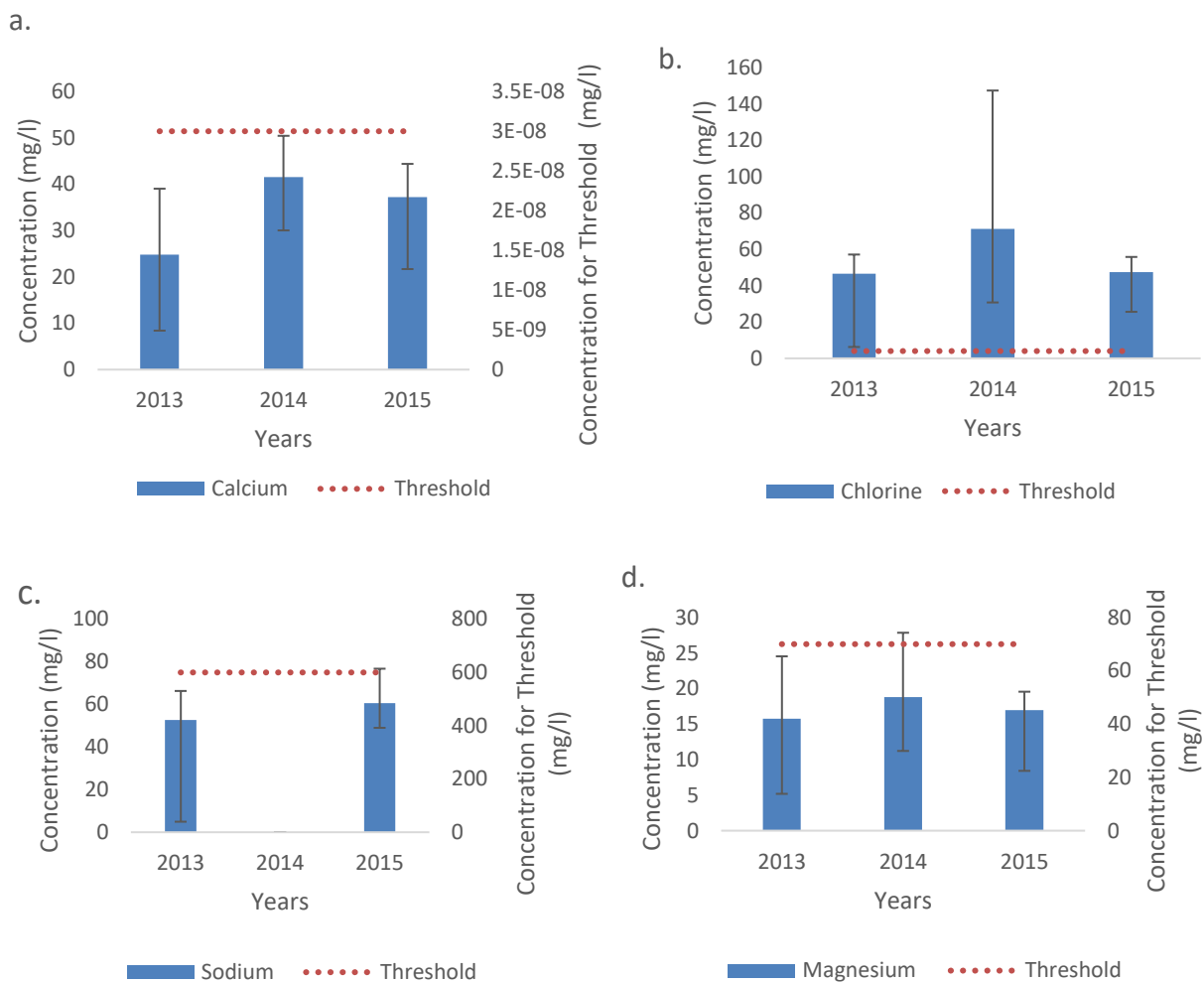


Figure 22. The mean (min/max) concentration for the six heavy metals analysed in the Hennops River, obtained from the Department of Water and Sanitation. Where the dotted line represents the suggested minimum risk threshold suggested by WHO (a. calcium, b. chlorine, c. sodium, and d. magnesium), there were no records for aluminium and copper for the Hennops River.

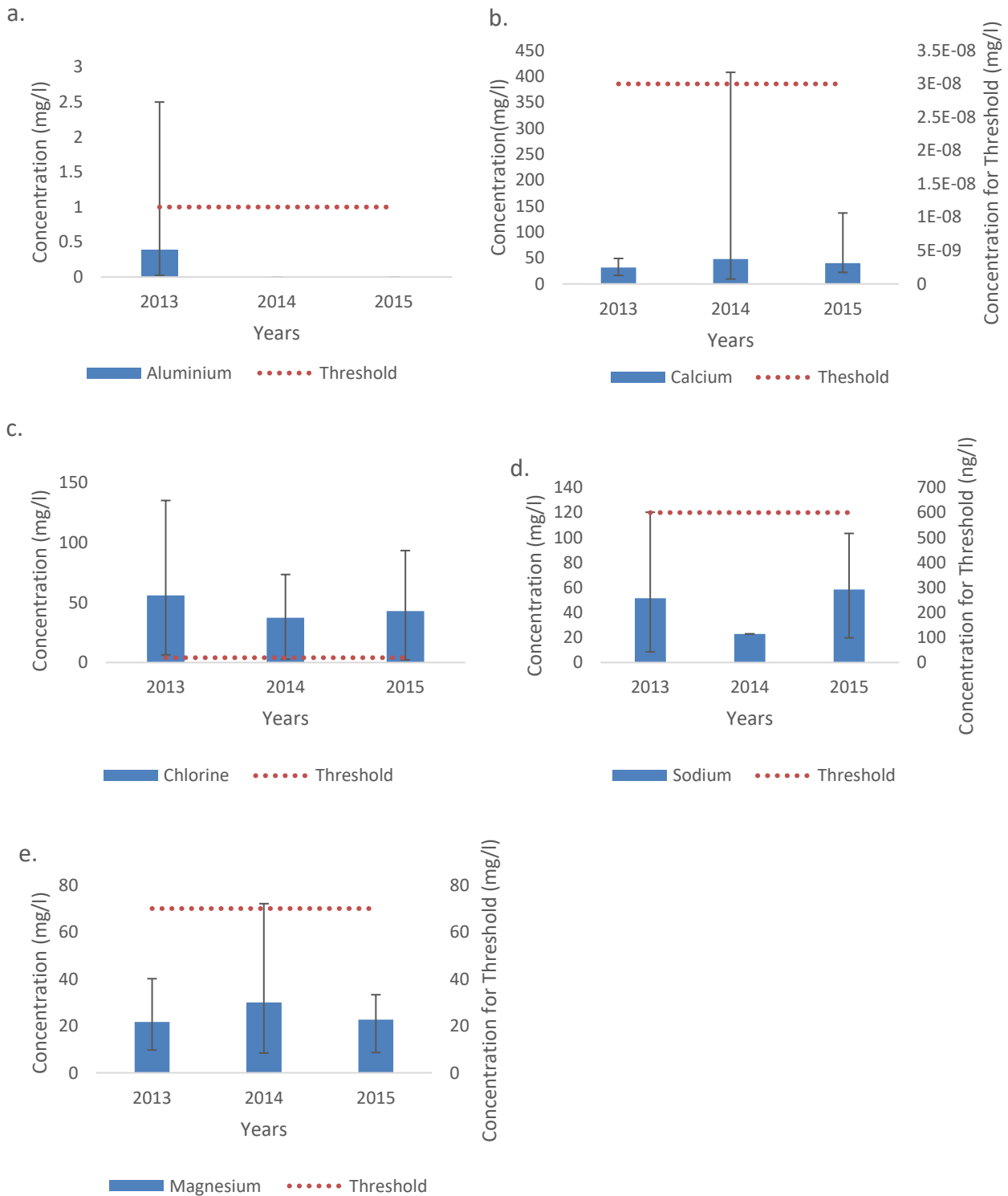


Figure 23. The mean (min/max) concentration for the six heavy metals analysed in the Blesbokspruit River, obtained from the Department of Water and Sanitation. Where the dotted line represents the suggested minimum risk threshold suggested by WHO (a. aluminium, b. calcium, c. chlorine, d. sodium, and e. magnesium).

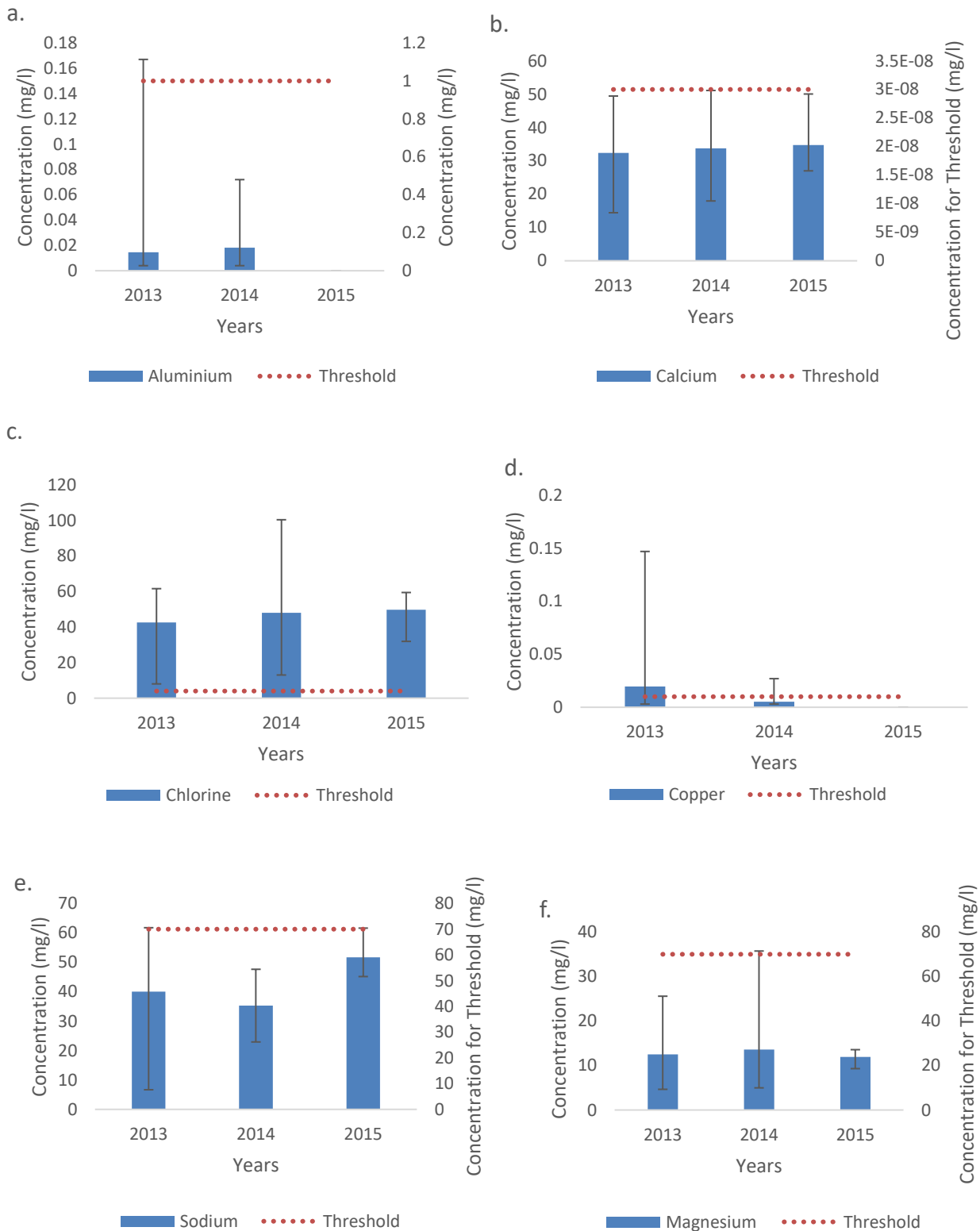


Figure 24. The mean (min/max) concentrations for the six heavy metals analysed in the Jukskei River, obtained from the Department of Water and Sanitation. Where the dotted line represents the suggested minimum risk threshold suggested by WHO (a. aluminium, b. calcium, c. chlorine, d. copper, e. sodium, and f. magnesium).

Table 5. The mean (min/max) concentrations of the EC and pH water quality measurements over the years 2013 – 2015 for the Klip River, Hennops River, Blesbokspruit River and the Jukskei River, obtained from the Department of Water and Sanitation.

River	Years	EC (mS/m)	pH
Klip River	2013	45.17 (5.0/71.0)	7.8 (6.2/8.7)
	2014	49.49 (15.75/88.1)	8.2 (7.3/8.9)
	2015	57.24 (2.82/99.6)	8.0 (5.9/8.8)
Hennops River	2013	59.68 (13.89/71.1)	7.9 (7.2/8.5)
	2014	69.60 (40.7/104.6)	8.1 (7.6/8.6)
	2015	60.89 (33.48/71.2)	8.0 (7.1/8.6)
Blesbokspruit River	2013	64.86 (12.58/119)	7.9 (7.0/9.4)
	2014	57.47 (19.84/91.5)	8.4 (7.8/8.8)
	2015	60.25 (33.5/82.6)	8.3 (7.6/8.7)
Jukskei River	2013	48.60 (15.46/68.1)	8.0 (6.8/8.5)
	2014	54.41 (20.37/137.38)	7.9 (7.0/8.6)
	2015	53.70 (34.6/61.6)	7.9 (7.0/8.3)

CHAPTER 5: RISK ASSESSMENT

Human and mammal illnesses associated with exposure to heavy metals have been extensively reported worldwide, specifically in more developed countries (Gheorghe *et al.* 2017). Water pollution is a worldwide problem which needs continuous monitoring and the implementation of solutions and conservation policies. In the aquatic environment, heavy metals are the most frequent detected pollutants (Gheorghe *et al.* 2017). The identification of their effects is essential to protect the ecosystems integrity as well as mammal health. The main concern for contaminated water resources is the health threat to all living organisms relying on these resources (Abdu-Kareem *et al.* 2011). Contamination of natural waters with heavy metals is increasing, most of these heavy metals are natural and essential constituents biologically as well as to the aquatic environment when present at low concentrations (Abdul-Kareem *et al.* 2011). However, some heavy metals become toxic when they exceed the threshold concentrations (Abdul-Kareem *et al.* 2011), leading to poisoning and in some cases to death. These toxic effects can impact on an individual's growth, metabolism or reproduction which eventually results in mortality of the individual, leading to serious consequences to the entire trophic chain and mainly to the top level feeders such as the otter (Abdul-Kareem *et al.* 2011; Gheorghe *et al.* 2017).

Terrestrial and aquatic food chains are susceptible to accumulating environmental contaminants which reach toxic concentrations (Clarkson 1995). Focus on the aquatic ecosystem is essential as there is less control of the accumulation of the contaminants into the food chain (Clarkson 1995). Even though the contaminant concentration levels are not significantly increasing, according to the data analysed from the Department of Water and Sanitation, the present concentrations of the contaminants in Gauteng rivers are high and still pose a threat to the species within the aquatic ecosystems. However, this does not suggest that no action plan should be set in place regarding the state of the Gauteng rivers. Instead this should be the starting point to improve the contamination levels within these ecosystems allowing the environment to enhance the quality of life for these species to thrive in these regions. The present condition of the water quality in the rivers of Gauteng with high heavy metal concentrations in the aquatic ecosystem indicates poor environmental health. This increases the potential risk of accumulation of these metals in aquatic mammals (Gutleb 2000). The position of

the individual in the food chain also determines the risk of heavy metal exposure as seen in a study by Gutleb (2000) showing that fish-eating mammals tend to accumulate high mercury concentrations from contaminated fish sources. Studies conducted on indicator species in highly contaminated water confirms this risk to mammals, showing that species are affected based on how the indicator species reacts to the high levels of contaminants (Ates *et al.* 2013). Thus, through this study it is seen that due to the increase in contaminant concentrations in the main food sources of the otter, there is a greater potential for bioaccumulation of these contaminants through the food chain, which in turn may have detrimental effects to the health of these individuals. This risk assessment aims to determine if the bioaccumulation of the contaminants analysed in this study is accumulating in the otter as well as the resulting health effects posed to the individual due to these accumulating contaminants.

5.1. Physical variables can increase risk

In metal monitoring, pH is one of the most important parameters to analyse, as chemical speciation and thus the bioavailability of many metals are greatly affected by this parameter (Meador 1991). In turn conductivity influences the toxicity of the metals due to the changes in the dissolved salt levels in the water and temperature affects the solubility of the metals and therefore the organism's tolerance levels and ability to take up these toxic contaminants. In this study the rivers tested were on average at a pH of 7.2, conductivity was low (the Klip River had an EC reading of 63.35 mS/m and the Hennops River had a reading of 55.25 mS/m) and temperature of both rivers had an average of 18.3 °C (where the Klip and Hennops rivers had temperatures of 16.5°C and 20.1°C respectively).

The pH levels from the Hennops and Klip rivers did not show any apparent trends, with average pH around 7.4 and 7.1 respectively. These pH levels fall within the preferred limits for freshwater rivers. The natural pH range is 6 – 8, where 8 is a high alkaline state at which some metals are toxic (Morrison *et al.* 2001). Other metals are insoluble in neutral/basic pH (pH 7 or above) waters, which makes them more difficult to detect in aquatic systems. Therefore, in some cases one may significantly underestimate the total metal concentration in the water (Lenntech 2016).

The electrical conductivity of a water body provides a good indication of the total dissolved salts in the water. Total dissolved salts can influence the organisms either directly due to sudden changes in salt levels, or indirectly as it influences the metal toxicity in the water. With lower conductivity levels due to less salts available for the metals to bind, an increase in toxicity levels can be detected in the waters (Sanders 1997).

Temperature is another important parameter in the assessment of metal bioavailability in an aquatic system. An increase in temperature can result in an increase in the metal uptake by the organisms, suggesting that certain metal concentrations may be more toxic to organisms during the summer seasons and may reach the organisms' tolerance limit (Sanders 1997; Buchwalter *et al.* 2003). The ideal temperature for freshwater rivers is 25°C (Lenntech 2016). Increased water temperatures do not only increase the solubility of toxic compounds but can also influence the tolerance levels of aquatic organisms to these toxic compounds (Bhadja and Vaghela 2013). At temperatures higher than 25°C, an organism's metabolic rate, tissue permeability as well as their oxygen consumption, is seen to increase compared to that seen at lower temperatures (Bhadja and Vaghela 2013).

The combination of physical variable parameters seen in the two rivers studied were neutral pH, low conductivity and a low temperature. While the risk of some heavy metals dissolving in low temperature and low conductivity waters is low, their toxicity may well be high based on the neutral pH levels. Both rivers analysed fall below the ideal temperature as the average temperature of the two rivers range between 16.5°C to 20.1 °C. The temperature of the water greatly affects the salinity of the water (Oyem *et al.* 2014), as salts are seen to be more soluble at higher temperatures. Warmer waters can dissolve salts and other minerals more easily than that seen in colder waters (Oyem *et al.* 2014). The temperature of the water varies throughout the year resulting in different effects to the toxicity level of the contaminants during different seasons (Kuzmanovic *et al.* 2017). In this study, temperature would not have a significant contribution to the increased potential for heavy metals to accumulate, as the temperature was low, reducing the solubility of metals in the water. At lower temperatures, minerals and salts are not as easily dissolved as in warmer water, and the ionic concentration is lower. Temperature would however have a more significant during the warmer seasons of the year, as the toxicity of the contaminants

will increase (Kuzmanovic *et al.* 2017). Therefore, lower water temperatures reduce the conductivity of the water (Buchwalter *et al.* 2003), which is what was evident in this study.

The average conductivity of all sites analysed was 59.3 mS/m, which is far below the expected EC values for freshwater rivers which is 156 mS/m (Morrison *et al.* 2001). The slight decreases along the river may be explained by a lower conductivity of an incoming source being either rain water or runoff, resulting in a dilution effect (Sanders 1997). These incoming water sources would be in contact with the land's surface for less time and would therefore have a reduced time to dissolve materials from the surrounding land flowing into the river. Slight increases in the conductivity levels along the river may be a result of reduced inflow and evaporation of water from the river. This leads to an increase in the concentration of dissolved salts per unit volume (Sanders 1997). Organisms inhabiting the Klip River as well as the Hennops River could have been affected during either the increases or decreases, since organisms are more vulnerable to the sudden changes in conductivity of the water (Morrison *et al.* 2001).

The pH levels have different effects on each of the metals studied. The solubility of lead, for example, decreases with an increase in pH (see Kim *et al.* 2011). Therefore, in my study, the current risk of lead contamination is low, as the pH levels remained around 7 (neutral) for the duration of the study period, this is supported by the low levels of lead detected in the water (more than 100 times lower than the minimum risk threshold level). However, mercury and titanium concentrations were measured to be high in the water samples for both the Klip and Hennops rivers and above recommended threshold limits. Literature shows that large increases in mercury uptake by aquatic organisms are evident with small changes in pH levels (7.3 - 6.3), suggesting that mercury may be bioavailable at neutral levels as found in this study (Kim *et al.* 2011; Kelly *et al.* 2003).

In the case of titanium, it was suggested by Avramescu *et al.* (2017) that titanium in the form of titanium dioxide (TiO₂), is most soluble at low pH levels. The dissolved concentrations of titanium are generally seen to decrease with an increase in salinity of the water (Skocaj *et al.* 2011). According to Skocaj *et al.* (2011), TiO₂ may differ significantly over a wide range of pH values (3.5 to 8; Skocaj *et al.* 2011). An associated increased rate of bioaccumulation was seen to be directly proportional to the concentrations of hydrogen

ions present for both mercury as well as titanium in the aquatic systems (see Kelly *et al.* 2003). With low water temperatures there is a decrease in the potential for these metals to accumulate. However, even though the conductivity was low, metals are sensitive to slight changes and thus mercury and titanium may change in toxicity according to the conductivity changes in the rivers. It appears that the data in this study support the fact that pH and EC may play a key role in contributing to the increased risk of titanium accumulation.

The current concentration levels for arsenic and uranium contamination in the water samples are low and below threshold levels. Wei *et al.* (2016) found that the maximum absorption for arsenic in form of arsenite (As (III)) and arsenate (As (V)) is seen between pH levels of 7 - 8 and 4 - 5 respectively. In the current study arsenic concentrations were seen to be low in the water samples, however the pH measured falls within the range of absorption for arsenic. Therefore, depending on the form in which arsenic is present as well as the low temperature and conductivity, there is currently a high risk of toxicity for arsenic at pH neutral levels.

Literature shows that uranium is greatly influenced by pH levels in the water in terms of absorption and uptake rate in aquatic organisms (Park *et al.* 2007). In my study uranium was measured in low concentrations and there is an increase in uranium solubility in waters with a pH range of 6 – 8 (see Brunskill and Wilkinson 1987). Therefore, there is currently a high risk of bioaccumulation as pH levels are neutral in this study and fall within this pH range for uranium solubility. Despite this, low concentrations of uranium were found, possibly as a result of the influences of low temperature and the conductivity of the water. The concentrations of uranium will be expected to further increase with the increase in temperature and conductivity levels.

While lead is currently at low risk of uptake with regards to pH and mercury, titanium, arsenic and uranium are at high risk for the uptake and bioavailability of heavy metals to aquatic organisms, all three physical parameters determine the overall risk and bioavailability of each metal. The EC was recorded at low levels, which increases the toxicity of each metal. Therefore, the overall level of risk in this study will increase from what was seen in pH levels and may result in all five contaminants being at higher risk levels for aquatic organisms according to the physical variable of the rivers.

5.2. Current risks to otters

In the analysis of water and sediment contamination, it is important to assess the risk due to the accumulation of contaminants within the sediment (Sanders 1997), as these can affect animals either directly or indirectly. In order to determine the bioaccumulation of contaminants through the food chain of the otter, indirect sampling techniques were conducted by analysing crab and scat samples. Zwiernik *et al.* (2008) conducted a study to determine if scat could be used to predict the contaminant concentrations in the liver and adipose tissues of mink species based on their diet. It was found that contaminant concentrations in scat and the liver and adipose tissues are significantly correlated. Indicating that it is possible to use the non-invasive scat analysis to determine the metal concentrations which have accumulated through the otter and thus resulting in several health effects (see Mason and MacDonald 1987; Elliott and Wilson 2003; Zwiernik *et al.* 2008).

The water samples for both rivers had little to no mercury, as mercury was only recorded at one site along the Klip River which was in the urban area and was recorded at a level 15 times higher than the minimum risk threshold concentration. Water samples also had high levels of lead and titanium however lead levels were lower than the minimum threshold levels. In the sediment, there were high levels of titanium and mercury, and unlike the water samples, very little lead was present in the sediment. Contrary to what was expected, arsenic and mercury were the two heavy metals which had higher concentrations in the food sources than in scat, suggesting that these two heavy metals have the potential to accumulate within the otters' body. Lead, titanium, and uranium were the other heavy metals for which concentrations in scat were higher than in the crab, suggesting that the otters are eliminating most of these heavy metals.

In Gauteng the main sources of pollution for the three heavy metals of concern in the water and sediment samples (lead, mercury and titanium) are by means of industrial waste, mining, urban runoff, sewage and other human wastes (Goving and Madhuri 2014; Lenntech 2016). In South Africa these activities are not seen to decrease significantly in the near future, which may result in increased heavy metal concentrations if these present levels in these ecosystems are not managed or treated (Rand Water 2017). In South Africa

sewage waters are often not treated properly before they are released back into the rivers, thus increasing the levels of the pollutants in the rivers (Musingafi and Tom 2014). In turn, an important titanium contributor could be urban runoff into the rivers as industrial activity contributes titanium and other hazardous contaminants through discharge into the sewer, rivers or wetlands (Buettner and Valentine 2011; Lenntech 2016). Titanium is mined as ilmenite which is a titanium oxide ore, during the mining process titanium dioxide dust can also settle into the rivers, resulting in higher titanium levels (Utembe *et al.* 2015).

Lead is very rare in nature (Lenntech 2016), thus potentially explaining the low concentrations of lead found in the sediment. Most of the lead concentrations present in the environment is a result from anthropogenic activities (Lenntech 2016) which explains the high levels of lead measured in the water samples. The chemical properties of lead and how it binds with water and sediment to make it bioavailable for organisms, may further explain the low lead concentration in the sediment. There are two possible explanations to support this. Firstly, lead is protected by a thin layer of lead oxide which makes it less available for aquatic organisms to take up, lowering the risk that lead may accumulate to top predators from the sediment (Sanders 1997). Secondly, lead may be present in a form that dissolves easily in water and therefore lead would not bind to sediment. In this study due to the literature as well as the physical variables discussed above, the overall risk of bioaccumulation of lead is low in both the water as well as the sediment samples. Lead may result in toxic effects to mammals which may cause severe health effects such as the synthesis of haemoglobin, effects on the kidneys, gastrointestinal tract, joints and the reproductive system as well as causing acute or chronic nerve damage depending on the lead levels and the length of exposure (Govind and Madhuri 2014). Lead is also found in compounds with other heavy metals which are generally toxic (Lenntech 2016). Even though in this study it appears that lead has a low risk of accumulation due to the physical variable of the environment, its effects on animals should not be discounted.

In contrast to lead, titanium and mercury concentrations were present at high levels in sediment. The main explanation to why this is evident for mercury may be due to the turbulence fluctuations in an aquatic system (Roberts and Webster 2002). The samples were taken during the winter season, during which the rain subsides, therefore lowering the turbulence of the rivers in South Africa. This decrease in the movement of the water

reduces the amount of mixing of contaminants that occurs between the water column and the sediment (Hosseini *et al.* 2015). This allows the contaminant particles to settle on the river bed, reducing their concentration in the water, which is confirmed by the low levels of mercury found in the water as mercury was only recorded at one site during the water analysis. However, these contaminants cannot be ruled out as a potential risk to species because the risk will increase again during the summer season (Hossen *et al.* 2015). South Africa experiences high rainfall during the summer season, resulting in an increase in the turbulence experienced within the river system. The increase in turbulence will cause mixing of the contaminants at the bottom of the riverbed which are then absorbed back into the water column, allowing these contaminants to be bioavailable (Hosseini *et al.* 2015).

Mercury is present in high concentration levels which far exceeds the minimum thresholds in water and low concentration levels in the scat analysis, suggesting that it is bioavailable and possibly accumulating in both the crab and otter. Mercury is known to be a very toxic heavy metal as well as being exceedingly bioaccumulative (see Jaishankar *et al.* 2014). Mercury is transformed in the aquatic ecosystem and is known as methylmercury which has the ability to undergo biomagnification, causing significant disturbance to aquatic lives (Jovanovic 2014; Parsa *et al.* 2014). It is mostly consumed through contaminated aquatic organisms such as aquatic invertebrates, fish or crabs (Trasande *et al.* 2005; Jaishankar *et al.* 2014). Therefore, the fact that mercury was seen to have high concentration levels in the sediment samples analysed poses a great risk to the otters as the organisms at the bottom of their food chain feed on invertebrates living in the sediment of the river (see Snyman *et al.* 2002; Parsa *et al.* 2014). A strong negative relationship was found between sediment and scat in this study (see Chapter 4). The findings in this study concur with Gutleb's (2000) assessment that fish and crab eating mammals tend to accumulate higher mercury concentrations than meat-eating mammals. Therefore, there is great potential for the bioaccumulation of mercury through the food chain of aquatic animals, leading to health effects. It was further found that the relationship between crab and scat has a strong positive regression, however the analysis of the concentrations show that mercury is lower in the scat suggesting that mercury concentrations are potentially starting to accumulate in the otter. The possible accumulation and long term exposure of mercury in the otter presents several health risks, these include reproduction,

neurochemical receptors, neurotoxicity, the central and peripheral nervous system, liver and kidney failure, tumours, sensory impairments and ultimately death (Wiener *et al.* 2003; Scheuhammer *et al.* 2015). Mercury poisoning is considered a risk factor for the survival of the otter as the primary targets for toxicity are the central and peripheral nervous system, as the methylmercury is mainly absorbed through the diet of an individual and can readily pass the blood-brain barrier (Aschner and Aschner 1990; Scheuhammer *et al.* 2015).

Titanium is seen to be at high concentrations in the water as well as in the sediment for the rivers in Gauteng. Titanium concentrations in the water samples, were 100 times higher than the minimum risk threshold levels, resulting in high risk to the mammals in the aquatic ecosystem. This suggests that titanium has the potential to biomagnify as well as bioaccumulate through an animal's food chain and thus potentially negatively affect the health of top consumers (Snyman *et al.* 2002; Jovanovic 2014; Parsa *et al.* 2014). The heavy metals in the sediment may not directly affect the mammals within the aquatic ecosystems, but instead an indirect effect, where the contaminants from the sediment bioaccumulate through the food chain to aquatic organisms at higher trophic levels (Parsa *et al.* 2014). Small organisms such as invertebrates or small crabs are able to take up contaminants such as heavy metals directly from the sediment (Snyman *et al.* 2002). Aquatic invertebrates, which are a main food source for fish, are known to accumulate heavy metals (Snyman *et al.* 2002; Parsa *et al.* 2014). Therefore, according to these findings and the corresponding literature, the bioaccumulation of titanium at higher trophic levels may result in health impacts for the otter (Jovanovic 2014; Parsa *et al.* 2014). The high titanium concentrations in the sediment could be explained by the titanium present in the bedrock of the streams in Gauteng. Titanium is held in minerals such as rutile, ilmenite and shene (Jovanovic 2014). These are relatively insoluble, thus, the concentrations of titanium present in these minerals will remain in the sediment rather than dissolve into the water (Jovanovic 2014). In this case the titanium concentrations are most likely due to natural factors. The concentration of titanium in the water depends on the amount that has been dissolved and the quality of the titanium particles which are dispersed in the water (Liu *et al.* 2013).

The crab and scat analysis revealed that the titanium concentration levels are found at higher levels in the scat than in the crab, suggesting that the titanium is being eliminated by the otter and thus not accumulating. It has been shown that titanium dioxide (TiO₂) is available (see Bell *et al.* 2001; Binning and Baird 2001; Pone *et al.* 2007) and has the potential to be absorbed as well as stored mainly in the liver, spleen and kidney in mammals after ingestion (Jovanovic 2014). In a review of the toxicological effects of titanium dioxide, Iavicoli *et al.* (2012) proposed that the respiratory tract is considered to be one of the most affected systems, inhalation of the TiO₂ nanoparticles may translocate to the central nervous system (CNS) via the axons of the sensory neurons within the respiratory tract (Iavicoli *et al.* 2012). This is a vital aspect for the effects of TiO₂ as this then introduces a direct route for the nanoparticles to link to the CNS, which allows the nanoparticles to cause toxic effects (Iavicoli *et al.* 2012). This link to an individual's brain was reported to induce a number of changes in the release and metabolism of neurotransmitters (Hu *et al.* 2010). As seen in the study conducted by Jovanovic (2014), there is substantial evidence to suggest that titanium dioxide can be absorbed and passed through the mammalian gastrointestinal tract, as well as bioaccumulate within tissues of mammals and other vertebrates. In addition, TiO₂ does interact with other elements but this knowledge is limited. A review conducted by Liu *et al.* (2013) focused on the combined effects or toxicities of the interaction of TiO₂ with metals such as lead, arsenic, copper and cadmium in solution. This interaction may result in the increased toxicity to aquatic organisms (Liu *et al.* 2013).

Arsenic is one of the most important elements causing concern for both ecological and individual health (Hughes *et al.* 1988; Jaishankar *et al.* 2014). Unlike mercury, arsenic was seen to have low concentration in the water samples and levels fell just under the minimum risk threshold levels. However, the presence of high concentrations in the crab and low concentration levels in the scat suggests that arsenic is bioavailable and accumulating in both the crab and otter as very little is being eliminated through the otter. Arsenic is extensively available in forms of sulphides, oxides or as salt of iron, sodium, calcium and copper (Singh *et al.* 2007). Therefore, arsenic does not only have health effects as its own individual element but is also able to have an impact on individuals, as it combines with other elements in the aquatic system. The most toxic compounds of arsenic are arsenite and arsenate which are lethal to the environment and living creatures (Jaishankar *et al.* 2014). It

has been reported that the major source of arsenic accumulation in aquatic mammals is through their diet (Kubota *et al.* 2001). Kubota *et al.* (2001) found that otters feeding on crabs had higher arsenic concentrations than those feeding primarily on fish. This study therefore supports this statement as seen by the potential arsenic accumulation through the otters' diet.

It was unexpected that uranium, which was present in both water and sediment, was not found in the crab or scat samples. This could be due to uranium not meeting the requirements for bioavailability. The bioavailability of uranium depends greatly on its speciation and physiochemical form (Markich 2002). In the aquatic ecosystem uranium appears in a variety of different forms, including free metal ions and complexes with inorganic ligands (i.e. uranyl carbonate or uranyl phosphate) (Markich 2002). The free ion activity model (Campbell 1995), suggests that metal toxicity in the aquatic system is not only represented by the total metal concentration but also by the concentration of free ions available for bonding. Depending on the pH levels and the presence of the different ligands, this model suggests that uranium is not bioavailable (Fortin *et al.* 2004). Studies have also shown that, although uranium is able to bioaccumulate in aquatic organisms, it does not biomagnify due to its low assimilation efficiency (Simon and Garnier-Laplace 2005). Simon and Garnier-Laplace (2005) suggest that the trophic transfer rates of uranium are low.

There is a great risk of bioaccumulation due to the accumulation of contaminant within the sediments of the Gauteng rivers. In sediment it is thought that only a small amount of total metal contaminants is available for bioaccumulation by organisms (Salomons and Forstner 1984; Sanders 1997). The bioavailability of these metals in the sediment depends on the metal speciation as well as the physiochemical characteristics of the sediment (Sanders 1997; Zimmerman and Weindorf 2010). Although many organisms may not be able to take up the contaminants from the sediment, it does serve as a source of metals for invertebrates and other organisms in lower trophic levels within the aquatic ecosystem. This then leads to the bioaccumulation from lower trophic levels through to the top feeding individuals (Jovanovic 2014; Parsa *et al.* 2014). Therefore, there is a growing concern for the otters being top level feeders, as smaller organisms living in the sediment will be taken up by the crustaceans in the rivers. These crustaceans are then eaten by the otter, resulting in accumulation of these contaminants within the otter. Even though only two contaminants

were seen to have the potential to accumulate in the otter, it is possible that the combination of all five contaminants being exposed to the otter may result in acute health concerns, therefore the otters' exposure to the different contaminants cannot be ruled out (Lenntech 2016).

5.3. Additional risks based on water quality data from the Department of water and sanitation

The water quality data from the Department of Water and Sanitation were obtained in order to determine any possible trends for the contaminants in the Gauteng rivers. The data obtained were analysed for trends over the past 3 years (2013-2015), which was the same period in which this study was conducted, as well as over the past 10 years (2005-2015). No clear trends were seen for the different contaminants over the past three years.

Over the three year study period calcium and chlorine displayed the highest concentrations for all the rivers analysed in Gauteng. These two contaminants had concentrations which far exceeded the suggested minimum risk threshold stated by the World Health Organisation. Once again, in the analysis of water quality, it is important to measure the physical variables (pH, conductivity and temperature) of the water samples from the rivers being analysed. It was seen from the water samples tested in this study that these two rivers in Gauteng have an average pH of 7.2, low conductivity and an average temperature of 18.3 °C, which is considered lower than the average normal river water temperature of 25 °C. In water quality analysis, pH is one of the most important parameters in order to determine the bioavailability of any contaminant within the river (Meador 1991).

Calcium is known to naturally appear in water as it occurs from rocks such as limestone, marble, calcite, dolomite, gypsum, fluorite and apatite (Lenntech 2016) which could be an explanation for the high levels seen in the rivers. Calcium is responsible for water hardness as it is found in water in the form of Ca^{2+} ions and may negatively influence the toxicity of other compounds. Copper, lead and zinc are far more toxic in soft water. Aquatic organisms are influenced by water hardness regarding contaminant toxicity. In soft water the

permeability of a fish's gills is increased, whereas in hard water, the presence of calcium better protects the fish from direct contaminant uptake (Lenntech 2016). According to the physical parameters analysed in this study, the Gauteng rivers studied have soft water. This is a result from the low pH levels in the water samples as well as the average conductivity being low (59.3 mS/m). Thus, contaminants will be more toxic to the aquatic individuals. As shown in the literature the uptake of these toxic contaminants by fish and other organisms will increase thus bioaccumulating through the food chain to top feeding individuals.

Chlorine is also a naturally occurring element. Chlorine enters the body by inhaling contaminated air or by consuming contaminated food or water. The effects of the chlorine taken in depend on a number of things; the amount the individual is exposed to, the length and frequency of exposure as well as the individual and the environments health when exposure occurred (Lenntech 2016). Once chlorine is in the air or in water, it reacts with other chemicals, it combines with inorganic materials to form chlorine salts, as well as combining with organic materials in water to form chlorinated organic chemicals (Lenntech 2016). In the making of organochlorines, dioxins are produced (Reindl *et. al.* 2015). Dioxins from organochlorines are extremely potent as the dioxin masquerades as a hormone in the cells of the individual. Dioxins influence the DNA to produce enzymes and other hormones which control growth and cell division, as well as causing birth implications and cancer (Reindl *et. al.* 2015). These effects of organochlorines along with the physical parameters and other pollutants taken in by the otter the combination of water quality and presence of contaminant exposure are suggested to be the main reason for the decline in the otter species (see Roos 2013). Even though the contaminant concentration levels for the three year period are not increasing significantly, the present concentrations of the contaminants are high and still pose a threat to the otter species within the aquatic ecosystems.

CHAPTER 6: DISCUSSION

Environmental pollution is currently one of the key areas of concern in environmental research today (Kaile and Nyirenda 2016). Urban development results in increased exposure to a variety of different pollutants, placing further stress on the urban wildlife (Ditchkoff *et al.* 2006). As a result of anthropogenic activities in and around cities, a number of different pollutants have entered aquatic ecosystems; many of these are known to be harmful to animals at high concentrations. Due to the exposure to and bioaccumulation of toxic pollutants, top predators mainly dependant on the aquatic ecosystems have suffered population declines and even local extinction (Gutleb 2000; Khayatzadeh and Abbasi 2010).

The water quality of South Africa's river systems has noticeably deteriorated over the past decade (Durand 2012). Unlike seawater, freshwater systems are highly variable in the amount of suspended material as well as chemical composition (Rainbow and Dallinger 1993). The aim of my study was to assess the risk of bioaccumulation of five common urban contaminants (lead, mercury, titanium, arsenic and uranium) in southern African otters inhabiting the surrounding areas of Johannesburg in Gauteng. This was conducted by applying non-invasive scat analysis to determine heavy metal accumulation through the otter. Data suggest that there is heavy metal contamination within the rivers of Gauteng, namely the Klip and Hennops rivers. Results have shown signs of impact on otter species where mercury and arsenic are likely to bioaccumulate and the other three contaminants (lead, titanium and uranium) posing serious threats due to physical variables. Over the past few years contamination levels are seen to be increasing at a slow rate, however this is still of concern to the otter and other aquatic wildlife's health.

Extensive studies are being conducted on the numerous health impacts heavy metals have on wildlife worldwide. The main concern around the toxicity of these contaminants, besides that of health effects to the individual, is their ability to bioaccumulate rapidly through the food chain to apex predators (Govind and Madhuri 2014), resulting in direct and indirect health effects to wildlife dependant on species that rely on the aquatic ecosystem (Hamilton 2004). Studies have shown the importance of concern for the rapid uptake of toxic elements such as those analysed in this study; arsenic, lead, mercury as well

as cadmium and chromium, and their possibility to biomagnify through complex food chains (Peralta-Videa *et al.* 2009).

In this study mercury and arsenic were two of the contaminants which were found to potentially bioaccumulate within the otter. Both mercury and arsenic can be exposed and taken up through drinking water, air, and more commonly via food sources, resulting in a build-up of these metals within the tissues and organs of these individuals. In addition to the chronic diseases associated with these two metals reproductive effects have recently been identified (Henson *et al.* 2016). In males, arsenic is seen to affect the control of hormones for reproductive processes, by altering the enzymatic regulating steroidogenesis (Wang *et al.* 2013; Henson *et al.* 2016). Many sources suggest that mercury does have profound affects to mammalian reproduction (see Apostoli *et al.* 2007; Henson *et al.* 2016). The exposure to mercury in males results in significantly depressed levels of sex-hormone-binding globulin levels within the individual with the effect levels dependant on the length of mercury exposure (Henson *et al.* 2016). Mercury also causes subnormal fertility in exposed male individuals, as it is seen that mercury accumulates in the testis, resulting in increased rates of cellular abnormalities. Female individuals exposed to mercury for extended periods of time have unfavorable effects to their menstrual cycle, as well as having prolonged oestrous cycles and altered steroid profiles (Apostoli *et al.* 2007; Henson *et al.* 2016). More research needs to be conducted in order to address the physiological mechanisms responsible for the detrimental effects on the embryonic and foetal development during the reproduction process of an individual (Henson *et al.* 2016).

6.1. Water quality concerns in Gauteng

The water quality data obtained from the Department of Water and Sanitation does not suggest definite increases of contaminant concentrations through the present trends. However, it can be seen from the findings in this study that there are potential risks for contaminant bioaccumulation, posing health risks to the mammals within Gauteng. As South Africa is a developing country, large scale development is likely to take place, therefore these input sources may not decrease in the near future (Geissen *et al.* 2015). This could also be due to poor

implementation of management plans to reduce the waste input into the aquatic environment (Geissen *et al.* 2015). Overall, if no alternative plan for waste disposal is found, all contaminants present in the river under the right conditions will be bioavailable, regardless of high or low concentration, will potentially bioaccumulate through the mammals' food chain. The increase in bioaccumulation through the mammals food chain will pose a severe health threat to mammals which are top consumers such as for the otter. Therefore, this is a great starting point to improve and enhance the quality of life for the species within the aquatic ecosystem.

There was no significant difference seen between the urban and peri-urban regions along both rivers for any of the sources analysed. Initially one would expect the peri-urban areas to be less polluted than that of the urban areas of the rivers. However, this result could be explained by the factors affecting the rivers equally through the entire length of the river as the water flows thus washing the contaminants down into the other areas, rather than having a higher impact in one specific area. The main sources of pollution to the rivers in these areas are industrial and domestic waste. The disposal of these waste waters is not well documented in urban areas in Africa (Showers 2002). In Johannesburg the waste water is thought to undergo a nutrient activated treatment process or treated through sludge plants. The disposal of this water is then either purified effluent into the Vaal Dam or barrage via the Klip River (See Showers 2002). In the peri-urban areas in Gauteng there is no treatment plan for the waste waters and these waters are thought to be disposed into the rivers running through area (Showers 2002; Wang *et al.* 2013). However, it is important to remember that my sample size was very small in this study (6 sites per urban and peri-urban area). This may not have been a large enough sample size to result in a conclusive and accurate comparison between the urban and peri-urban areas along the rivers. Therefore, it is suggested that future studies would need to be conducted focusing mainly on what effect the urban and peri-urban areas have on the Gauteng rivers.

The present condition of the water quality in the rivers of Gauteng with high heavy metal concentrations in the aquatic ecosystems indicate poor environmental health. This increases the potential risk of accumulation to otters and other aquatic mammals. Studies conducted on indicator species in high contaminated waters confirm this risk to mammals, by inferring that species are affected due to how the indicator species reacts to heavy metals at these levels (Ates *et al.* 2013). This is evident as mammals are influenced by the accumulation of contaminants

through their food chain. Fish are good environmental health indicators as they are sensitive to a variety of foreign chemicals (Ates *et al.* 2013). The position of fish in the aquatic food chain means that the assessment of their populations and the health of the fish can provide an indication of the health of lower levels in the food chain (Ates *et al.* 2013). Sanders *et al.* (1999) suggested that crustaceans could also be included as good bioindicators for environmental health, particularly in areas with elevated heavy metal contaminant concentrations in aquatic ecosystems. Snyman *et al.* (2002) suggests that crabs are used in biomonitoring processes as well as have a role in assessing the exposure and bioavailability of heavy metals. The ecotoxicology relevance is enhanced by the fact that crabs serve as a main food source for otters in South Africa and therefore are considered as an indicator species for the accumulation risk of heavy metals to otter species (Snyman *et al.* 2002). Understanding the effects of the nanoparticles on indicator species such as fish and crab is an important aspect to assess when understanding the effects of the heavy metal nanoparticles on the aquatic environment's (Ates *et al.* 2013).

6.2. Implications for urban wildlife

In a developing country such as South Africa, large scale development is most likely to take place. With this development there is an increase in mining, industrial, agricultural and domestic activities which unfortunately lead to an increase in water pollution (Durand 2012). In this study, this increase in pollutants in the river system in Gauteng was however not significant apart from titanium and mercury concentrations, found in the water and sediment samples, which were substantially higher than the minimum risk threshold. Both contaminants, more specifically mercury, is one of the more serious contaminants threatening aquatic ecosystems because it is a potent neurotoxin in fish, wildlife as well as humans, which bioaccumulates and persists in the environment (AJUA Environmental Consultants 2011). Once mercury has been changed from inorganic mercury to an organic methylmercury, the toxicity of the mercury is greatly increased and thus has a higher potential to accumulate in aquatic biota and through the aquatic food chain (Scudder *et al.* 2009). Heavy metals found in the sediment is known to have an indirect effect on mammals in the aquatic ecosystem (Parsa *et al.* 2014). Smaller organisms such as crabs, being the

otters main food source, will directly take up these contaminants, thus allowing it to bioaccumulate through the food chain (Snyman *et al.* 2002). The size of the crabs caught did not play a role during the field work, as crab numbers in the rivers were very low therefore I used what I could get. Furthermore, the possible moulting of the crabs were not considered an issue as once the crab has moulted the crab will bioaccumulate the same contaminants in its new shell, thus having the same risk to the otters. This results in more significant contamination to the individuals at higher trophic levels, resulting in health impacts (Jovanovic 2014; Parsa *et al.* 2014).

In this study the prey availability in the rivers studies was very low. The analysis of fish would have been optimal as fish is another primary food source for otters. However, in the field, the availability of fish was very low and no fish were caught in order to analyse. Crabs, another primary food source for otters, were then selected to be analysed. Crab numbers were also low and therefore only a small sample size was able to be collected. Once the prey is exposed to an increased level of heavy metals, the bioaccumulation of these contaminants will have lethal effects on the prey available to these predators. This supports the findings in this study, that crabs are exposed to the high concentrations of metals in the water and sediment (titanium, mercury and lead), therefore there is a high risk for the accumulation of these contaminants. This may further result in a change in predator's diet which may be unsuitable for long term survival and thus affecting the overall predator population's survival (Petchey 2000).

In this study a non invasive sampling technique was applied in order to determine the accumulation of contaminants to the otter (see Mason and MacDonald 1987; Elliott and Wilson 2003; Zwiernik *et al.* 2008). Arsenic and mercury were the two contaminants which had concentrations higher in the food samples compared to the scat. Therefore, they are most likely to accumulate through the otters body, resulting in a number of severe health risks. Mercury as well as arsenic poisoning is considered to be of great concern for the ecological and individual health resulting in a huge risk factor for the otter's survival as these contaminants readily bioaccumulate within their food chain (Peterson and Schulte 2016). Otters who had mercury and arsenic poisoning showed erratic behaviour before dying (Roos 2013), therefore further suggesting that mercury and arsenic could lead to possible declines in the otter populations (Roos 2013).

Over the last three year period, 2013 – 2015, high heavy metal concentrations were also recorded by the Department of Water and Sanitation. Calcium and chlorine displayed concentrations higher than the suggested thresholds, and copper was close to the thresholds in some rivers and over the thresholds in the Klip and Jukskei rivers in Gauteng. Thus, it is highly likely that these trends are seen in all rivers throughout Gauteng. It is also possible that more heavy metals as well as organic contaminants are above or close to the suggested thresholds for mammal individual exposure to contaminant concentration in aquatic environments. The risk of bioaccumulation of heavy metals affects a number of factors in the aquatic environment and not just the health of the individual. These areas include prey availability, individual reproduction, health and most importantly the physiological effects to the individual being exposed to high levels of contaminants (Snyman *et al.* 2002).

The main issue regarding water pollution is that the majority of the contaminants are soluble in water and thus the effect of contaminates once introduced to the water body is not immediately visible. Thus, the impact of the contaminants to the aquatic environment may only be noticed once the damage has already been done to the environment as well as to the health of the aquatic organisms who rely on this ecosystem. It is therefore essential that management strategies are put in place for the monitoring of the water quality of the Gauteng rivers in order to prevent or limit the health and quality impacts of these ecosystems. The need to understand past species populations is important in order to inform the current management about the significance of quantifying the impacts of urbanisation and pollution on the species to encourage conservation action to take place (Longcore 2010).

Due to the deteriorating conditions of the otters habitat, there is an increase risk posed to the species as they are more likely to take up contaminants from the water. The Gauteng Department of Agriculture and Rural Development is particularly concerned about the conservation of these species as they are higher level predators and due to the increasing rates at which their population numbers are declining (Seegers, *pers. Comm.*)⁵. It is therefore important that a conservation plan is put in place, not only for the survival of the

⁵ Christina Seegers, Biodiversity Officer: Biodiversity Stewardship, Gauteng Department of Agriculture and Rural Development.

otter population within Gauteng but also for the survival of the remaining waterways and wetlands in which they inhabit.

In conclusion, the general quality of the water from both rivers was better than expected for rivers based in Gauteng running through both urban and peri-urban areas. Otters are at risk of exposure to pollutants due to their dependence on freshwater habitats. The results obtained in the present study suggest that mercury and arsenic were the two contaminants with a high potential of bioaccumulation through the otters food chain as well as accumulating in the otter as they were not eliminated from the body, causing a variety of health risks. These results therefore support my hypothesis that, due to the increase of contamination in the Gauteng rivers, otters are at high risk of accumulating some level of contaminants through their food chain. The potential health risks of major concern to specifically the otter species in Gauteng are damage to the liver, kidney and spleen, DNA damage, reproduction affects, neural damage and effects to the CNS and PNS systems. In severe cases it has been seen that most of these conditions may be fatal to the otter individual. If no alternative plan for waste disposal is found, there may be a large increase in all pollutants found in the aquatic ecosystem, thus increasing the potential to bioaccumulate as these concentrations are most likely to exceed the minimum threshold levels. This poses a severe threat to the otter population and to other mammals which are top consumers as there will be an increase in the bioaccumulation of contaminants through their food chain.

The otter is an aquatic top predator and is consequently heavily exposed to a number of environmental contaminants. It is thus important to continue monitoring the possible risks posed to otters by the accumulation of heavy metal contaminants. I have indicated the possibility of two heavy metals, mercury and arsenic, posing a risk to the otter species occupying the rivers within Gauteng. Although the other heavy metals did not display signs of accumulation in this study, the combined effects from the exposure of a number of contaminants over a long period of time cannot be ruled out. The otter is a good indicator species indicating the quality of the environment, namely the aquatic ecosystems. Thus, it is important to continue the health effect studies on the otter species. The potential risks to the otter species in Gauteng are not fully understood throughout the province. It is therefore suggested that detailed assessments to determine the potential risk of

accumulation and health effects of freshwater contaminants for all rivers in Gauteng continue to be conducted in order to further conserve the other species in Gauteng.

6.3. Limitations of the study and recommendations for further research

There were a number of limitations to my project, firstly the main weakness to this study was the small sample size. It would be suggested that future studies increase not only the number of sites along each river but also the number of rivers studied. Lastly, my study was only conducted in the winter periods, therefore less turbulence for the contaminants to mix into the water column therefore less contaminants to accumulate. It would thus be suggested that future studies look at the seasonal differences for contaminant concentrations in order to obtain a more accurate concentration of the accumulation of heavy metals through the food chain of Gauteng otters.

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APPENDIX A

Chemical Analytical Procedure

The chemicals which were used for analysis of trace levels of the contaminants were of analytical grade or higher. Concentrated analytically pure nitric acid (65% w/v, supra pur grade) and hydrogen peroxide (30% w/v, supra pur grade) were obtained from Merck in South Africa. Hydrochloric acid, acetic acid, sodium chlorate, magnesium sulphate, acetonitrile pur were obtained from Sigma, Aldrich. Stock solutions from ICP-OES and ICP-MS, were supplied by De Bruyne, South Africa, at the concentration of 10mhl⁻¹. All standard solutions were prepared on the day before use.

1.1. *Cleaning Protocol*

In trace analysis, contamination is a major source of error in environmental analysis. Clean up can reduce or minimise of any contamination in the sample. The best way to control the degree of contamination at any step of sample treatment is to use a blank. It is important to work under a clean environment for successful analysis at a very low concentration levels. A rigorous cleaning procedure for all vessels was used in sampling and sample preparation. The following cleaning procedure was used (Rosamond 2014):

1. running tap water was used to initially rinse all vessels (plastics and glassware) to remove all solids and liquid dirt;
2. all vessels were washed with soap, after which they were then rinsed thoroughly with running tap water followed by being rinsed with de-ionized water, with an electrical resistivity of 18.2 MΩcm (Millipore, USA);
3. all vessels were then soaked in a 10% (v/v) HNO₃ analytical grade (Merck) solution for 3 days after which they were thoroughly rinsed with de-ionized water.

1.2. Microwave Digestion

The samples first went through a vital pre-treatment step called the mineralization process, after which the analytical determination could be performed. This step aided to prevent any unwanted residues that could settle on the equipment which could potentially affect the spectral interferences, thus resulting in the inaccuracy of the measurements in the analytical techniques (Soceanu *et al.* 2007). There were several methods in which the sample pre-treatment could be done, these being wet ashing, dry ashing, and microwave or ultrasound influences (Alvarez *et al.* 2017). Wet digestion of organic matter was mostly applied for the analysis of biological samples (Ekholm *et al.* 2007; Soceanu *et al.* 2007).

The crab and scat samples were wet digested in the microwave digestion system as follows: a mass of 0.25 ± 0.005 g of homogenized scat and crab samples were weighed using an analytical balance (Precisa 180A, Switzerland) and placed into acid washed digestion tubes (PTFE-TEM liners) onto which 16ml HNO₃ and 4ml H₂O₂ were added before being placed into the Multiwave 300 microwave digester (Anton Paar, Switzerland).

1.3. Inductively Coupled Plasma –Optical Emission Spectroscopy (ICP-OES)

Inductively coupled plasma-optical emission spectroscopy (ICP-OES) (Genesis, Spectro Instruments, Germany) was used to determine the total metal concentration in the solutions. Measurement conditions: argon pressure: 800 KPA; pump speed: 2 steps; plasma power: 1400W; coolant flow: 14 l/min; auxiliary flow: 1.00 l/min; and nebulizer flow: 1.00 l/min. Working standards of 0.1, 0.2, 0.5, 1 and 2mgL⁻¹ were used for calibrating the instrument. The samples were diluted before analysis, in order to fit them into the calibration curve.

1.4. Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)

An ICP-MS (Agilent technology, 7700 series ICP-MS) instrument was used for trace metal determination. Acid washed torch and spray chamber were used in order to minimize contamination. The nebulizer was also rinsed with de-ionized water, and silicosteell tubing (Restek) was used to reduce the mercury absorption in the sample introduction system.

ICP-MS parameters such as ion optics voltages, mass scan, time scan, pump speed, and argon flow were optimized to obtain a better resolution and analyte-background intensity ratio. A standard solution was prepared on the day of analysis from a stock solution of 10 mgL⁻¹ (De Bruyn Spectroscopic Solution, SA) for the instrument calibration and analyses were performed on triplicates.

Appendix B

Table 1. A summary of the number of samples collected for water, sediment, scat and crab at each site along the Hennops and Klip River in Gauteng.

River	Site	Water	Sediment	Scat	Crab
Hennops	H1	1	1	1	2
Hennops	HT	1	1	0	2
Hennops	HC	1	1	0	0
Hennops	HSd	1	1	1	0
Hennops	H2d	1	1	1	2
Hennops	HD	1	1	1	0
Klip	K7	1	1	1	2
Klip	K8	1	1	1	2
Klip	K9	1	1	1	4
Klip	K11	1	1	1	0
Klip	K4	1	1	1	4
Klip	K6	1	1	1	0
Total Samples per source		12	12	10	7

Appendix C

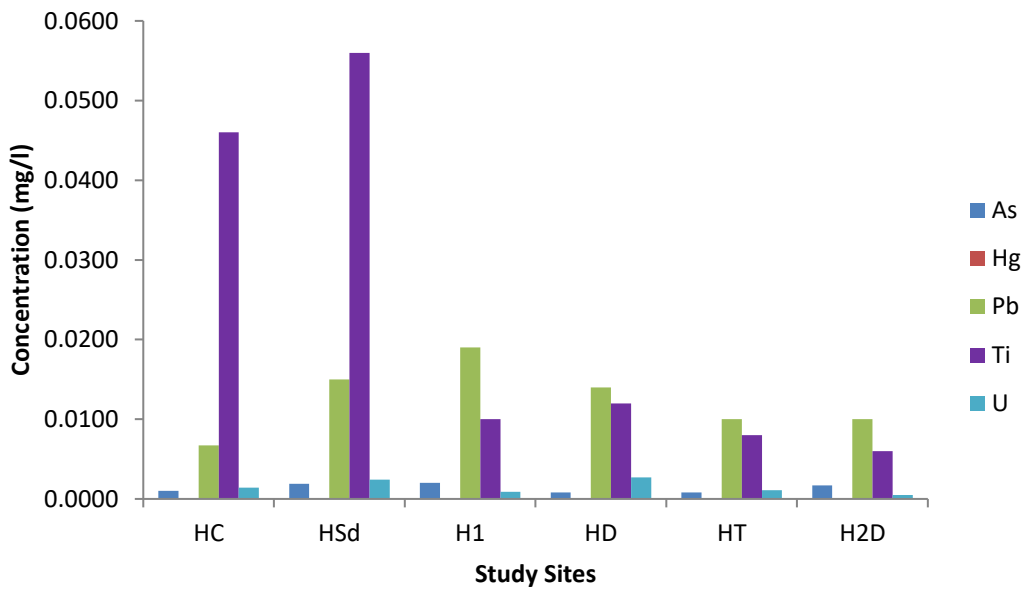


Figure 1. A representation of the 5 heavy metal concentration levels found in the water samples from the Hennops River.

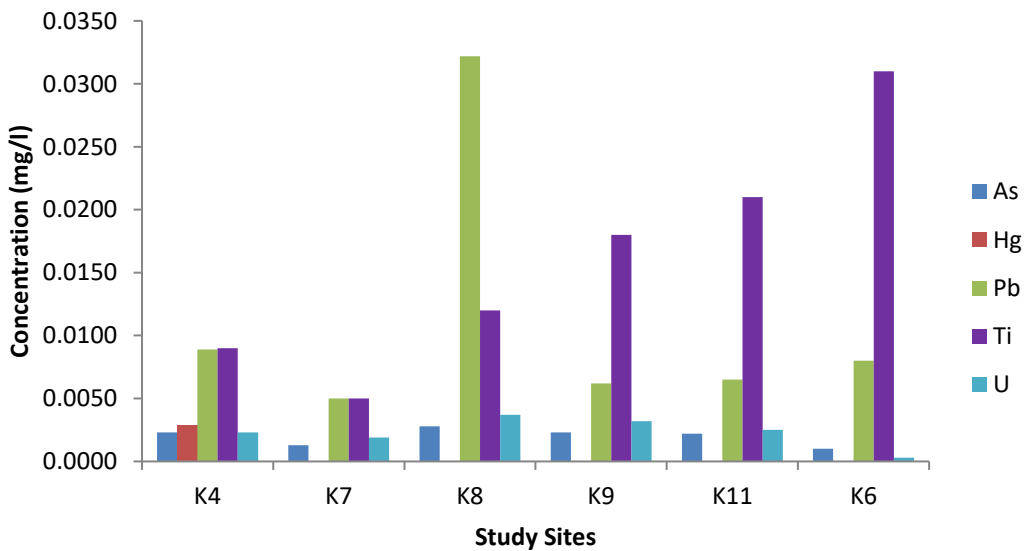


Figure 2. A representation of the 5 heavy metal concentration levels found in the water samples from the Klip River.

Appendix D

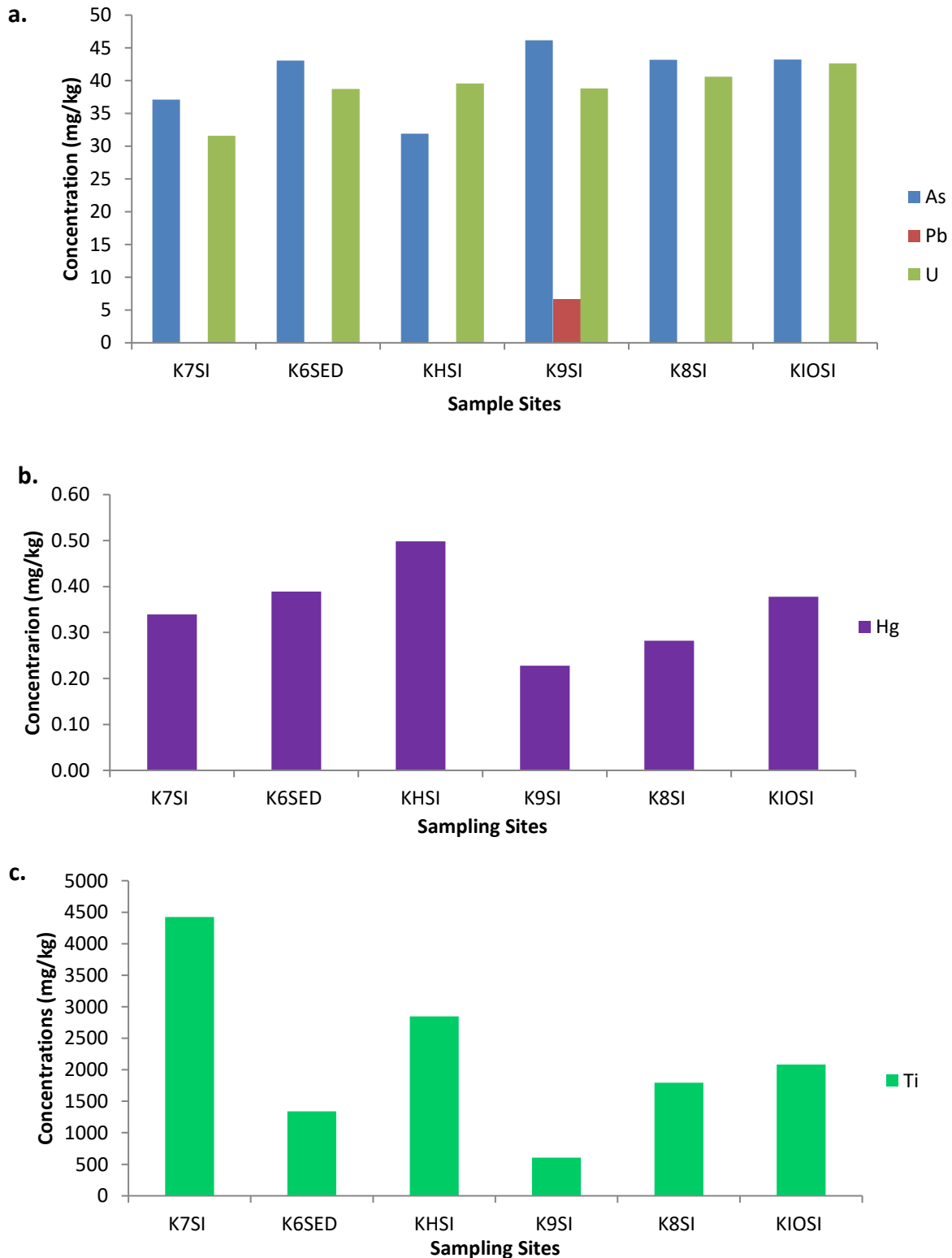


Figure 1. A representation of the contaminant concentrations for the sediment samples collected along the Klip River (a. representing arsenic, lead and uranium. b. Representing mercury and c. representing the concentrations for titanium).

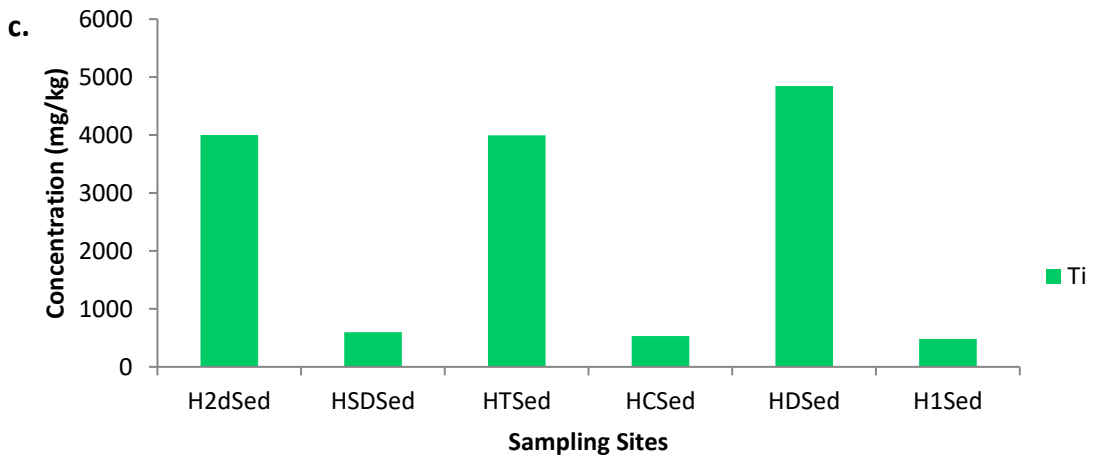
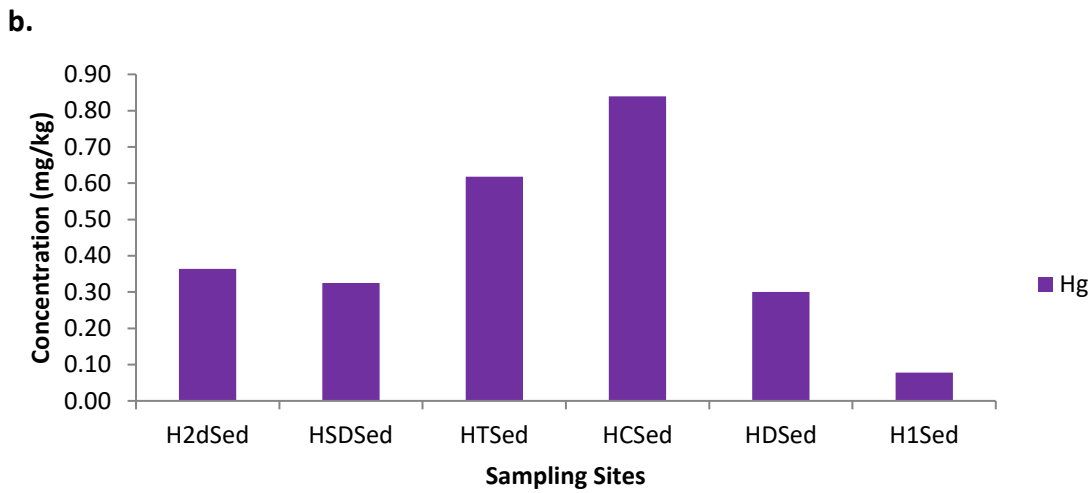
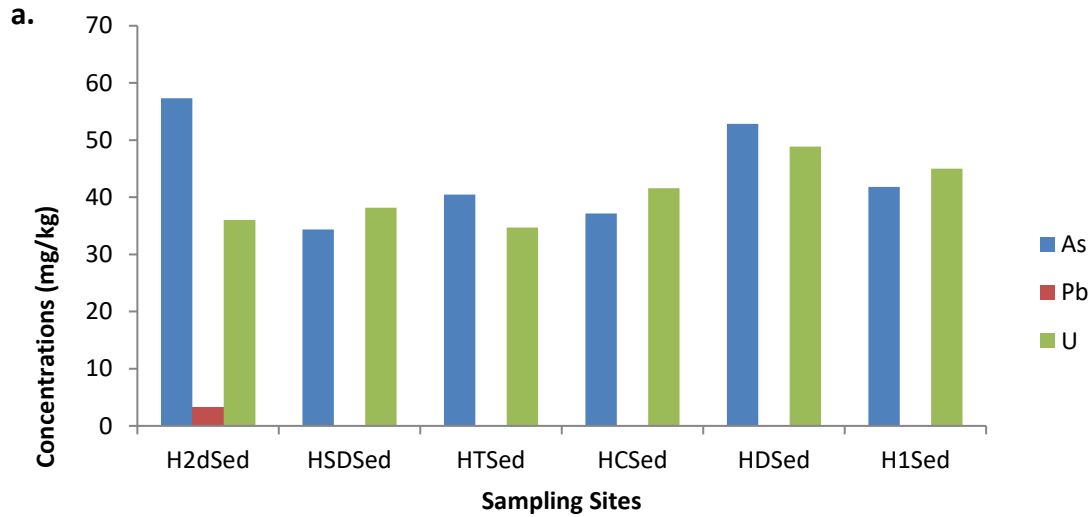


Figure 2. A representation of the contaminant concentrations for the sediment samples collected along the Hennops River (a. representing arsenic, lead and uranium. b. Representing mercury and c. representing the concentrations for titanium).

Appendix E

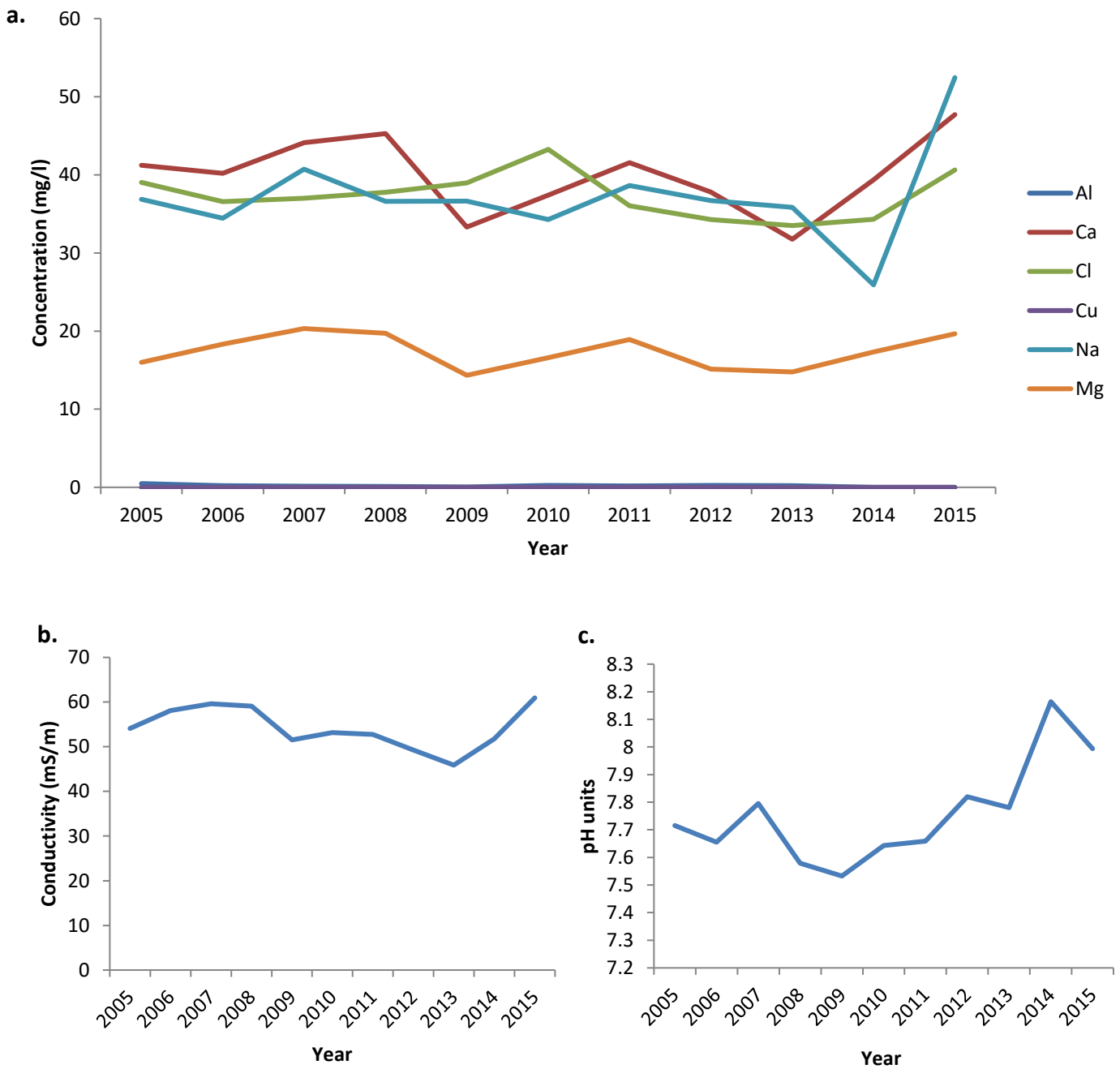


Figure 1. A representation of the water quality data recorded over the last ten years for the Klip River, obtained from the Departments of Water and Sanitation (a. the six heavy metals analysed, b. EC and c. pH).

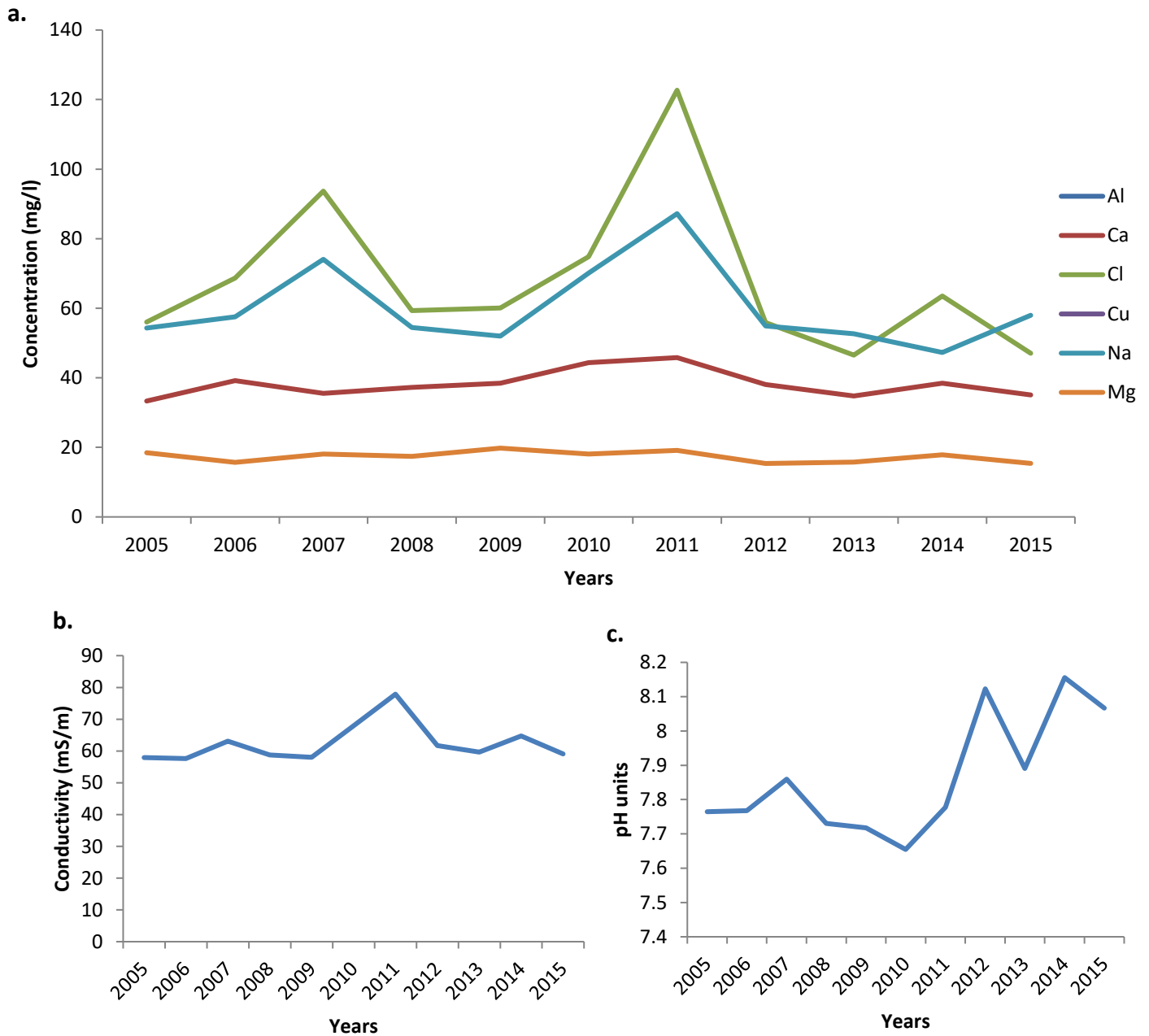


Figure 2. A representation of the water quality data recorded over the last ten years for the Hennops River, obtained from the Department of Water and Sanitation (A. the six heavy metals analysed, b. EC and c. pH).

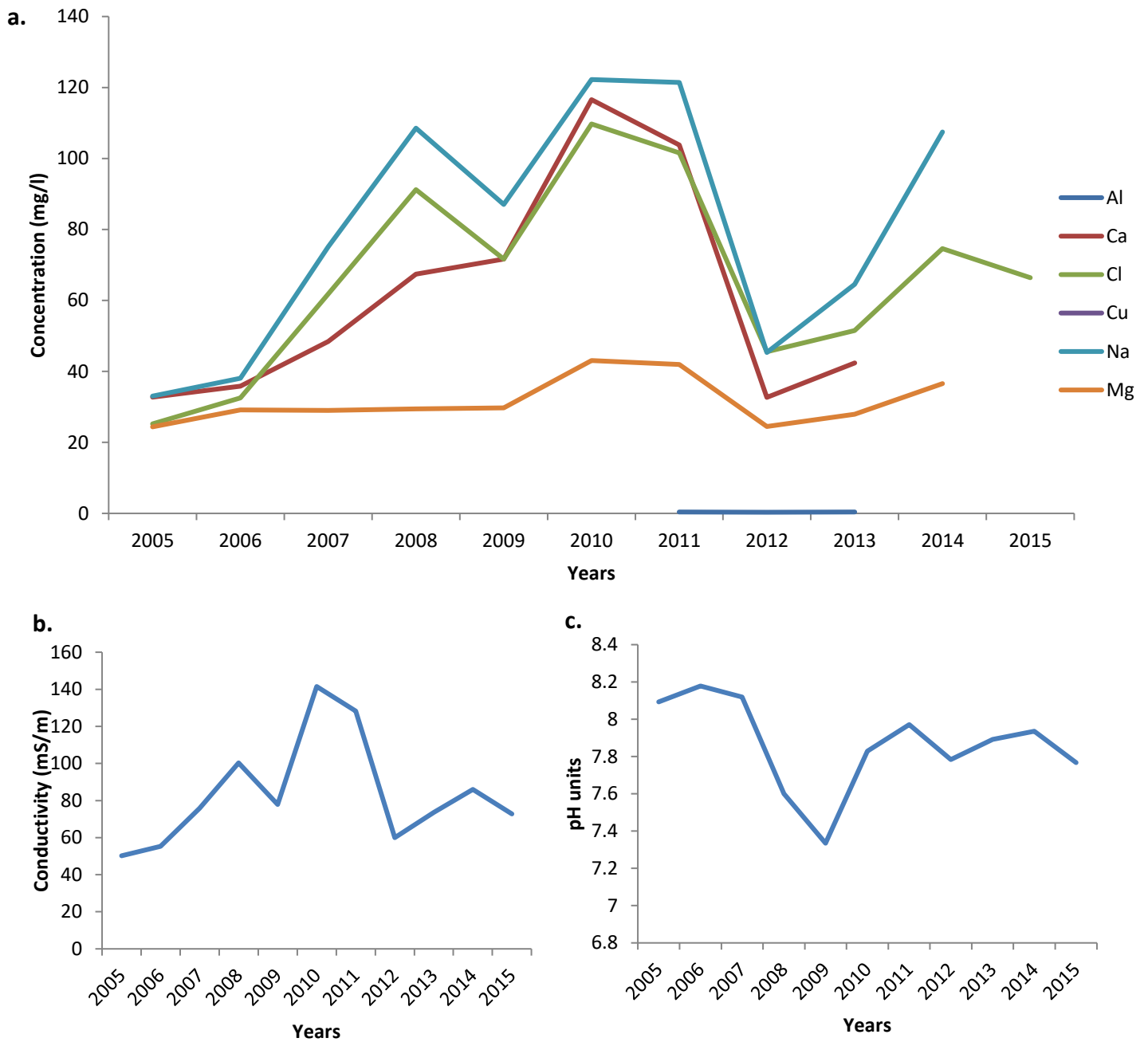


Figure 3. A representation of the water quality data recorded for the past ten years for the Blesbokspruit River, obtained from the Department of Water and Sanitation (a. the six heavy metals analysed, b. EC and c. pH).

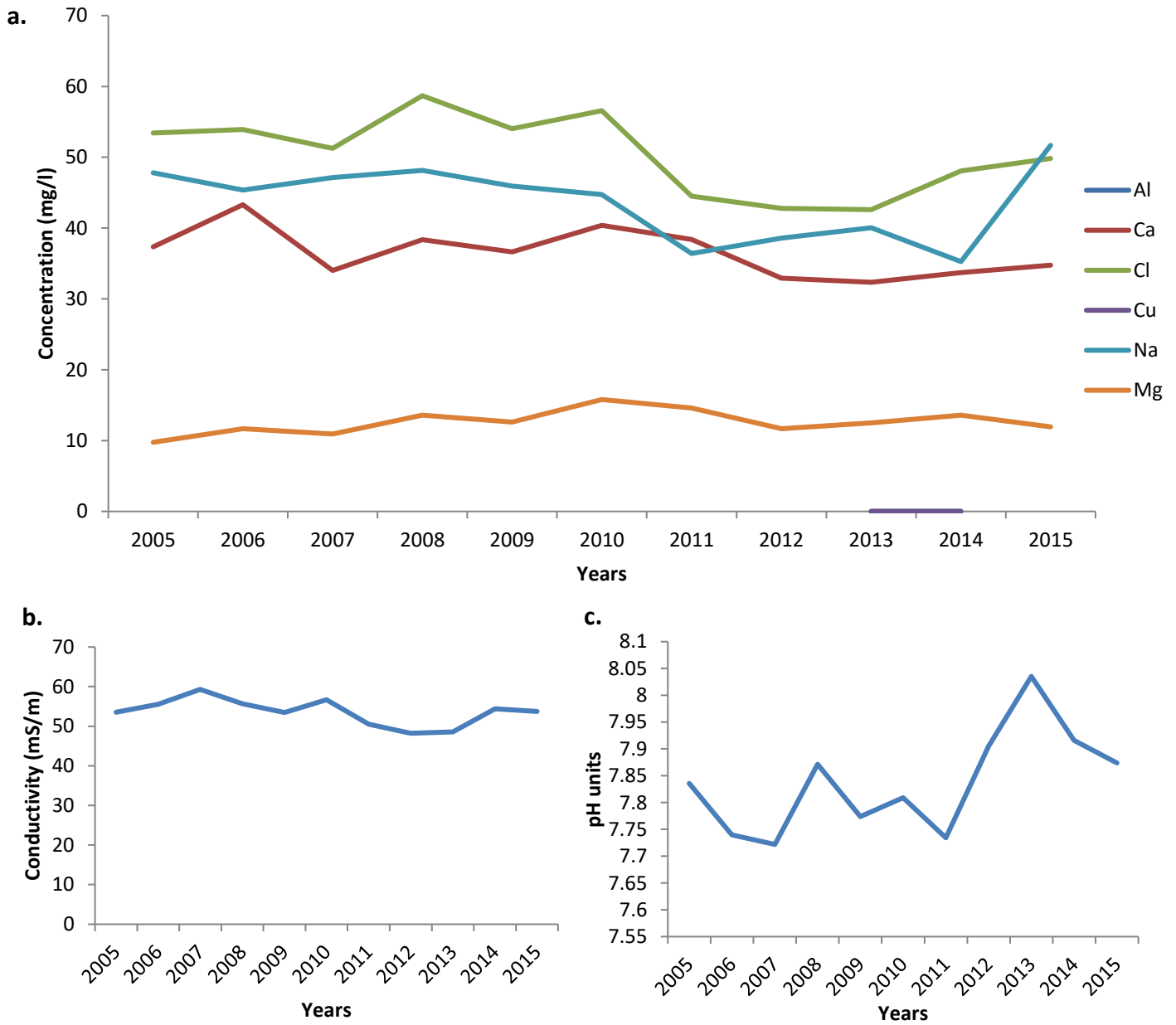


Figure 4. A representation of the water quality data recorded over the past ten years for the Jukskei River, obtained from the Department of Water and Sanitation (a. the six heavy metals analysed, b. EC and c. pH).