The presence of persistent organic pollutants and heavy metals in sediment samples from rivers in the Kruger National Park

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20104580

Dissertation submitted in fulfilment of the requirements for the degree *Magister Scientiae* in Environmental Sciences at the Potchefstroom Campus of the North-West University

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May 2015

Acknowledgements

"We do not inherit the earth from our ancestors; we borrow it from our children"

~ Native American Proverb ~

First and foremost, I would like to acknowledge my Lord and Saviour, for without His blessings, grace and love, everything I have achieved would not have been possible.

Research of this magnitude cannot be accomplished by one person; it requires a team of experts, with a passion for nature. I would like to thank each and every person that played a part in this project. To all my colleagues at the North West University, through the years, thank you for the weekends, late nights and endless efforts, the laughter, fun and friendship. It made the difficult times more bearable.

I am grateful for the financial support that I have received for the duration of my studies from the Water Research Commission (WRC), North West University, and my study promoters (Dr Rialet Pieters & Prof. Henk Bouwman). It is greatly appreciated. Without these grants I would not have been able to complete my studies.

I would especially like to thank the following people and institutions that have contributed to this project:

The North West University

- Prof. Henk Bouwman, Dr Rialet Pieters, Laura Quinn, Claudine Roos, Lieb Venter, Belinda Venter and Prof. Martie Coetzee for their input regarding the Crocodile project.
- Dirk Cilliers, for the shapefile of the Kruger National Park.

Norwegian Institute for Air Research

Kruger National Park

Many thanks to my mother and family, which include: all the Van Gessellens', Jouberts' and Roos', and to all of my friends who always motivated and supported me during the course of my studies.

Without the continued support and motivation from everyone in my life, I would not have been able to make such a success of this project.

This thesis is dedicated in loving memory of my father (JAD van Gessellen).
I will always remember his love for the Kruger National Park and nature. He has inspired me and has made this more than just a project. All that I have learnt is thanks to him; such knowledge cannot be obtained from books. He is sorely missed.

Crocodiles basking on a sand bank at Crooks Corner that forms part of the Limpopo River.

Opsomming

Vanaf 2008 is daar groot hoeveelhede Nylkrokodilkarkasse (*Crocodylus niloticus*) opgemerk in die Nasionale Krugerwildtuin (NKW) van Suid-Afrika. Die verskynsel is hoofsaaklik in die Olifantskloofgedeelte waargeneem, net na die sameloop van die Letaba- en Olifantsriviere. Die is die gedeelte voordat die twee riviere vanaf die Suid-Afrikaanse kant in die Massingirdam in Mosambiek inloop. Die dam dien as ’n hulpbron vir die inwoners van Mosambiek.

Nadoodse ondersoeke van die krokodilkarkasse het getoon dat die liggaamsvet van wit na ’n geelagtige kleur verander het. Pansteatitis is ’n simptoom van lipiedperoksidase en word gekenmerk deur ’n tekort aan vitamien E. Dit veroorsaak dat die vetweefsel verhard en verkleur. Die toestand word meestal geassosieer met akwatiese diere wat in besoedelde water voorkom. Vermoedelike oorsake van die krokodilsterftes het te doen met die verandering van die ekostelsel na die herbou en verhoging van die Massingirdam se wal aan die begin van 2008. Die Olifantskloof is voorheen gekenmerk deur die vinnig vloeiende water wat nou feitlik tot stilstand gebring is. Die vertraging van die water in die kloof veroorsaak dat die gesuspendeerde sediment vinniger uitsak. Sediment, wat ’n algemene vervoermeganisme vir besoedelstowwe is, hoop op in die area en kan dus lei tot ’n konsentrasie van besoedelstowwe in die direkte omgewing.

Sedimentmonsters van verskeie riviere en poele in die NKW is versamel. Die monsters is geanaliseer vir die teenwoordigeheid van verskeie swaarmetale, persisterende organiese besoedelstowwe (POB), en polisikliese aromatiese koolwaterstowwe (PAK). Die sedimentmonsters is in Noorweë geanaliseer vir POB en PAK met hoë-resolusie gaschromatografie/massaspektrometrie (GC/MS). Die swaarmetale is in Suid-Afrika geanaliseer met induktief-gekoppelde plasma-massaspektrometrie (ICP/MS).

Om te bepaal welke swaarmetale wel ’n rol kon speel in die krokodilmortaliteit is daar gebruik gemaak van verskeie sedimentkwaliteitsindekse (SKI). Hierdie indekse maak dit moontlik om vas te stel watter metale se konsentrasies moontlik ’n impak op die stelsel kon gehad het. Hierdie metale is gelys vanaf die metaal met die hoogste na die laagste moontlik invloed: Se>As>Ni>Cr>Cu>I>V>Mn>Co>Fe>Cd>Hg>Zn>Pb>Ba>U. Die data is ook met verskeie internasionale riglyne vergelyk. Al hierdie inligting is gebruik om vas te stel dat die sediment van die Krokodil-, Nkomati-, Olifants- en Letabariviere die hoogste konsentrasies besoedelstowwe gehad het.

Die volgende elemente Fe, Cu, Cr, Pb, V, Co, As en Ni, het hoë konsentrasies gehad, veral in die Olifantskloofarea, wat moontlik ’n negatiewe invloed op die krokodille kon veroorsaak het. Hierdie verhoogde konsentrasies van elemente, saam met die dramatiese verandering aan die fisiese omgewing as gevolg van die dam, kon moontlik bygedra het tot die krokodilmortaliteit in die Olifantskloof.

Sleutelwoorde: Nasionale Krugerwildtuin; Nylkrokodil; Olifantsrivier; Pansteatitis; Swaarmetale; persisterende organiese besoedelstowwe; polisikliese aromatiese koolwaterstowwe; sediment.

Summary

Since 2008, large numbers of Nile crocodile (*Crocodylus niloticus*) carcasses were found in the Kruger National Park (KNP), South Africa. Most of the crocodile carcasses were found in the Olifants Gorge, which is situated below the Letaba and Olifants river confluence, before the Mozambique border and Massingir Dam. The Massingir Dam is an important resource and it plays a significant role in the welfare of the local Mozambican population.

Autopsies performed on the crocodiles indicated that the adipose tissue colour changed from normal white to yellow and this is usually a sign of pansteatitis. Pansteatitis is caused by lipid peroxidation in an organism and it is characterised by the lack of vitamin E. This disease is recognisable by the hardening of the fatty tissue and yellow discolouration, and is mostly associated with aquatic organisms from polluted ecosystems. There are speculations that the crocodile fatalities may be associated with the Massingir Dam that backed up into the Olifants Gorge after flooding. After the dam was reconstructed, it flooded the Olifants Gorge, causing it to act like a localised sediment trap as the water flow slowed down and as a result, caused pollutants to build-up.

Sediment samples were collected from selected rivers and ponds within the KNP. These samples were analysed for selected elements, persistent organic pollutants (POPs), and polycyclic aromatic hydrocarbons (PAHs). The sediment samples were analysed in Norway for POPs and PAHs with the use of a high-resolution gas chromatography/mass spectrometry (GC/MS) and the heavy metals were analysed in South Africa with the use of inductively-coupled plasma mass spectrometry (ICP/MS).

In order to identify which elements may have affected the health of the crocodiles, a series of sediment quality indices were used. These indices made it possible to determine which elements may have been involved. The order of probability of heavy metals causing harm was Se>As>Ni>Cr>Cu>I>V>Mn>Co>Fe>Cd>Hg>Zn>Pb>Ba>U.

The data was compared to selected international guidelines. All the information was used to determine which of the sampled sites had the highest contamination. The sites sampled with the highest concentrations were in the Crocodile, Nkomati, Olifants, and Letaba Rivers. Concentrations of the elements, POPs, and PAHs were also quantifiable in the Olifants Gorge.

The following elements (Fe, Co, Cu, Cr, Pb, V, As, and Ni) were quantified at elevated levels and may therefore have caused negative effects on the crocodiles in the Olifants Gorge. These elevated concentrations, in combination with the dramatic change in the physical environment due to the dam, could have added additional stress that may have contributed to the observed crocodile mortalities in the Olifants Gorge.

Keywords: Kruger National Park, Nile crocodile, Olifants River, pansteatitis, elements, persistent organic pollutants; polycyclic aromatic hydrocarbons, sediment.

Abbreviations and Acronyms

α-HCH	**α-Hexachlorocyclohexane**
ANZECC	**Australian and New Zealand guidelines for fresh and marine water quality**
β-HCH	**β-Hexachlorocyclohexane**
BFRs	**Brominated flame retardants**
Cd	**Cadmium**
CCME	**Canadian Council of Ministers of the Environment**
CF	**Contamination Factor**
Cr	**Chromium**
Cu	**Copper**
CROC	**Consortium for the Restoration of the Olifants Catchment**
DWS	**Department of Water Affairs and Sanitation**
DWA	**Department of Water Affairs**
DWAF	**Department of Water Affairs and Forestry**
DDT	**Dichlorodiphenyltrichloroethane**
DDE	**Dichlorodiphenyldichloroethylene**
DDD	**Dichlorodiphenyldichloroethane**
DL	**Dioxin-like**
EDCs	**Endocrine disrupting chemicals**
EF	**Enrichment Factor**
FAO	**Food and Agriculture Organization of the United Nations**
γ-HCH	***γ*-Hexachlorocyclohexane**
Igeo	**Geoaccumulation index**
GC/MS	**High-resolution gas chromatography/mass spectroscopy**
Hg	**Mercury**
HMW PAHs	**High Molecular Weight Polycyclic aromatic hydrocarbons**
HCB	**Hexachlorobenzene**
HPLC grade	**High-Pressure Liquid Chromatography grade**
KNP	**Kruger National Park**
LEF	**Life Extension**
LWM PAHs	**Low Molecular Weight Polycyclic aromatic hydrocarbons**
NKW	**Nasionale Krugerwildtuin**
NEMA	**National Environmental Management Act**
NILU	**Norwegian Institute for Air Research**

NDSQG	**New Dutch Target and Intervention Values**
OMP	**Organic Micro Pollutants**
PFOS	**Perfluorooctane Sulfonic Acid**
Pb	**Lead**
PBDE	**Polybrominated Diphenyl Ethers**
PeCB	**Pentachlorobenzene**
PLI	**Pollution Load Index**
PAH	**Polycyclic Aromatic Hydrocarbons**
PCB	**Polychlorinated Biphenyls**
PCDD	**Polychlorinated Dibenzo-*P*-Dioxins**
PCDF	**Polychlorinated Dibenzo Furans**
RC	**Rotterdam Convention**
ROS	**Reactive Oxygen Species**
SANParks	**South African National Parks**
SAWQG	**South African Water Quality Guidelines**
SC	**Stockholm Convention**
SQG	**Sediment Quality Guidelines**
SQP	**Sediment Quality Parameters**
SEPA	**Swedish Environmental Protection Agency**
TOC	**Total Organic Carbon**
USEPA	**United States Environmental Protection Agency**
UNEP	**United Nations Environment Programme**
UV	**Ultra Violet**
UC	**Upper Continental Crust**
V	**Vanadium**
WHO	**World Health Organisation**
WRC	**Water Research Commission**
WNA	**World Nuclear Association**
Zn	**Zinc**

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Chapter 1: Introduction

More than one billion people in the world still lack access to potable water and only two and half billion have inadequate sanitation (WHO, 2006). Since the 1980's, water quality has declined in South Africa and it has become a great concern (Botha *et al.,* 2011).

In South Africa, there are many large industries, from mining, to agriculture. These industries need to generate income and this often comes at a cost to the environment. Agriculture is the greatest consumer of freshwater utilising 70% for irrigation (UN WATER, 2009). Large amounts of acid mine drainage, pesticides, sewage effluent and industrial effluent are released into the aquatic environment, sometimes without treatment and this can render the aquatic ecosystems unsafe for human and animal use (Oberholster *et al*., 2010; Aneck-Hahn *et al*., 2009; Adler *et* al., 2007; Bornman *et al*., 2007; CCME, 1999a). These effluents consist of complex mixtures with different chemical characteristics (Bouwman *et al*., 2008; Leusch, 2008; Binning & Baird, 2001). The mixtures that may have an impact on humans and animals include organic chemicals such as personal care products, pharmaceuticals, herbicides, insecticides and inorganic chemicals such as nitrite, sodium arsenate, and heavy metals. (Newman, 2010; Hibberd *et al*., 2009; Esolugas *et al*., 2007; Sonneveld *et al*., 2005; Hilscherova *et al*., 2000).

A pollutant can be described as a substance that is introduced into the environment as a result or part of manmade activities, which can cause detrimental effects to living resources, affect human health, or reduce the quality of resources (Sciortino & Ravukumar, 1999; Moriarty, 1983). Contamination is the presence of elevated concentrations of a natural substance released because of manmade activities (FAO, 1999; Moriarty, 1983). Xenobiotic compounds are foreign chemicals or materials in the environment that are not produced in nature, nor are they the product of natural biological processes found in the environment (Rand & Petrocelli, 1985).

Crocodiles are large, predatory reptiles that have a long lifespan and can be used as bio-indicators of aquatic ecosystems (Van Vuuren, 2011). During the winter of 2008, dead crocodiles were spotted at the confluence of the Olifants and Letaba rivers in the KNP. Thereafter, recurring deaths took place from 2010 until 2011. More than 215 crocodile deaths were recorded from 2008 to 2011 (KNP 2014). This was the first record of mass crocodile deaths in South Africa. Various research institutions in South Africa and elsewhere became involved to try to solve the mystery of these crocodile mortalities.

The current study focuses on persistent organic pollutants (POPs), polycyclic aromatic hydrocarbons (PAHs), and selected elements. The aim was to assess which of the aforementioned may be affecting the health of the Nile crocodile (*Crocodylus niloticus*) in the Kruger National Park (KNP) of South Africa. POPs and PAHs are hereafter collectively referred to as organic micro pollutants (OMPs).

This study was undertaken to determine whether the presence of OMPs and selected elements in the river systems of the Kruger National Park may have contributed to the episodic mortalities of crocodiles since the winter of 2008. Sediment samples from rivers in the KNP were collected, with attention placed on the area where the crocodile mortalities occurred.

1.1 Rivers of the Kruger National Park

Most of the rivers flowing through the KNP have their origin outside its boundaries, carrying their potential contaminant load into the relative pristine environment of the park (Figure 1). The geology of the KNP largely consists of granite, basalts, rhyolite, sandstone and shale (KNP, 2014). In this section, a brief overview of some of these rivers is provided to understand the possible types of contaminants that might be transported into the KNP.

The Limpopo River is on the northern border of the KNP. It consists of numerous small tributaries, streams and pools, forming part of a large drainage system of the northern part of SA. The Limpopo River catchment receives effluent from large cities such as Johannesburg and Pretoria, but also from agricultural and mining activities (Winde, 2009; Roychoudhury & Starke, 2006). The various mining industries within the Limpopo River catchment include diamonds, emeralds, coal, nickel, chrome, vanadium, manganese, dolomite, gold, arsenic, pyrite, lead, iron, tungsten, cobalt, silver, platinum, and copper (Ashton *et al*., 2001). The Luvuvhu River enters the KNP as a separate river, and later joins the Limpopo River inside the KNP. The Luvuvhu River also flows through urban, agricultural, and mining areas before entering the KNP.

The Selati River originates on the slopes of the Drakensberg Mountain region and flows eastwards towards the KNP. This river travels through small villages and large-scale commercial irrigation farms. Domestic effluent and seepage from tailing dams of mines are discharged into the upper reaches of the Selati River. The river joins the Olifants River 10 km east of Phalaborwa (WISA, 2012;

DWA, 2001) practically on the western border of the KNP. Phalaborwa is a city with various industries that include leather tanning, distilleries, steel manufacturing (Van Vuren *et al*., 1994), as well as a large copper mine, smelter, and refinery complex that produces about 80 000 tonnes of refined copper per year (Phalaborwa Mining Company Limited, 2012). Phosphate rock (foskorite and pyroxenite) is also mined in the vicinity (Foskor, 2011).

The Olifants River originates near the towns of Bethal and Breyten and flows in an easterly direction through the Drakensberg Mountain before crossing the KNP into Mozambique, where it flows into the Indian Ocean after its confluence with the Limpopo River (Van Vuren *et al*., 1994). It is considered one of the most polluted rivers in Southern Africa (Heath *et al*., 2010; Myburgh & Botha, 2009). The Olifants River catchment is divided into four sub-catchment areas namely; the upper, middle, lower Olifants River and Steelpoort catchments (DWA, 2009a). The Olifants River catchment is approximately 54 500 km^2 and covers 4.3% of the total surface area of South Africa (Grobler *et al.*, 1994). The annual run-off is approximately 2 400 million m^3 per year (WISA, 2012). The diverse group of economic activities along the Olifants catchment include gold, chromium, platinum, zinc, silver, titanium, tin, manganese, coal, vanadium, thallium, and copper mining (Van Vuuren, 2010; Van Vuuren, 2009; Asthon *et al*., 2001), manufacturing industries, electricity (power generation), government activities, and agricultural activities (DWAF, 2005). The lower Olifants River enters the KNP at the Phalaborwa area and joins the Letaba River before flowing in the direction of the eastern border of the KNP (Van Vuuren, 2010; Van Vuuren, 2009). The flow dynamics in the catchment have been significantly obstructed, thus restricting the flow.

The Letaba River catchment covers 13 670 km^2 and is divided in two major rivers namely the Lesser Letaba River and the Greater Letaba River. The Greater Letaba River has 20 major dams that are used for agricultural and domestic purposes. On the eastern side of the KNP, the two rivers flow together where they join the Olifants River approximately 30 km up-stream of the Mozambique border at the Olifants Gorge. Upstream of the KNP, the Letaba Rivers flow through gold, phosphate and vermiculite mining areas (Asthon *et al*., 2001).

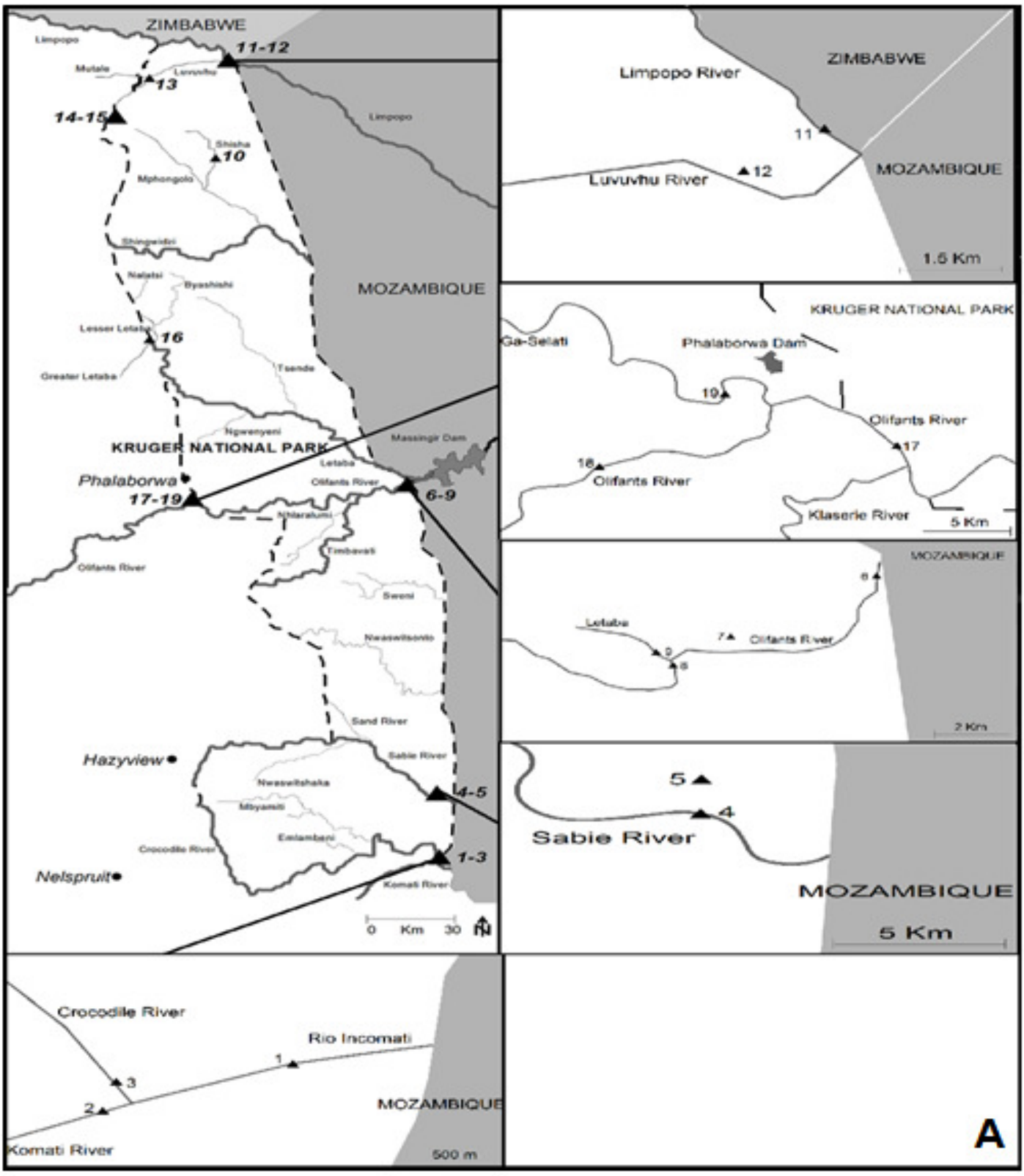


Figure 1: Major river systems that flow through the KNP.

The Sabie River originates in the Drakensberg at 2 130 m above mean sea level, where it drops into the Lowveld and joins the Sand River inside the KNP; this forms the Sabie-Sands Catchment area (RHP, 2008). This catchment forms part of the larger Inkomati System. The Komati River (also referred to as the Nkomati River) and its tributaries pass through large agricultural areas (mainly sugar cane) in both South Africa and Swaziland and numerous small settlements. It joins the Crocodile River, which forms the southern border of the KNP (DWA, 2009b; CCCE, 2000). The southern banks of the Crocodile River are lined with farms that mainly cultivate sugar cane. Sources of contaminants can be from urban run-off, agriculture, paper mills, timber manufactures as well as nickel and gold mines (Van Vuuren, 2010; Asthon *et al*., 2001).

1.2 The Massingir Dam

The KNP Rivers flow towards Mozambique where they ultimately flow into the Indian Ocean. The Olifants and Letaba Rivers, flow into the Olifants Gorge where it enters the upper reaches of the Massingir Dam (Heath *et al*., 2010). The Massingir Dam was built in the 1970s to supply water and food to the Mozambican locals. The Massingir Dam wall was restored in 2008 and later additional top wall sluices were added to the dam wall (ADF, 2007). The restoration of the dam wall potentially provided Mozambique with an estimated 2 480 million m^3 of stored water (ADF, 2007).

After the Massingir Dam wall was raised, the water flow slowed down even more than before and larger quantities of the fine suspended particles of the two rivers settled in one area (Osthoff *et al*., 2010). The Olifants Gorge was then covered with fine sediment deposits, with only a few seasonal sandbanks where crocodiles could bask in the sun. These few remaining seasonal sandbanks could not be used as permanent nesting sites for the crocodiles (Huchzermeyer *et al*., 2011). This was also the area where the most infected crocodiles were found (Heath *et al*., 2010).

One of the current working hypotheses for the crocodile mortalities (as stated by Osthoff *et al*., 2010) is that the Olifants Gorge, 8.3 km downstream of the confluence of the Olifants and Letaba Rivers, acts as a sediment trap for the contaminants from the two rivers.

Other hypotheses include environmental impacts such as microcystins from cyanobacteria in the water and food of the crocodiles (Myburg and Botha, 2009); consumption of local catfish (*Clarias garpienus*) with steatitis (Huchzermeyer *et al*., 2011), consumption by dead and rancid fish

(Ashton, 2010), or large scale anthropogenic ecosystem changes. Predators tend to feed on unhealthy fish as they make easier prey and subsequently, they become affected (Huchzermeyer, 2012; Woodborne *et al*., 2012). Lastly, Oberholster *et al*. (2012), found very high concentrations of aluminium in the fat of the Nile tilapia (*Oreochromis niloticus*), which indicates that this element may have influenced the health of the crocodiles.

During the rainy season, the river flow rate increases and the volume of water that is transported increases. With a strong water current, large amounts of pollutants and sediment can be transported from both the Olifants and the Letaba rivers and deposited in the Olifants Gorge, where the water current slows down, thus causing an accumulation of sediment in which various xenobiotics and contaminants might be trapped. Another explanation is that during the dry season, pollutants accumulate upstream from the Olifants Gorge. Following high rainfall, the pollutants are likely flushed into the Olifants Gorge, causing a spike in the contaminant concentrations in the area (WISA, 2012).

The raising of another dam wall may also explain the crocodile deaths in the in Lake Flag Boshielo, downstream from Lake Loskop, which was raised by 5 m in 2005. After enough rainfall, the banks flooded, and eliminated most of the basking sites used by large crocodiles. The absence of suitable nesting sites made it difficult for the crocodiles to produce offspring (Ashton, 2010). According to a study done by P.J. Botha, in the Loskop area, the changes to the dam caused a decline of crocodiles from approximately 135 individuals in 1995, to 98 in 2009, with far less reproducing individuals (Ashton, 2010).

1.3 Crocodile mortalities

The Nile crocodile (*Crocodylus niloticus*) is a large reptile that used to populate most of the waterways of Africa, but their numbers have declined as a result of the destruction of their natural habitat by humans, persecution, as well as the lack of prey. Habitat destruction has had a negative impact on their population growth by destroying breeding grounds; it can take up to a year for a female to find new breeding sites (Musambachime, 1987).

Crocodiles can be viewed as important biological indicators of aquatic ecosystem health, because they spend their lives both on land and in water, and this makes them good sentinels for understanding the ecotoxicological effects of both terrestrial and aquatic environments (Van Vuuren,

2011). The mass mortalities of these ancient predators have never before been recorded in South Africa. Little research is available on the deaths except for the data that was derived from this project or similar projects related to the same events in South Africa.

The largest secure Nile crocodile populations in South Africa can be found in the KwaZulu–Natal province and the KNP. There are two other major game reserves that also have protected areas for the Nile crocodile, namely, Ndumo Game Reserve and Lake St Lucia (Combrink *et al*., 2011). The Nile crocodile has been regarded as endangered or vulnerable species for the last three decades (Groombridge, 1982). Large numbers of unexplained crocodile mortalities were recorded in two regions of the Olifants River (Heath *et al*., 2010), namely, the Loskop Dam, and confluence of Letaba and Olifants Rivers in the KNP.

The Loskop catchment, located south of Groblersdal in the Mpumalanga province and forms part of the Olifants River system. In 2007, the Loskop Dam was in the media, because of fish and serrated hinged terrapin (*Pelusios sinuatus*) mortalities, and shortly thereafter, large numbers of crocodile mortalities were also reported (Oberholster *et al*., 2010). On the 27th of May 2008, a bloated crocodile was spotted at the confluence of the Olifants and Letaba River in the KNP. The Olifants and Letaba rivers join each other 10 km upstream of the Mozambique border (Huchzermeyer *et al*., 2011). After closer inspection of the area, more carcasses in different phases of decomposition were found (Figure 2). At the end of November 2008, 170 crocodiles were reported to have died in that part of the KNP. During the winter of 2009, another series of deaths occurred in the same area as well as in a section of the Sabie River. The mortalities in the Olifants and Letaba River recurred in the winters of 2010 and 2011 (Ferreira & Pienaar, 2011). Over the past four years, 215 dead crocodiles were found within the Olifants River gorge area. Both apparently healthy and affected crocodiles were always present in the same area. After an aerial survey to assess the summer floods in 2012, several infected crocodiles were spotted yet again by the KNP game rangers (personal communication: Mr Danie Pienaar, Head of the Department for Scientific Services at the Kruger National Park).

Figure 2: Crocodile carcasses in different phases of decomposition. (A) A bloated crocodile carcass. (B) A crocodile in advanced stage of decomposition. (C) A carcass floating belly-up in the water.

After the initial mortality reports of 2008, researchers from different institutions in South Africa including various universities (especially the North-West University), South African Police Service's (SAPS) forensic science, private sector and the Scientific Services of South African National Parks (SANParks) started investigating the possible causes of these deaths. As a result of this, a multidisciplinary research programme under the support of the Consortium for the Restoration of the Olifants Catchment (CROC) initiative was established to address the cause of the crocodile mortalities in the KNP (Huchzermeyer *et al*., 2011, Van Vuuren, 2009).

1.4 Pansteatitis

Autopsies performed on crocodiles carcasses revealed that they were affected by pansteatitis. When fat necrosis (damage caused by disease or infections to the fat cells) occurs in all of the fat deposits

in the body it is called 'pansteatitis' (Huchzermeyer, 2003). This disease causes the fat colouration to change from white (Figure 3–A) to yellow or brown (Figure 3–B) and is used as an indicator of the disease. Pansteatitis may apparently be caused, inter alia, by lipid peroxidation due to oxidative damage in an organism (Kotin & Falk, 1963).

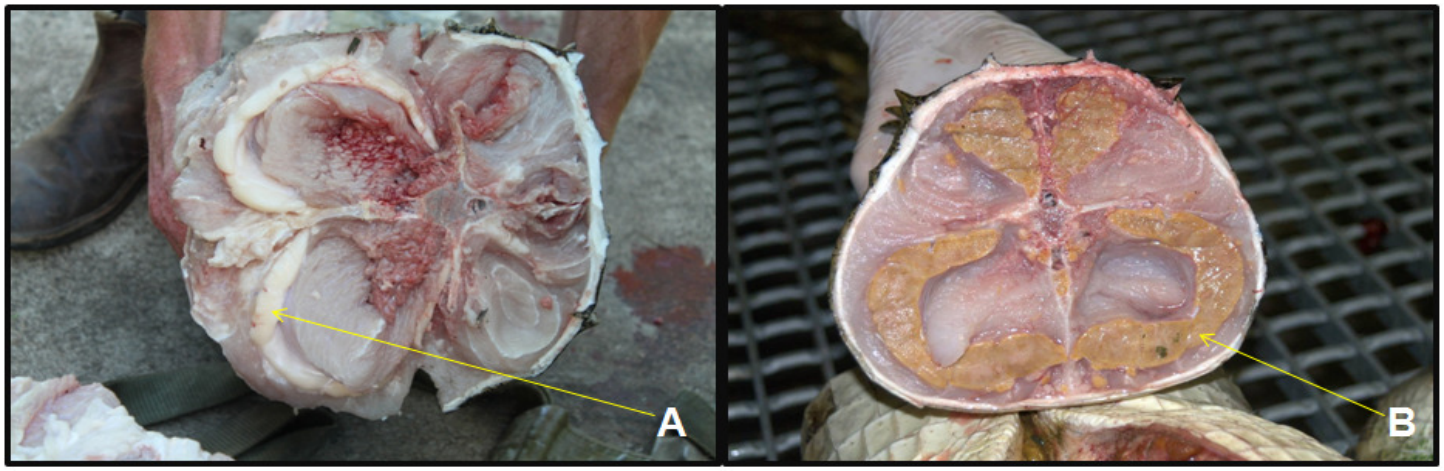


Figure 3: Comparison between healthy fat and affected fat. (A) The tail fat of a healthy crocodile without steatites. Note the healthy white colour of the fat. (B) The fat of an affected crocodile. Note the yellowish appearance of the fat layer.

The fatty tissue dies and undergoes saponification or also known as hardening of the fat. Due to these characteristics, saponified fat can no longer be used by the crocodile as a source of energy (Huchzermeyer, 2003). If the tail fat is saponified, it can also inhibit movement and the affected crocodile has difficulty swimming and walking. Inflammation and discolouration of the fat often seems to result from vitamin E deficiency (Osthoff *et al*., 2010).

Vitamin E functions as a biological antioxidant that protects the tissues (primarily the membranes) against waste products produced by oxidation and/or metabolism of lipids. Factors such as diet, environmental stressors such as extreme temperatures and contamination, plasma cholesterol levels and seasonal variation, have an impact on the dietary availability of vitamin E in animals (Dierenfeld, 1989). In laboratory studies, research has found that animals with vitamin E deficiency can be supplemented with Se, as it is both an antioxidant and an O_2 scavenger. The O_2 scavenger function is to remove reactive oxygen species (ROS), thus protecting the organisms from oxidative stress (Doytte *et* al., 1997; Klaunig *et al*., 1998).

Pansteatitis has been recorded in various instances, affecting wild, captive and domestic animals such as domestic cats (Fytianou *et al*., 2005), captive blue fin tuna *Thunnus thynnus*, (Roberts &

Agius, 2008), captive white sturgeon, *Acipenser transmontanus* (Guarda *et al*., 1997), wild heron *ssp*. (Myburgh & Botha, 2009; Pollock *et al*., 1999), red-tailed hawk, *Buteo Jamaicensis* (Wong *et al*., 1999) and captive marmosets *Callithrix spp*. (Juan-Sallés *et al*., 2012). Most of these cases can be associated with the ingestion of either rancid fish oils or pansteatitis infected fish (Roberts & Agius, 2008; Wong *et al*., 1999).

In 2008, the pansteatitis of the crocodiles in the KNP did not appear to be linked to fish mortalities as there were none seen. However, in July 2009, fish mortalities did occur in the Olifants Gorge. The fish species mostly affected were the sharp tooth catfish, *Clarias gariepinus* (Burchell). According to Huchzermeyer *et al*. (2011), characteristic brown/yellow fat cells were noted in the dead fish. The studies indicated that the fish in that specific area were also affected with pansteatitis. The crocodile and fish mortalities occurred at the onset of winter. There were higher numbers of deaths in the middle of the winter when the temperatures were at their lowest. The characteristics of the disease, in addition to the low winter temperatures, caused the crocodiles to become less mobile, making them vulnerable and incapable of accessing food (Ashton, 2010; Osthoff *et al*., 2010; Belgrano *et al*., 2005; Guggisberg, 1972).

1.5 Crocodile Toxicology

Very little is known about the chemical toxicology of crocodiles that will lead to mortality. The authoritative book on crocodiles by the well-known South African crocodile veterinarian Mr Fritz Huchzermeyer (2003) does not refer to this aspect. Bouwman et al. (2014) lists all the known published sources dealing with environmental pollutants and crocodile eggs in the wild, and none of these were associated with mortalities, nor could any studies be found about environmental pollutants associated with crocodile mortalities. This dearth of information has hampered the search for potential chemical causes of the mass mortality in the KNP. The current study is under the same restrictions.

The only option to investigate this matter with the available data is to compare the contaminant concentrations in sediments from the sites where the crocodiles died with sites from sites where they did not and apparently healthy, as well as comparisons with international sediment quality guidelines. Small differences in concentrations are not expected to cause mortalities, so only large differences and exceedances of guideline limits may give an indication. The extent to which elevated

concentrations of pollutants will cause or contribute towards crocodile mortality is unknown, but order-of-magnitude differences and limit exceedances may provide indications about contaminants to be further investigated.

It should be noted though, that in the search for possible chemical causes of pansteatitis, that the mediation of the effects is assumed to be via food and not direct exposure to water or sediments. Given the mobile nature of crocodiles and their prey, the stationary sedimentary concentrations are here considered as proxy of the concentrations likely to be found in the food of the crocodiles, and therefore indicative of possible causation.

1.6 Study objectives

1.6.1 Hypotheses

The concentrations of OMPs and selected elements in the sediment of the Olifants Gorge contributed to the localised mass mortality in its Nile crocodile population.

Because so little is known about crocodile toxicology, the main premise for this study is that the sites where the Crocodiles died should have markedly higher concentrations and exceedances of guideline values of the OMPs and selected elements compared with other sites in the KNP where crocodiles have not died *en masse*.

The null-hypothesis (H_0) of this study therefore is that there are no marked differences in concentrations of the OMPs and selected elements between sites with affected and non-affected crocodiles. The alternate hypothesis (H_1) is that there are statistically significant differences between the pollutant and contaminant concentrations at the sites where the crocodiles died and at the sites where they did not die.

1.6.2 Aims

1. To compare the concentrations of selected elements in the sediment of various rivers in the KNP.
2. To compare the concentrations of organic micro pollutants in the sediment of various rivers in the KNP.
3. To compare *in situ* water quality variables of various rivers in the KNP.
4. To assess whether the concentrations of the pollutants in the sediment can be associated with the crocodile mortalities.

1.6.3 Objectives

I. Determine and compare the total levels of 17 selected elements in the sediment of 18 sites in 11 different rivers in the KNP.

II. Determine and compare the concentrations of DDT, HCH, HCB, PeCB, chlordane, heptachlor, mirex, PCBs, PCDD/Fs and PBDEs (POPs) in the sediment of 11 sites in seven different rivers.

III. To determine and compare the concentrations of the 16 priority PAHs in the sediment of the sites in objective II.

IV. To determine and compare the physical water quality (pH, EC, TDS and temperature) of the sites with existing guidelines for a once-off assessment.

V. To compare the concentrations of the compounds at the different sites with international sediment quality guidelines.

VI. To assess on an overall basis whether chemical pollutants can be linked with the mass mortality events.

Chapter 2: Literature Review

2.1 The Nile crocodile (*Crocodylus niloticus*)

The largest extant reptilians known to man are collectively known as crocodilians. Between the three families' alligators, crocodiles and gavials, there are 23 species. The Nile crocodile (*Crocodylus niloticus*) is one of largest (Figure 4). An adult individual can reach a maximum size of about six meters and a mass of up to 780 kg (NGS, 2011). The body of a Nile crocodile is dark olive to grey with dark cross bands (Figure 4–A). They are covered with thick scales consisting of keratin, except on their backs where the scales are strengthened by bony plates called osteoderms (Burnie, 2004).

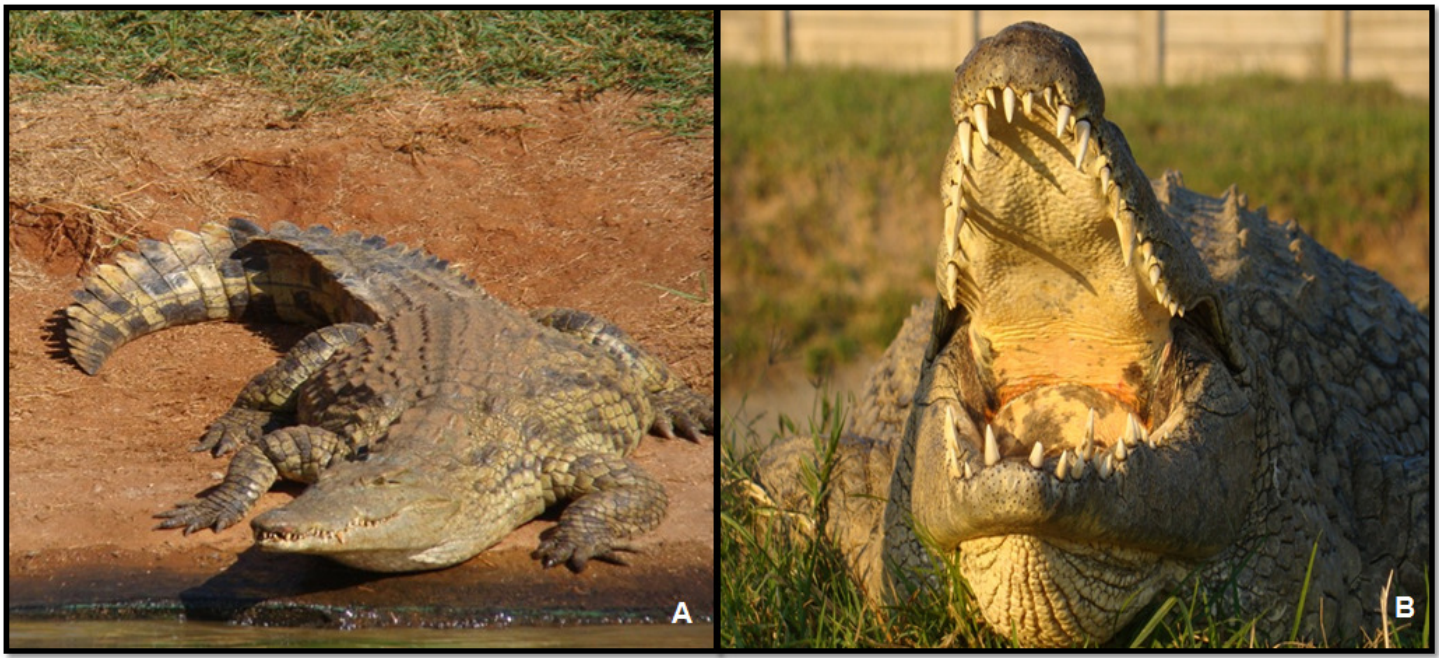


Figure 4: (A) The body of a crocodile is protected by bony-like structures which are flattened dorsoventrally from heat to tail. (B) Crocodiles have long jaws with sharp teeth.

The head and body is flattened dorso-ventrally and is protected with hard osteoderms. The eyes are on top of its head and protected with three eyelids. Its long jaw enables it to bite with great force. The stomach acid of crocodiles is so effective that not only the soft tissues, but also the bone fragments are digested. This allows for these reptiles to swallow large fragments of meat at a time (Njau & Blumenschine, 2006).

The Nile crocodile is a semi-aquatic species and lives throughout sub-Saharan Africa and Madagascar in freshwater rivers and swamps. Nile crocodiles are exothermic reptiles, which mean that they are unable to tolerate low temperatures. They use thermo-gradients in the water to regulate their body temperatures in addition to the sunlight, when basking on river banks (Figure 5; Huchzermeyer, 2003; Guggisberg, 1972). During 2008-2011 crocodile carcasses were found in the water and river banks of Olifants Gorge (Figure 5–the yellow line indicates such an event). In the history of the crocodiles of the KNP, there have been no records of mass mortalities such as those in 2008.

Figure 5: Nile crocodiles basking on a riverbank in the Olifants Gorge. The arrow indicates a crocodile carcass lying near the water's edge.

2.2 The health of the SA environment

The sources of chemical contamination found in SA can be associated with an industrialised country that includes mining, smelting, transport as well as chemical and synthetic manufacturing plants (Combrink., *et al* 2011; Heath., *et al* 2010; Scutte & Pretorius, 1997). Mining activities contributes to chemical and physical impacts on the environment. Chemicals used in ore treatment and smelting can be associated with the change in acidity and/or alkalinity of water systems. The physical impacts include the damming of natural rivers or streams, and the deforestation or de-vegetation of sites

(Ashton *et al*., 2001). Agricultural practises use large areas of land in SA and Africa for commercial and subsistence farming. These activities can have widespread effects in and on the environment (Botha *et al.,* 2011; Bornman *et al*., 2007; Gulumian *et al*., 2005).

The inland rivers that flow into the KNP, and towards the Mozambique border into the Indian Ocean, are some of the largest rivers in SA, which include the Limpopo, Crocodile and Olifants rivers (Ashton *et al*., 2001). These rivers form part of the essence of life for the KNP, and are not just important for the ecology of the KNP but also play an important role in the socio-economic status of this area (Pimbert & Pretty, 1995). The KNP is also one of South Africa's largest tourist attractions and plays an important role in the economic welfare of the country (SANParks, 2006). Protected areas such as the KNP are not only important for the protection of the natural ecosystem, but also represent many cultural, aesthetic and spiritual values to many human communities (Pimbert & Pretty, 1995).

2.3 Water quality guidelines for South Africa

Aquatic ecosystems are defined by the abiotic (physical and chemical) and biotic components. Habitats and ecological processes contained within rivers and their riparian zones, reservoirs, lakes, wetlands and their fringing vegetation are all considered aquatic ecosystems by the South African Water Quality Guideline (SAWQG) (DWAF, 1996a). Volume 7 of the SAWQG was used to assess the *in situ* water quality variables of the studied rivers. The abiotic components such as pH, temperature, electrical conductivity (EC), and total dissolved solids (TDS) are briefly discussed to explain their usefulness as water quality parameters.

The pH of water is measured to determine the activity of hydrogen ions (H^+), since the equilibrium between H^+ and OH^- influences the acidity of the water system. Three major factors that influence the pH is temperature, and inorganic and organic ions. When any of these factors causes a change in pH, it may have severe consequences for the aquatic environment (WHO, 2011; DWAF, 1996a). This also means that pH and temperature are co-variants of each other.

Temperature affects the rate of chemical reaction within an organism, which makes it an important factor in the aquatic environment. Increased temperature of an aquatic environment causes the viscosity, surface tension, compressibility, surface heat, the ionisation constant and the latent heat of

vaporisation to decrease. Conversely, the thermal conductivity and vapour pressure will rise with increased temperature. These factors play important roles in the natural responses of organisms, for example, spawning, migration, hatching and overall development (Dallas & Day, 2004; DWAF, 1996a). Water temperature can be influenced by anthropogenic activities such as irrigation return water, water from heated power stations, and heated industrial discharge waters (Dallas & Day, 2004). The unnatural raising of water temperature has also shown to influence the biochemical and physiological processes that are associated with bioaccumulation within biota (Newman, 2010).

The total dissolved solids (TDS) represent the total quantity of dissolved material and include organic and inorganic, ionised and unionised particulates (Dallas & Day, 2004). Electrical conductivity (EC) is the ability of water to conduct an electrical current. The higher the conductivity, the greater the number of ions dissolved in solution. This means that EC and TDS are co-variants and can be used to determine the number of charged particles in a solution. The TDS value can then be used to determine the salinity of water systems, the higher the TDS value the higher the salinity of the water (Dallas & Day, 2004; DWAF, 1996a).

The surface water quality in the current study was compared to the SAWQG, but there are no SA guidelines for sediment and soils, therefore international sediment quality guidelines had to be used (Gordon & Müller, 2012). According to the Canadian Council of Ministers of the Environment (CCME) 1999a, guideline concentrations aim to protect all forms of aquatic life and all aspects of the aquatic life cycles, including the most sensitive life stage of the most sensitive species over the long term. Sediment quality guidelines are derived from different approaches for example the mechanistic, empirical or consensus approach. The empirical approach is the most popular and includes observed biological responses from single or mixed contaminants from toxicity tests or data from field-collected sediments (Gordon & Müller, 2012).

The Dutch guideline has been chosen because it is more conservative (the highest concentrations which are acceptable to regulators). This means that any exceedance of the most conservative concentrations (i.e. the Dutch guidelines.) implies toxic involvement to the highest trophic levels, in this case the Nile crocodile.

A word of caution should be expressed about the use of SQGs from countries where crocodiles do not occur or have not been taken into account when setting these limits. Crocodiles represent a

trophic level higher than the normal fish predators (e.g. pike, trout, eel, and bass) common to European, Canadian, and New Zealand's freshwaters. It is not clear whether crocodiles were taken into account with the determination of the Australian guidelines. This additional trophic level could account for additional orders of magnitude in bio-accumulation, meaning that protective guidelines elsewhere might not be protective of ecosystems with "super", long-lived, predators such as the Nile crocodile. Further research is therefore warranted to determine if these guidelines are indeed applicable to freshwater systems that host crocodiles.

The Canadian (CCME, 2012), Australian and New Zealand (ANZECC, 2000) and Dutch (NDSQG, 2011) sediment quality guidelines were compared with the data from this study. In order to use the guideline limits for comparison of the organic chemicals as well as the inorganic chemicals, the analysed data needed to be normalised to 1% or 10% total organic carbon (TOC) (as indicated in CCME, 2012; ANZECC, 2000 and NDSQG, 2011). This means that the data needed to be normalised to either 1% TOC for comparison to the Australian/New Zealand and Canadian guidelines; for the Dutch guidelines, the data had to be normalised to 10% TOC.

2.4 Pollutants, contaminants, and xenobiotics

Although the effects of compounds are studied on a single compound basis, humans and wildlife are usually exposed to a mixture of compounds (De Wit, 2002; Davies et *al.,* 1991). It is therefore possible that the mixture of organic compounds and elements in the Olifants and Letaba Rivers may have had an additive or synergistic effect on the crocodiles of the KNP (Heath *et al*., 2010, Van Vuren *et al*., 1994). The Department of Water Affairs and Sanitation (DWS) has an active water monitoring programme within Limpopo and Mpumalanga provinces and although the National Toxicity Monitoring Programme (NTMP) covers organic and inorganic compounds, there are compounds that are not part of the monitoring programme (Heath *et al*., 2010).

Research performed in South Africa showed the presence of organic chemical compounds such as PAHs in sediment and soil (Nieuwoudt *et al*., 2011), brominated flame retardants (BFRs), mirex, HCHs, DDT, and PCBs in bird eggs (Bornman *et al*., 2007, Kimbrough, 1985). High levels of DDT have been reported in breast milk of women living in areas where DDT is still sprayed (Bouwman *et al*., 2006; Bouwman *et al*., 1990). Banned pesticides namely aldrin, chlordanes, hexachlorobenzene and dieldrin were detected in ambient air samples in Durban, South Africa

(Batterman *et al*., 2008). In South Africa, heavy metal contamination has been reported in freshwater fish, soil and sediment samples (Oberholster *et al.,* 2012; Oberholster *et al.,* 2010; Winde, 2009; Roychoudhury & Starke, 2006; Ashton *et al*., 2001).

2.4.1 Persistent Organic Pollutant (POPs)

The persistence of a compound refers to the duration of time it will remain in the environment before it is broken down or degraded into another or less harmful substance (Ritter *et al*., 1995). This means that substances might survive in the environment long after their emissions have ceased (SEPA, 2004). Persistent Organic Pollutants (POPs) are characterised as mostly anthropogenic organic compounds that resist photolytic, chemical and biological degradation in the environment and have the ability to be transported over long distances (SEPA, 2004; Bouwman, 2003, Lerche *et al*., 2002; Osibanjo *et al.,* 2002). These chemicals are everywhere and are even found in parts of the world such as the Arctic, where there is no record of their usage (UNEP, 2011; Fielder, 2003; Lerche *et al*., 2002; Ritter *et al*., 1995). Persistent compounds also have the ability to affect the environment by bioaccumulation and biomagnification in an ecosystem (Bouwman, 2003; Giesy *et al*., 2002; Lerche *et al*., 2002).

After the publication of Rachel Carson's book Silent spring (1962), the public and national governments became more aware of how pesticides can affect non-target organisms, including humans. Out of concern for the possible effects of pesticides, environmental protection agencies started to act against contaminants and the production of hazardous chemicals.

In the mid 1980's the United Nations Environment Program (UNEP) and the Food and Agriculture Organization of the United Nations (FAO), started a voluntary information-exchange program on pesticides and hazardous chemicals. This led to the formation of the Rotterdam Convention on the prior informed consent procedure for certain hazardous chemicals and pesticides in international trade from the early twentieth century (Rotterdam Convention, 2011).

With the rising concern of persistent organic pollutants (POPs) that pose risks to the health of humans and the environment, UNEP requested an international assessment of an initial list of compounds known as the "dirty dozen", to determine if international action was needed. These contaminants included aldrin, dieldrin, endrin, chlordecone, chlordane, hexachlorobenzene (HCB),

heptachlor, mirex, dichlorodiphenyltrichloroethane (DDT), polychlorinated dibenzo-*p*-dioxins (PCDD) and polychlorinated dibenzofurans (collectively PCDD/Fs), polychlorinated biphenyls (PCBs) and toxaphene. Persistence or high environmental stability is one of the important characteristics of POPs, while semi-volatile and high lipophilicity also plays a role in the compounds' ability to enter the atmosphere and be moved over long distances (Corsolini *et al*., 2002; Ritter *et al*., 1995). In June 1996, UNEP requested international action against the aforementioned initial 12 POPs (Stockholm Convention, 2009).

The Stockholm Convention's (SC) aim is to limit and/or eliminate specific POPs on an international level to protect the health of the environment (Bouwman, 2004). South Africa became part of the Convention in September 2002. Although DDT is a toxic compound (Ritter *et al*., 1995), it still has restricted use in some countries to control mosquitoes, which are vectors of malaria (Basel Convention, 2011; Stockholm convention, 2011; Bouwman *et al*., 2006). One of these countries is South Africa.

The original list of 12 POPs has increased since this study was initiated in 2008. New pesticides that were added to the Convention were chlordecone, alpha- and beta hexachlorocyclohexane, lindane (although part of the initial study) and pentachlorobenzene (Stockholm Convention, 2011). Industrial chemicals that were added included hexabromobiphenyl, hexabromodiphynel ether, heptabromodiphenyl ether, pentachlorobenzene, perfluorooctane sulfonoic acid its salts, perfluorooctane sulfonyl fluoride, tetrabromodiphenyl ether and pentabromodiphenyl ether. On the list of by-products, alpha- and beta hexachlorocyclohexane as well as pentachlorobenzene were added (Stockholm Convention, 2011). The main reason all of these compounds are of such a concern is because of their ability to harm humans and animals by, *inter alia*, causing DNA mutations, reproductive defects, and increased risk of cancer (USEPA, 2008).

The different compound classes can be classified by the chemical and compound structure and whether or not the compound is released as an unintended by-product or as an intended end product (Stockholm Convention, 2011; SEPA, 2004). The unintended by-products are PCDD/Fs, PCBs and HCBs. PCDD/Fs and (mono-and non-ortho-substituted congeners) PCBs are collectively known as dioxin-like chemicals (DL). DL chemicals form part of the planar tricyclic aromatic compounds, a class with similar chemical properties containing varying amounts of chlorine (USEPA, 2000). These compounds can be formed, *inter alia*, from inadequate burning of landfills, waste, fossil fuel, leaded

gasoline, incineration process, smouldering of copper cables and waste oil refineries (Stockholm Convention, 2009). Intended end products are compounds that are formulated for a specific reason and these include chemicals such as aldrin, mirex, DDT, heptachlor and lindane, to name a few (Stockholm Convention, 2011).

2.4.2 Polycyclic Aromatic Hydrocarbons (PAHs)

PAHs are a complex hydrocarbon group and can comprise of two or more fused benzoid rings with approximately 100 different combinations. Within this complex group of compounds, there are 16 PAHs regarded as detrimental to the health of humans and animals and are listed as priority pollutants by the United States Environmental Protection Agency (USEPA; 2008). PAHs are categorised into low and high molecular weight PAHs (CCME, 2008). The low molecular weight PAHs (LMW PAHs) has two or three aromatic rings and is mainly released in the gaseous phase. In contrast, the high molecular weight PAHs (HMW PAHs) consist of five or more rings, and is emitted mainly as particulates (LEE & VU, 2010; Tsymbalyuk *et al*., 2011). They occur naturally in the environment but can also be from anthropogenic origin.

The major pathways for PAHs into river ecosystems can be either from atmospheric fallout, urban and industrial discharges, spills and surface runoff. Inadequate burning of products such as coal, wood, tobacco, gas, oil, and garbage can create different types of PAHs (CCME, 2008). Different PAHs occur in mothballs, tar preservatives, blacktop and creosote wood. The molecular weight of PAHs can be used to predict their hydrophobicity, tendency for bioaccumulation, resistance to biodegradation and its overall persistence, because these factors increase with an increase in molecular weight. The compound properties enable them to be transported over long distances (CCME, 2008; USEPA, 2008). The simplest of the 16 USEPA priority pollutants is naphthalene with only two aromatic rings; but PAHs can have up to seven rings such as coronene (Newman, 2010). Human and animal exposure to PAHs to humans and animals may lead to increased rates of DNA mutation (Tsymbalyuk *et al*., 2011), reproductive defects and the increased risk of cancer as these compounds have the ability to act as a carcinogens (CCME, 2008).

2.4.3 Elements

Since the 1850s, the production and use of metals increased at an exponential rate, due to the manufacturing of cars, ships, buildings, and industrial and household equipment. The demand for the metals grew and more mines and factories lead to more environmental contamination (Gaur *et al*., 2005; Müller, 1977). Elements are not only released by factory waste outlets, but also as land surface runoff and from rain precipitation. This can lead to an increase in essential, possibly beneficial, or non-essential metals in water and sediment (Sherameti & Varma, 2010; Barka *et al*., 2001; Winchester & Nifong, 1971). These metals can be active in water bodies or taken up by organisms such as plankton, fish and animals. Metals can be found in the natural environment at small or trace amounts and are necessary for a healthy diet. However, large amounts of any heavy metal or element may cause acute or chronic toxicity (LEF, 2011).

All elements can be classified as metals or non-metals based on loosely defined criteria. Metals usually have high electrical conductivity and lustre, are malleable, and readily form cations (Newman, 2010). However, the term elements will be used here to refer to those elements with toxic effects and that occur in such high concentrations that they cause contamination in the environment. Examples of metals are copper (Cu), cadmium (Cd), total chromium (Cr), lead (Pb), mercury (Hg), zinc (Zn) and vanadium (V) (Newman, 2010; WHO, 2007; Barka *et al*., 2001, Van Vuren *et al*., 1994). Some non-metals also have toxic effects on the ecosystem such as uranium (U), arsenic (As) and selenium (Se), which are often released into natural aquatic systems as a product or waste from anthropogenic activities (WNA, 2011). Monitored discharge points in the West Rand, South Africa showed an annual average discard of 3.5 tons of dissolved uranium from gold mines over the last 10 years (Winde, 2009). Elements such as Cd, Pb, Zn, Cr and Hg can have toxicity even at trace amounts found in pipes, drains, old paint supplies, batteries and pesticides. These compounds are often by-products from mines and industries and occur in domestic run-off (LEF, 2011; Fatoki & Mathabatha, 2001).

2.5 Characteristics of elements and OMPs and their behaviour in the environment

The nature of the OMPs and elements studied in this research project are summarised in table format (Tables 1-3). Included in these tables are the indicative applications and uses of the compounds. Many of the compounds are ingredients of everyday products used in industries, agriculture and

household applications. The POPs are summarised in Table 1, with the 16 priority PAHs in Table 2 and Table 3 discusses the 17 selected elements.

The chemical characteristics of the organic pollutants are (Table 1 & 2) these compounds have the ability to bioaccumulate, bioconcentrate, biotransfer and biomagnify in trophic levels in the aquatic and terrestrial environment (MOEW, 2006). The bioaccumulation of contaminants in the food web is a systematic assimilation of contamination from microorganisms to plants to herbivores and predators to such a magnitude where the concentration of contaminants is higher in the organism than in the environment. The contaminant concentration in the organism increases as the amount of contaminants increase in the environment (Arnot & Gobas, 2003). Biotransformation is the biological process where a compound is transformed from one chemical compound to another. This type of transformation involves enzymatic catalysis that can lead to the activation of an inactive compound or the deactivation of an active compound. Biomagnification is defined as the increase of the contaminant concentration from one trophic level to the next. This means that the contaminants are more likely to end up in organisms that are at the top of the food web such as carnivores (UNEP, 2011; Ritter *et al*., 1995). DDT is one of the most studied organic pollutants. The breakdown of *p,p'*-DDT forms two other substances *p,p'*-DDE and *p,p'*-DDD. These two substances are chemically similar to DDT and are very persistent as they bind with lipid-rich tissues in all living organisms. From different parts of the world, data shows that DDT is widely distributed and found in biota, from human breast milk and serum, to bird eggs, animal tissue, sediments and marine fish (Bouwman *et al*., 2006; Corsolini *et al*., 2005; Smit, 1999; Ritter *et al*., 1995; Bouwman *et al*., 1990).

PCBs were manufactured since the early 1930s for industrial purposes, but were banned in the 1980s because of their toxicity. PCBs have 209 different congeners, meaning the composition of the compound can change as there are many combinations of the total number and position of chlorine atoms on the molecule (Basel Convention, 2011). In general, the persistence of different PCB congeners increase according to the number of chlorine atoms, therefore degradation in the environment depends on the degree of chlorination of the biphenyls (UNEP, 2011, Kimbrough, 1985). Some POPs are formed because of incomplete combustion and have the same physical and chemical characteristics as intentionally produced chemicals such as the pesticides and PCBs. Hexachlorobenzene (HCB), pentachlorobenzene (PeCB), PCBs, and dioxins are formed by thermal processes involving organic matter and chlorine.

Dioxin and DL-PCB compounds are semi volatile and can occur in both gaseous and particulate phases in the atmosphere. Dioxin like chemicals (DLC) are not just persistent in the atmosphere, but they are also associated with particulate matter, and can be transported over long distances before being removed from the atmosphere, mostly by precipitation (UNEP, 2011; Mackay *et al*., 1992).

The chemical characteristics of the OMPs (Table 1 & 2) allow them to be transported over large distances via biota, air, precipitation and water. The OMPs are mostly hydrophobic (water solubility listed in Table 1 & 2) explaining their long half-lives. Atmospheric transport is a dominant pathway that determines the fate and distribution of PAHs and POPs (Tanabe *et al*., 1982). The compounds are released into the atmosphere at low latitudes to be returned to the earth via precipitation at mid-latitudes; from there they are volatilised again and transported to even higher latitudes at the Polar Regions. They are then returned to the earth mainly via snow, and because of the cold, stay trapped in the ice for long periods, and/or get taken up by polar biota. This movement of the pollutants: consecutive evaporation and deposition, has been termed the grasshopper effect (Corsolini*et al*., 2002).

As indicated, the chemical compounds have different characteristics. It is because of their hydrophobic nature (low water solubility; Table 1 & 2) that the organic chemical compounds bind to or dissolve in fats or lipids rather than remain dissolved in water. This means that when a compound is released into water, it will strongly bind to substances in this environment with high lipid content, than stay in the water phase. Substrates in the environment with high lipid content include plants, animals, and sediment (ANZECC, 2000).

The high lipophilicity of the OMPs results in biomagnification and bioconcentration from the environment into organisms (UNEP, 2011, MOEW, 2006, SEPA, 2004). Metals and metalloids are also subjected to a type of biological transformation which results in the elimination or sequestration within the individual. These inorganic compounds may also bind to plasma-associated ligands and becomes available for removal from the organism without any transformation (Newman, 2010).

Table 1: The chemical characteristics of the POPs studied as well as some of their applications

Compound name and abbreviation	Water solubility (µg/ℓ)	Application	Half-life in the environment	References
Chlordanes (Chlor)	56	Used as a pesticide for both residential and agricultural applications.	37–3500 days in soil	ATSDR, 2012; Standberg *et al,* 1998
Dichlorodiphenyltrichloroethane (p,p'-DDT)	25	DTT was commercially used as a pesticide over the world is now restricted to malaria control. DDT breaks down to from metabolites	7–15 yr in soil 14–21 yr in sediment	UNEP, 2011; CCME, 1999b
Dichlorodiphenyldichloroethylene (p,p'-DDE)	120			
Dichlorodiphenyldichloroethane (p,p'-DDD)	90			
α-Hexachlorocyclohexane (α-HCH)	0.695	Mainly used as insecticide to protect seeds, and as treatment for poultry and livestock. α-HCH and β-HCH are major by-product in the manufacturing of lindane (γ-HCH). Lindane used in creams, lotions and shampoos as a scabicide and pediculicide for humans.	88–1146 days	ATSDR, 2012; UNEP, 2011; WHO, 2011; MOEW, 2006; Blakley *et al*., 1999
β-Hexachlorocyclohexane (β-HCH)	5 000			
γ-Hexachlorocyclohexane (γ-HCH)	17 000			
Heptachlor	50	Acts as a stomach and contact insecticide. Was used agriculturally and in households. Is an acute toxicant that has the ability to effect the environment even when used with in regulations	±2 yr in soil	ATSDR, 2012; Basel Convention, 2011; UNEP, 2011; WNO, 2011 MOEW, 2006; WHO, 2004.
Mirex	0.07	Stomach insecticide. In SA used to combat harvester termites. Globally used as fire retardant in plastics, rubber, paint, paper and electrical goods.	<10 yr in soil	Basel Convention, 2011; UNEP, 2011; Ritter *et al*., 1995.

Table 1 (continue): The chemical characteristics of the POPs studied as well as some of their applications

Compound name and abbreviation	Water solubility (µg/ℓ)	Application	Half-life in the environment	References
Polybrominated diphenyl ethers (PBDE)	0.0009-30	Brominated flame retardants that are widely used by industries and the general public. To prevent combustion of consumer goods	130 – 700 days in soils	ATSDR, 2012; US EPA, 2010; De Wit, 2002
Pentachlorobenzene (PeCB)	180	Fungicide, flame retardant, dyestuff carriers and as heat transferring materials. Traces can be found in pesticides; endosulfan, pentachloronitrobenzene and atrazine.	up to 6 years in temperate organic soil and sediments	UNEP, 2012; Stockholm convention, 2011
Hexachlorobenzene (HCB)	40	By-product and used as a fungicide	Aerobic: 2.7 – 22.9 yr. Anaerobic:0.5 – 4.2 yr.	UNEP, 2012; Basel Convention, 2011; UNEP, 2011; MOEW, 2006
Polychlorinated -dibenzo-*p*-dioxins (PCDDs)	0.12 -7.4 x 10^{-8}	PCDD released through the production of pesticides and other chlorinated substances, whereas PCDFs are released by the production of PCBs and thermal processes. There are 75 PCDD and 135 PCDF congeners where the most toxic are the 17 PCDD/F congeners that have chlorine atoms on the 2,3,7 and 8 lateral positions	10 – 100 yr	ATSDR, 2012; SEPA, 2004; CCME, 2001; Sinkkonen & Paasivirta, 2000.
Polychlorinated dibenzofurans (PCDFs)				
Polychlorinated biphenyl (PCBs)	0.01 -0.1	PCBs had valuable industrial purposes because of the stability of the chemical. Used in electrical equipment, -fluids and containers. They consist of two benzene rings joined with a carbon-carbon bond, with chlorine atoms on any or all of the remaining 10 carbon atoms.	Up to 3 years in soil	UNEP, 2011; MOEW, 2006

Table 2: Chemical characteristics and application of the 16 priority PAHs

Compound name and abbreviation	Water solubility (µg/ℓ)	Usage	Half-life in the environment	References
Acenaphthylene (Acy)	3930	Forms part of the incomplete combustion of coal, wood, petroleum, municipal trash-medical- incineration, metalworking, oil refining, mining, chemical production and the electrical industry.		ATSDR, 2012; EC, 2001; EC, 2001; EA, 1999; ATSDR, 1995 Mackay *et al*., 1992
Acenaphthene (Ace)	1930	Processing of certain food, intermediate compound in the pharmaceutical & photographic industry. Small amounts in soaps, insecticides, fungicides, plastics, pigments and dyes.		ATSDR, 2012; BCME, 2012; EC, 2001; ATSDR, 1995; Mackay *et al*., 1992
Anthracene (Ant)	76	Wood preservatives and dyes.		ATSDR, 2012; BCME, 2012; EC, 2001; ATSDR, 1995; Mackay *et al*., 1992
Benzo(a)anthracene (BaA)	10	Forms part of the incomplete combustion of coal, wood, petroleum, municipal trash-medical- incineration, metalworking, oil refining, mining, chemical production and the electrical industry.	3 – 300 weeks	ATSDR, 2012; EC, 2001; EA, 1999; ATSDR, 1995; Mackay *et al*., 1992
Benzo(a)pyrene (BaP)	2.3			
Benzo(b)fluoranthene (BbF)	1.2			
Benzo(bjk)fluoranthene (BkF)	0.76			
Benzo[ghi]perylene (BgP)	0.26			
Chrysene (Chr)	2.8			
Dibezo(*a,h*)anthracene (DaA)	0.5			

Table 2 (Continue): Chemical characteristics and application of the 16 priority PAH's

Compound name and abbreviation	Water solubility (µg/ℓ)	Usage	Half-life in the environment	References
Fluoranthene (Fla)	260	Manufacture of dyes, pharmaceuticals and agrochemicals		ATSDR, 2012; BCME, 2012; EC, 2001; ATSDR, 1995; Mackay *et al*., 1992
Fluorene (Fle)	1980	Forms part of the incomplete combustion of coal, wood, petroleum, municipal trash-medical- incineration, metalworking, oil refining, mining, chemical production and the electrical industry		ATSDR, 2012; EC, 2001; EA, 1999; ATSDR, 1995; Mackay *et al*., 1992
Indeno[1,2,3]pyrene (IcP)	62			
Naphthalene (Nap)	31700	Production of phthalic anhydride, carbaryl insecticide, beta-naphthol, tanning agents, moth repellent & surfactants. It is also found naturally in fossil fuels.		ATSDR, 2012; BCME, 2012; USEPA_a, 2012; EC, 2001; ATSDR, 1995 Mackay *et al*., 1992
Phenanthrene (Phe)	1200	Manufacture of pesticides and resins		ATSDR, 2012; EC, 2001; ATSDR, 1995; Mackay *et al*., 1992
Pyrene (Pyr)	77	Manufacture of pigments		ATSDR, 2012; EC, 2001; ATSDR, 1995; Mackay *et al*., 1992

Metallic emissions into the atmosphere have a short atmospheric residence time of a few days at most. Metals do not accumulate in the atmosphere and do not (with some exceptions) travel far from the point of origin. Therefore, because of the relative short distance of airborne travel, metals tend to fall out and accumulate on land surfaces close to the emission source (WHO 2007, Ayres, 1992). Chemicals such as Cd, Pb and Hg are released into the atmosphere mainly by industries and subsequently settle out to non-emission point source areas where they may bioconcentrate (WHO, 2007). As mentioned, crocodiles are mega predators and play an important role in maintaining the structure and function of freshwater ecosystems (Van Vuuren, 2011, Frank *et al.,* 2005).

Table 3: Chemical characteristics, application and health effects of selected elements that could conceivably influence the health of crocodiles in the KNP

Compound name	Usage	Health effects	References
Arsenic (As) Non-metal	Processing of ores and minerals and can be found in pesticides. Also used in wood preservative and special types of glass. Distributes easily in the environment	Causes anaemia, lung irritation, skin changes, hart disruptions, brain damage and it can damage the DNA. Carcinogenic and can cause skin, lung, liver and lymphatic cancers	Lenntech 2011; Ayres, 1992; Pershagen, 1981
Barium (Ba) Metal	Rubber production, lubricating oil additives, fuel synthesis, fuel combustion, phosphate fertilizer, sewage sludge	Causes cardiovascular, gastrointestinal and reproduction defects	ATSDR, 2012; Lenntech 2011
Cadmium (Cd) Transition metal	Non-essential toxic element that enters the environment through various industrial processes. Three-fourths is used in Ni-Cd batteries and the remaining is used for pigments, coatings and plating but in some cases as a stabiliser for plastics such as PVCs	Neurological, bone and cardiovascular diseases and is carcinogenic. Inhibits/ increases in testosterone production as well as modification of pituitary hormone secretion, thus acting as a endocrine disrupting compound (EDC)	Lenntech 2011; Henson & Chedrese, 2004, Hilscherova *et al*., 2000; Ayres, 1992
Chromium (Cr) Transition metal	Tanning of leather, dyes, paints, moulds for the firing of bricks. Metal plating to resist corrosion and gives a shiny finish.	Respiratory problems, weaken immune systems, lung cancer, alteration of genetic material, death	Lenntech 2011
Cobalt (Co) Transition metal	Mainly used as alloys, dying agents for paints or inks and as catalysts for the petroleum and chemical industries it is also a by-product from nickel and copper mining.	Vomiting, vision problems, heart problems and thyroid damage. It is also categorised as a carcinogenic compound. Inhibits/ increases testosterone production (EDC)	Lenntech 2011; Hilscherova *et al*., 2000
Copper (Cu) Transition metal	Used in electrical equipment and can be found in high concentrations in mine discharge. Fungicides and insecticides. Rubber, phosphate fertilizers and sewage sludge.	Inhibits/ increases in testosterone production (EDC)	Lenntech 2011; Hilscherova *et al*., 2000; Laskey & Phelps, 1991
Iron (Fe) Transition metal	Most used metal in the world and can be found in food containers, cars, tools, washing machines and cargo ships as to name some examples	It may cause conjunctivitis, choroiditis and retinitis if in long term contact with tissue. However it is considered a carcinogenic	Lenntech, 2011
Iodine (I) Non-Metal	Commonly used as water disinfectant, painting inks and dyes. It is added to table salt and as animal supplements. Some forms of iodine are used in medicines.	Different forms of I have different effects on the environment. The elemental I is toxic whereas the iodine in table salts are essential. Radioactive I can cause cancer	CANSA, 2012; Lenntech, 2011; WHO, 2011.

Table 3 (continue): Chemical characteristics, application and health effects of selected elements that could conceivably influence the health of crocodiles in the KNP

Compound name	Usage	Health effects	References
Lead (Pb) Metal	Is largely used in the petroleum industries. It is still used in lead-based paints, plumbing pipes, staining glass and moulding of fishing sinkers	Acute effects include anaemia, behavioural changes or sudden death. Neurological, bone and cardiovascular diseases and has carcinogenic effects. Delayed sexual maturation, suppression of sex steroid biosynthesis	Lenntech 2011; Pokras & Kneeland, 2009; Hilscherova et al., 2000; Ronis et al., 1998; Ayres, 1992
Manganese (Mn) Transition metal	Essential in the production of iron and steel. One of the most abundant metals in soils and is mined in South Africa	Fatness, glucose intolerance, blood clotting, skin problems, skeleton disorders, birth defects and neurological symptoms.	Lenntech 2011
Mercury (Hg) Transition metal	In the past it was used as a medical antiseptic, food preservative. To protect ship hulls, interior paint from mould and mildew, paper pulp from bacterial attack and in thermometers	DNA damage and chromosomal damage, disruption of nervous system. Depression or increases in testosterone production (EDC) and Carcinogenic	Lenntech 2011; Hilscherova et al., 2000; Ayres, 1992
Nickel (Ni) Transition metal	Preparation of alloys and the production of stainless steel. It is also used in rechargeable batteries, foundry products, boats propeller shafts and plating.	Lung embolism, respiratory failure, birth defects allergic reactions and heart disorders. It is also carcinogenic and can cause lung, nose, larynx and prostate cancer. Depression or increases in testosterone production (EDC)	Lenntech 2011; Hilscherova et al., 2000
Selenium (Se) Non-metal	Used in electronics such as photocells, light meters and solar cells. It is used to remove colour from glass. Metallic ore mining and smelting. Coal mining and usage as well as agricultural irrigation.	Contact exposure cause brittle hair and deformed nails, rashes, swelling of the skin. Can cause reproductive failure in fish. Uptake exposure of high levels can cause heart and muscle problems.	Lenntech 2011; WHO, 2011; May *et al*., 2008; Lemly, 2002 & 1999.
Silver (Ag) Transition metal	Old photographs and negatives were dispersed into silver. Long-life batteries, cutlery, jewellery and mirrors. It is also used in electrical industry for paints, circuits and computer parts.	Can cause anaemia as well as kidney, eye, liver, lung and brain damage	Lenntech 2011
Uranium (U) Actinide	Used by the military for weapons, fuel for nuclear power plants. Discharged from mining activities	Effects the estrogen receptors (EDC) and can cause kidney disease	Lenntech 2011; Winde, 2009; Craft *et al*., 2004; WNA,2011.
Vanadium (V) Transition metal	Alloys are used in nuclear reactors, high speed air-frames, steel alloys and jet engines as it doesn't deform under high temperatures	Cardiac and vascular disease, inflammation of stomach and intestines. Damage to the nervous system, skin rashes, weakening and DNA changes	Lenntech 2011
Zinc (Zn) Transition metal	Used in producing galvanized steel and iron also used as a pigment in plastics cosmetics, photocopier paper, wallpaper etc.	Depression or increases in testosterone production (EDC), it can biomagnify and accumulate in the environment	Lenntech 2011; Hilscherova et al., 2000

Heavy metals/elements have the ability to accumulate in food, soil, and sediment. More information is provided in Table 3. The accumulation pathways of these heavy metals and elements (e.g., selenium, antimony, arsenic, cadmium, copper, lead, mercury and similar elements) are comparable and can cause adverse effects on the aquatic biota (Ghrefat *et al*., 2006; Van Vuren *et al*., 1994; Long & Morgan, 1991). Studies have shown that anthropogenically-released elements are bioavailable in the receiving environment (May *et al*., 2008; Lemly, 2002; Zovko & Romić, 2011). The typical impacts on water quality caused by coal mining include different chemical reactions such as ion exchanges, mobilisation and precipitation of ions or groups of ions.

2.6 Health effects of organic and inorganic contamination

Studies done in South Africa show that POPs may affect the natural environment as well as humans (Nieuwoudt e*t al*., 2011, Quinn *et al*., 2009, Bouwman *et al*., 2006). Some of the effects of POPs and elements include neurological defects (SEPA, 2004), endocrine disruption, reproductive and developmental problems (MOEW, 2006), liver damage, skin and eye disease, effects such as urogenital birth defects and death (Borman *et al*., 2009; Corsolini *et al*., 2005). As mentioned, crocodiles are mega predators that play an important role in maintaining the structure and function of freshwater ecosystems (Van Vuuren, 2011). These mega predators are classified as carnivores because adult crocodiles will eat anything that is available, including fish, birds and land mammals (Van Vuuren, 2011). Findings from a study in the Olifants River concluded that metals bioaccumulate in the tissues of fish (Avenant-Oldewage & Marx, 2000). The younger crocodiles prey on different main food sources in different life stages which include insects, frogs and crustaceans (Huchzermeyer, 2003; Guggisberg, 1972). Biomagnification is greater in predators that change their diet with age e.g. crocodiles (Newman, 2010). Crocodiles have a long life span and they can reach an average age of 45 years in the wild and up to 80 years in captivity. The effects of bioaccumulation and biomagnification are higher in long-lived animals. Toxic chemicals in predators are relatively higher than in prey (Newman, 2010). Studies conducted on top-level predators in the Aarctic indicated that animals on the top of the food web are exposed to considerable concentrations of toxic chemicals (Corsolini *et al*., 2002).

2.6.1 Carcinogenicity

Carcinogenesis is the induction of a mutation in the genetic material (DNA) of the reproductive cells such as the ova and sperm of animals and humans. Effects that can be associated with carcinogenicity include disturbance of the endocrine system, immune system, reproductive system and the nervous system (USEPA, 2000). Dioxin- and DL-PCB compounds, DDT, cadmium, chlordane, HCB and benzo[*a*]pyrene are examples of known or suspect carcinogens in humans and animals (WHO, 2011; Bouwman, 2003; USEPA, 2000).

2.6.2 Immunotoxicity

POPs, PAHs, and elements as reported by MOEW, 2006; Qing, 2007; SEPA, 2004 can have adverse effects on the immune system function (MOEW, 2006; Qing, 2007; SEPA, 2004; Repetto & Baliga, 1997). The immune system plays a critical role in maintaining a healthy body. Suppression of this system may increase the number and severity of infections and cancers. The bioaccumulation of immunotoxic xenobiotics is more frequently reported in animals in higher trophic levels (Blakley *et al*., 1999; Ross *et al*., 1996). The equilibrium of the immune system is vulnerable to any chemical that is introduced in high concentrations, over long periods of time, or during the embryonic stage in animals (especially those in higher trophic levels) and humans (Blakley *et al*., 1999). Chemicals such as Cd, Pb, Hg, chlordane, heptachlor and PAHs affect the developing immune system, by affecting or destroying the immune system parent stem cells (Sunyer, 2008; Repetto & Baliga, 1997; Bernier *et al., 1995*). OMPs have been found in wild and captive harbour seals that showed significant alteration and suppression of the functioning of the immune system (Blakley *et al*., 1999; Repetto & Baliga, 1997; Ross *et al*., 1996). Xenobiotics can be immunosuppressive or immuno-potentiating which can cause histopathological effects in the immune tissues and organs (bone morrow, thymus, spleen and lymph nodes). Cellular pathology that can be modified includes; abnormal proliferation of stem cells; altered maturation of immuno-competent cells; changes in B and T cell subpopulations; and functional alterations of immuno-competent cells, which are classified as the altered humoral-mediated immunity, cell-mediated immunity, or nonspecific responses (Blakley *et al*., 1999).

2.6.3 Endocrine disruption

The exposure of organisms to OMPs and elements may cause interference of the synthesis, secretion, transport, metabolism, binding action or elimination of natural hormones that are present in the body of humans and animals (Diamanti-Kandarakis *et al.,* 2009; USEPA, 2000; Hilscherova *et al*., 2000). Compounds with the ability to manipulate or interfere with the natural endocrine systems are collectively known as endocrine disrupting compounds (EDCs) (Barceló & Kettrup, 2004).

If a compound with estrogenic or androgenic abilities enters an organism it could activate the endogenous processes. An example of oestradiol's role is sex determination in certain fish species and the preparation of females for reproduction (Baroiller *et al*., 1999). Problems are caused when an increased concentration of oestradiol-like compounds in an aquatic environment stimulates physiological processes in male fish that are usually only active in females (Tilton *et al*., 2002). Androgens play a critical role in the development and maintenance of the male reproductive system and other physiological targets, predominantly in males. The role of androgen receptors (AR) in males are very much the same as oestrogen receptors (ER) in females (Janošek *et al*., 2006). Anti-estrogenic or anti–androgenic compounds bind to the receptors but inhibit the transcription and translation of the endogenous processes (Giesy *et al.*, 2002, Sonnenschein & Soto, 1998). Compounds that can cause EDC effects in the receiving environment include; DDT (Kelce *et al*., 1994), endosulfan (Legler *et al*., 1999), lindane (Walsh & Stocco, 2000) Cu (Laskey & Phelps, 1991), U (Craft *et al*., 2004), Pb (Ronis *et al*., 1998), PCBs (Bolong *et al*., 2009) and Benzo(a)pyrene (Thompson *et al*., 2010) to name a few.

DDT is sprayed indoors in rural areas of Africa to control the *Anopheles* mosquito which act as the vector for malaria. Malaria is considered to be one of the main causes of mortality in Africa (Sadasivaiah *et al.,* 2007). *p,p'*-DDT is metabolised to *p,p*'-DDE (1,1–dichloro–2,2-bis(p–chlorophenyl)ethylene) and it is this metabolite that binds to the androgen receptor in the body of animals and humans (Kelce *et al*., 1995). Its potency as an endocrine disruptor is increased due to its long biological half-life estimated to be only slightly less than the average human life span (Stockholm Convention, 2009).

Chapter 3: Material & Methods

3.1 Site background

The KNP is situated on the north-eastern corner of South Africa (Figure 6) and borders Mozambique to the east and Zimbabwe to the north. Two of the Park's borders consist of large rivers; on the northern border, the Limpopo River and on the southern border, the Crocodile River. In addition to these, there are six major rivers that also flow through the KNP's boundaries. The KNP covers 1 948 528 hectares and is home to over 1 100 species of fauna and flora. This subtropical region has a summer rainfall. Annual precipitation varies between 700 mm in the south to 400 mm in the north (KNP, 2011; Gertenbach, 1983). Average temperature ranges between 19^0C–35^0C within the KNP area (SANParks, 2011).

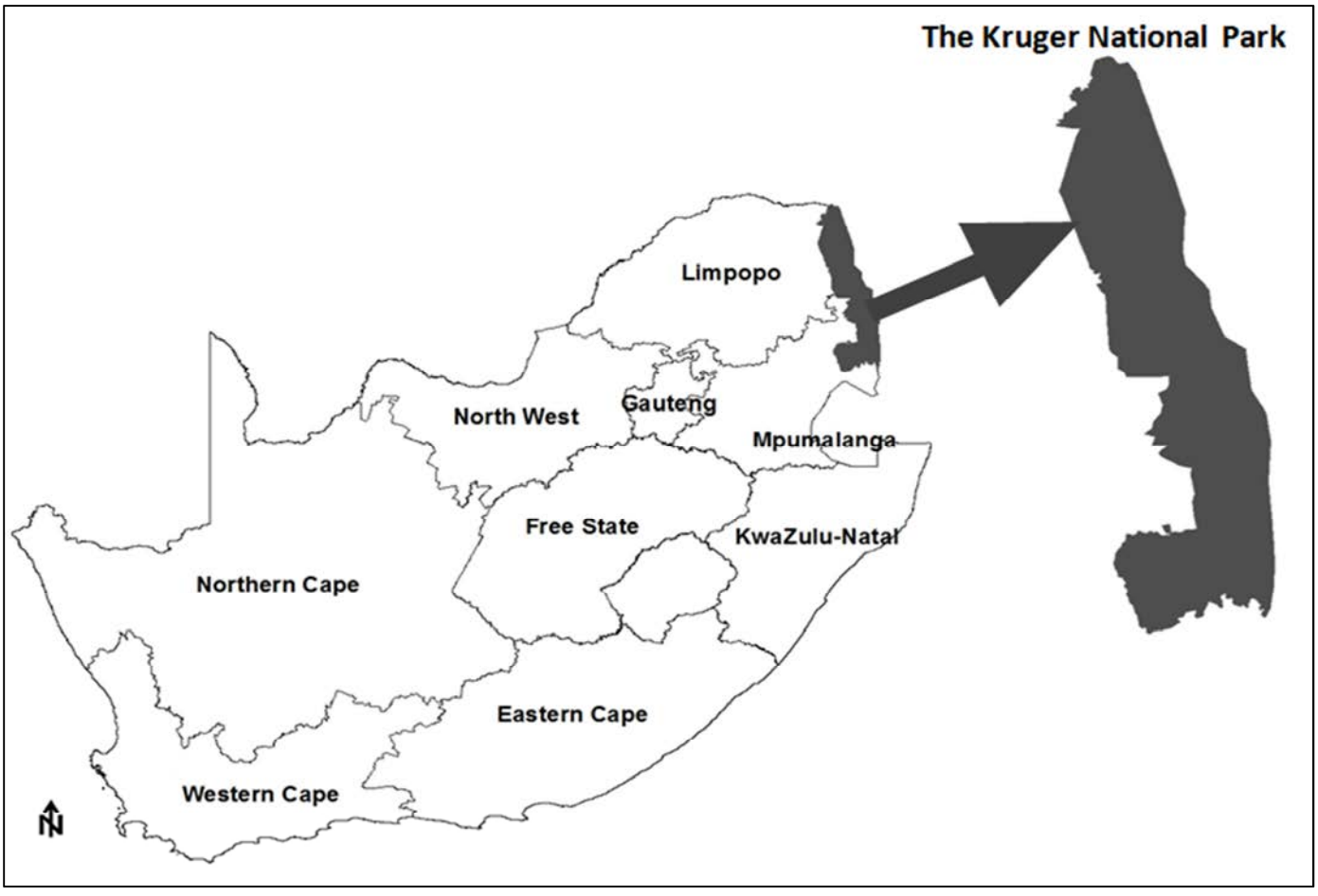


Figure 6: A map illustrating the location of the Kruger National Park on the eastern border of South Africa.

3.2 Sampling sites

The sampling campaign represented a single, cross-sectional, assessment of the major rivers in the KNP. Some of the sampling sites selected were from areas where high concentrations of dead or affected crocodiles were observed. Rivers where there were no recorded crocodile mortalities were also selected. Additional sites needed to be accessible by helicopter. This was the major mode of transport for collection. The premise of the present study was that if the selected chemical pollutants were in any major way involved in the crocodile mortalities, that the differences in concentration and composition between affected and non-affected sites would be marked. Therefore, widely separated sites throughout the KNP were chosen, including areas where there are healthy populations of crocodiles where no mortalities have occurred.

The sites are numbered from one to nineteen (Figure 1A); however only eighteen of these sites will be used as at Site 15 only water samples were collected. The rivers sampled were the Crocodile (1_CrocR, 3_CrocR), Nkomati (2_NkoR), Sabie (4_SabR), Olifants (6_OliR, 8_OliR, 17_OliR, 18_OliR), Letaba (9_LetR, 16_LetR), Limpopo (11_LimR), Luvuvhu (12_LuvR, 14_LuvR), Mutale (13_MutR) and Selati (19_SelR) rivers (Table 4). Two of the sites; Mlondozi pond (5_Mlzi pond) and Shisha River (10_ShisR) were sampled in semi-dry rivers with only a few pools remaining in the riverbed. Lastly site 7 (7_Po) was not listed as a river as this was an isolated rain-fed pond located near the area where the crocodile mortalities occurred. Sites 5_Mlzi pond, 7_Po and 10_ShisR were chosen to act as reference sites, because these were not rivers in full flow and had their entire catchments inside the KNP. The prediction here was that these sites would have lower concentrations and different composition of pollutants carried mainly by air and not water (Winchester& Nifong, 1971). Table 4 includes a brief summary on the coordinates, physical description, and abbreviations of the different sites.

Table 4: A summary of the sampling sites for this study with geographical location and their relation to each other if they occurred within the same river system.

Location	Site abbreviation	Description	Water body	GPS coordinates
Crocodile River	1_CrocR	South of the Lebombo Mountains on the Mozambique border, downstream of its confluence with the Nkomati River.	River	31º 5'89.35"E 25º 2'62.26"S
Nkomati River	2_NkoR	Nkomati before confluence with the Crocodile River.	River	31º 5'84.03"E 25º 2'63.53"S
Crocodile River	3_CrocR	Crocodile River upstream of the confluence with the Nkomati River.	River	31º 5'83.97"E 25º 2'62.03"S
Sabie River	4_SabR	Sabie River an upstream of the Mozambique border.	River	31º 5'96.41"E 25º 0'97.84"S
Mlondozi pond	5_Mlzi pond	Pond of water in a dried up riverbed.	Dry river	31º 5'96.93"E 25º 0'85.68"S
Olifants River	6_OliR	Samples collected from the Olifants Gorge where most dead crocodiles were found.	River	31º 5'29.67"E 23º 5'75.95"S
Pond	7_Po	Rain-fed pool on the side of the Olifants River close to Olifants Gorge. Isolated pool not directly linked to the Olifants River.	Pond	31º 5'04.47"E 23º 5'91.28"S
Olifants River	8_OliR	The confluence of the Olifants and Letaba River were sampled.	River	31º 4'95.53"E 23º 5'93.25"S
Letaba River	9_LetR	Letaba River upstream from the confluence with the Olifants River.	River	31º 4'92.76"E 23º 5'92.39"S
Shisha River	10_ShisR	Pool of water in an otherwise dry river bed.	Dry pool	31º 1'44.74"E 22 º5'06.80"S
Limpopo River	11_LimR	Limpopo Crooks Corner, upstream of the Mozambique border.	River	31 º1'81.57"E 22º 2'52.53"S
Luvuvhu River	12_LuvR	Luvuvhu River just upstream of its confluence with the Limpopo River.	River	31 º1'74.53"E 22º 2'56.99"S
Mutale River	13_MutR	Mutale River just upstream of its confluence with the Luvuvhu River.	River	31º 0'45.46"E 22º 2'69.73"S
Luvuvhu River	14_LuvR	Luvuvhu River just after it entered the KNP.	River	30º 5'41.49"E 22º 4'19.32"S
Letaba River	16_LetR	Where the Letaba River enters the KNP.	River	31º 0'89.06"E 23º 3'89.96"S
Olifants River	17_OliR	Where the Olifants River enters the KN P, directly downstream of its confluence with the Selati River.	River	31º 1'45.06"E 24º 0'40.02"S
Olifants River	18_OliR	Olifants River at the Phalaborwa barrage, upstream of its confluence with the Selati River outside the border of the KNP.	River	31 º0'44.39"E 24º 0'44.40"S
Selati River	19_SelR	Selati River, upstream of its confluence with Olifants River, outside the KNP border.	River	31º 1'02.61"E 24º 0'22.87"S

3.3 Sediment sampling

As previously mentioned, the cross-sectional assessment of all the sites was made possible by the use of a SANParks helicopter (Figure 7). The sample containers were pre-cleaned with a phosphate-free soap, rinsed with warm tap water, followed by rinsing with double distilled water (18 MΩ) and allowed to dry. Before sampling, all equipment was cleaned with high-pressure liquid chromatography grade (HPLC grade) acetone (Burdick & Jackson) to remove all polar organic compounds followed by HPLC grade hexane (Burdick & Jackson) to remove any non-polar organic compounds (USEPA, 2000; USEPA, 1999).

Figure 7: The SANParks helicopter provided access to otherwise remote and inaccessible areas of the KNP.

The samples were collected with a metal spade or cup and mixed in stainless steel containers to ensure homogenous samples (Simpson *et al*., 2005; Figure 8–A). The upper sediment layer was collected at three different locations within a 20 m radius at each sampling site to create a composite sample for each site (Kralik, 1999; Figure 8–B).

Figure 8: (A) Sediment sampled with a metal spade or cup and (B) mixed in pre-cleaned stainless steel containers to ensure homogenous samples.

The *in situ* water quality of the different sites was measured using a handheld multi-probe meter (YSI 569 multiprobe: Figure 9). The YSI 556 multi-probe simultaneously measures the pH, conductivity and temperature, at the same time as the sediment samples were collected. These parameters describe the physical characteristics of the water body on the day of sampling (DWAF, 1998). The basic water quality guidelines for South African aquatic ecosystems, as outlined in volume 7, were used to determine if the surface water had pH, conductivity and temperature variables within the guideline parameters (DWAF, 1996a). Because nitrate, sulphate, magnesium, calcium, fluoride and potassium, were not determined, the following equation was used to derive a TDS value from the measured EC value (DWAF, 1996a):

$$TDS\ (mg/l) = conductivity\ (mS/m)\ \times 6.5$$

Figure 9: The *in situ* water quality of the different sites was measured using a handheld multi-probe meter.

3.4 Preparation of sediment samples

Before chemical analysis commenced, the samples were air-dried in dark conditions, and homogenised through sieving (Simpson *et al*., 2005). All the equipment used was pre-cleaned as described in section 3.3.1. Air-drying of samples took between three to four days. The dry samples were ground with a pre-cleaned mortar and pestle and large organic materials such as sticks and leaves were removed. A copper sieve (mesh size of 0.5 mm) was used to obtain a homogenous sample. The sieving process also helped to remove large amounts of coarse material that could interfere with extraction (Sympson *et al*., 2005). The elemental concentrations for all the sediment samples were determined by Eco-Analytica, North-West University. Due to the high cost of POPs and PAH analysis, only selected samples were shipped to the Norwegian Institute for Air Research (NILU). The samples were mainly selected from areas where there was a high concentration of dead or infected crocodiles as well as sites upstream from these areas, and some reference sites.

3.4.1 Chemical analysis

3.4.1.1 Organic micro pollutants

The compounds analysed in Norway included the “dirty dozen” POPs, PAHs and PBDEs (see Chapter 2, Table 1 & 2). The method used by NILU was of the ISO/IEC-17025; “General requirements for the competence of testing and calibration methods”. The following quality assurances conditions had to be met for unequivocal identification and quantification of the analytes:

1) The retention time had to be in a window of +3 to 0 sec compared to the corresponding labelled isomer;
2) The signal-to-noise ratio had to be more than 3:1 for identification;
3) Blank values had to be determined for the complete clean-up and quantification procedures;
4) The recovery of the internal standards had to be within a 40% to 120% (ISO/IEC–17025).

PAHs were also extracted and analysed using the ISO/IEC-17025 accredited method at NILU as described in Nieuwoudt *et al*., (2011). In short, the samples were spiked with the relevant per–deuterated labelled isotopes (PAHs), and extracted with cyclohexane (pesticide grade, Merck) using a Soxhlet apparatus (Bucheli *et al*., 2004). The bulk of the sample matrix was removed with size

exclusion chromatography and solid phase extraction with silica and activated neutral aluminium oxide (USEPA,1996). The quantification of the 16 USEPA priority PAHs were performed with the use of high-resolution gas chromatography/mass spectrometry (HRGC/HRMS) with an Agilent 6890 N gas chromatograph coupled to an Autospec (Micromass Waters, Manchester UK) mass spectrometer for separation and identification.

For OMPs and dioxin-like compounds, extraction and analysis were performed according to Norwegian accreditation as described by Bengtson Nash *et al.* (2008); Knutzen *et al.* (2003) and Quinn *et al.* (2009). In short, samples were spiked with ^{13}C–labelled (2,3,7,8–chloro substituted PCDD and PCDF congeners, and other OMPs) standards and extracted with toluene. Clean–up and fractionation were done with a multi-column chromatography setup yielding fractions for different compound classes. Quantifications were done with HRGC/HRMS using an Agilent 6890N gas chromatograph coupled to an Autospec (Micromass Waters, Manchester UK) mass spectrometer. All concentrations are on a dry weight basis.

3.4.1.2 Elements

The elements were analysed using inductively-coupled plasma mass spectrometry (ICP/MS). The laboratory used a revised international EPA 3050B method to analyse samples for elements (USEPA, 2011). Briefly, 2 g of air dried sediment was weighed and treated with a mixture of nitric acid (HNO_3), hydrogen peroxide (H_2O_2), hydrochloric acid (HCl) and de–ionised water to completely digest the samples (USEPA, 2011). The metals selected for quantification were chosen due to their known detrimental effects to the environment at high concentrations (as listed in Table 3). All concentrations are on a dry weight basis.

3.5 Data analysis

Basic statistics were performed with Microsoft Office Excel 2007, supplemented with IBM SPSS statistics version 20. To be able to establish whether the null hypothesis or its alternative differs statistically significant, power analysis is needed to determine the number of samples. However, since this study was based on a cross-sectional sampling survey, only a large number of samples would have made effective testing of the hypothesis possible. This is why the international guidelines were used as comparisons to evaluate toxicity of the sediment. Maps, indicating the relative

concentrations of compounds at each of the sampling points, were created using Mapviewer 7 Version 7.0.1675–July 2006 (Golden Software).

Univariate analyses were not performed, due to the small number of samples, each with only one replicate. Principle Component Analysis (PCA) (CANOCO for Window Version 4.5) was therefore used to obtain and compare the fingerprint profiles of the contaminants at the various sites. This statistical analysis method allows one to investigate the distribution patterns for both sites and isomers in a multi-dimensional statistical space based on underlying commonality (Barona & Romero, 1996). In the investigation of contamination profiles using principle component analysis, the influence of concentration must be minimised so that the variation and relationship between pollutants can be highlighted (Howel, 2007). To minimise this effect, the data was log-transformed according to the method proposed by Howel (2007), prior to running the PCA. The log–transformation was done in accordance to the equation where p is the applicable data and g the geometric mean of the dataset.

$$Log\ (p_{ij}/g(p_j)) \text{ where } g(p_j) = (p_{j1}, p_{j2}, p_{j3}\ldots\ldots\ldots p_{jd})^{1/d}$$

Where: p = proportion and
g = geometric mean.

The PCA statistical multivariate method results in the clustering of variables into different groups. The different groups with strongest correlations will thus group together and variables that do not correlate will have different distributions on the factor graphs (Barona & Romero 1996). Variables that were used in the log–transformation were the selected OMPs, elements, and water quality constituents (pH, EC, TDS and temperature).

3.6 Calculation of oxidisable and total organic carbon

Determining the total organic carbon (TOC) content of a sample normalises the concentrations of the compounds to the carbon content of the area where the samples were collected (Simpson *et al*., 2005). Normalised concentrations allows for comparisons between sites, independent of its capacity to absorb the pollutants. The oxidisable organic carbon (OXC) content of the samples was determined using the Walkley-Black method (Schumacher, 2002). This method is fairly easy to perform and cost-effective. It is a quantitative and destructive technique based on the principle of

wet-oxidation followed by ferrous ammonium sulphate titration. To calculate the OXC content, Schumacher (2002) proposed the following equation:

$$OXC\ (\%) = \frac{[Fe(NH_4)_2(SO_4)_2\ used\ for\ blank\ (ml) - Fe(NH_4)_2(SO_4)_2 used\ for\ sample\ (ml)] \times M\ \times 0.3\ \times f}{Mass\ of\ sediment\ sample\ (g)}$$

Where *M* is the concentration of $Fe(NH_4)_2(SO_4)_2$ of 0.5 mol/dm^3 and *f* is the correction factor of 1.4. The TOC content was calculated from the percentage OXC, using the following equation (Sánchez–Monedero *et al*., 1996):

$$TOC\ (\%) = 1.23(OXC) + 0.35$$

3.7 Calculation of the Toxic Equivalents (TEQs)

International sediment quality guidelines report dioxin concentrations as TEQs. To compare the quantified concentrations of PCDD/Fs and DL–PCBs to the guideline concentrations, the concentrations were therefore converted to TEQs. In order to calculate the TEQs, a value describing how toxic each dioxin and dioxin–like congener is compared to the most toxic congener, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8–TCDD), which has a TEF–value of 1 (Van den Berg *et al*., 2006; Van den Berg *et al*., 1998). There are three lists of toxic equivalency factor (TEF) with comparable data sets that can be used for different types of biota (mammals, birds, and fish). There is no TEF-value for reptiles or sediments. Crocodiles hunt and spend time in both terrestrial and aquatic environments which makes it difficult to use either fish or bird TEF values. Thus the WHO 2005 TEF for mammalians were used in this study (as listed in Van den Berg *et al*., 2006). The TEQ is used by multiplying the toxic equivalency factor (TEF) for each congener (Van den Berg *et al*., 2006; Van den Berg *et al*., 1998) with its corresponding concentration. The sum of the TEQs at a site is reported as the TEQ of the site:

$$TEQ = \sum Ci \times TEFi$$

Where C*i* = concentration of each congener and TEF*i* is the corresponding TEF value for that congener.

3.8 Sediment quality parameters (SGPs)

The assessments used provide criteria for the evaluation of elements in freshwater sediment (Singh *et al*., 2003). Analysing the surface sediment can help with understanding the extent, distribution, source and the possible hazards of the elemental contamination. The sediment quality parameters (SQP) were used to quantitatively assess the pollution load for the elements at the sites.

A variety of indices were calculated to determine the quality of the sediment. Some of the indices made use of the quantified concentrations together with the background value of the area. Because there are no background values for SA (according to the SA Geological Survey), the universal upper continental crust (UC) values were adopted from Wederpohl (1995) and used as background values. The UC values are summarised in Table 5 and it includes all the elements that were used to calculate the enrichment factor, geoaccumulation index, as well as to assess the pollution load index. Only 17 elements (heavy metals, metal alloys and non-metals) will be discussed. Aluminium was the 18th element and was only used as the normalising factor.

Table 5: The continental upper crust (UC) values as published by Wederpohl (1995)

Symbol	Name	Upper crust value (µg/kg)
Ag	Silver	0.06
*Al	Aluminium	77 440
As	Arsenic	2.00
Ba	Barium	668
Cd	Cadmium	0.10
Co	Cobalt	11.60
Cr	Chromium	35.00
Cu	Copper	14.30
Fe	Iron	30 890
Hg	Mercury	0.06
I	Iodine	1.40
Mn	Manganese	527
Ni	Nickel	18.60
Pb	Lead	17.00
Se	Selenium	0.08
U	Uranium	2.50
V	Vanadium	53.00
Zn	Zinc	52.00

* The normalisation factor Al, is also added to the list of UP values

There is no consensus regarding the most appropriate sediment constituent to be used for normalisation (Rubio *et al*., 2000). The elements most often used for normalisation are Al, Li, Se, Zr, Ti, Fe or Mn (Roychoudhury & Starke, 2006; Reimann & De Caritat, 2005; Loska *et al*., 1997). What needs to be considered is how immobile the element is and if the sediment originated from freshwater or marine environments (Reimann & De Caritat, 2005). Aluminium was chosen as the sediment samples originated from freshwater sources and it was the most abundant element according to sediment mineral composition studies in all of the sampled sites (See Section 4.7). Therefore Al was not investigated as a contributor to crocodile deaths. The enrichment factor for Al was then used as the normalisation factor for all the other elements. Unpublished data showed no differences in Al concentrations between crocodile tissue from affected and non-affected crocodiles from the KNP.

3.9 Calculation of the contamination factor (CF)

The CF is used to evaluate the contamination in the environment with the use of single substances (Loska *et al*., 1997). It is the ratio between each trace element in the sediment and its background value (Galán *et al.,* 2002). The CF value was categorised according to concentrations of contamination (Table 6) (Bhuiyan *et al*., 2010). The CF was calculated according to:

$$CF = \frac{C_{Heavy\ metal}}{C_{Background}}$$

C $_{Heavy\ metal}$ = quantified concentration

C $_{Background}$ = corresponding background value (Table 5)

Table 6: The different classification levels of CF (Loska *et al*, 1997)

Values for CF	Level of contamination
CF <1	Low contamination
1≤ CF <3	Moderate contamination
3≤ CF < 6	Considerable contamination
6 < CF	Very high contamination

3.10 Calculation of the pollution load index (PLI)

The pollution load index (PLI) is the geometric mean of the concentrations of various trace metals within a specific site, thus providing a comparative mean for assessing the different sampled sites

(Usero *et al*., 1996; Chakravarty & Patigri, 2009). The CF as discussed above for each element was used for the calculation of the pollution load index (PLI) (Galán *et al.,* 2002).

$$PLI\ for\ a\ site = (CF_1 \times CF_2 \times CF_3 \times \ldots\ldots\ldots \times CF_n)^{1/n}$$

If the calculated value for PLI = 0 it indicates pristine; PLI = 1 indicates only baseline levels of pollutants and PLI>1 indicates a deterioration of the site's sediment quality (Mohiuddin *et al*., 2010).

3.11 Calculation of the Geoaccumulation index (Igeo)

Igeo is defined as the quantification of metal accumulation in sediments (Mohiuddin *et al*., 2010). The level of pollution is indicated in Table 7 with values less than 0 as pristine (no pollution) and greater than 6 as extremely polluted. The upper crust values listed in Table 5 were used in this equation as the background value. The values were calculated according to the following equation (Müller, 1969):

$$I\text{geo} = \log_2 \frac{C_{HM\ (Sample)}}{1.5 \times C_{HM\ (Background)}}$$

Where: $C_{HM\ (Sample)}$ = concentration of the heavy metal/element in the sediment samples

$C_{HM\ (Background)}$ = concentration of the heavy metal/ element in the upper crust

A factor 1.5 was introduced to include possible differences of the background values that arise from lithological differences. To interpret the degrees of enrichment above the background values, Müller (1981) introduced seven classes for Igeo values (Table 7).

Table 7: The different Igeo classes to describe pollution severity attributed to any single element (Müller, 1981)

Igeo Classes	Values	Level of pollution
Class 0	Igeo ≤ 0	Pristine
Class 1	0 < Igeo < 1	Unpolluted to moderately polluted
Class 2	1 < Igeo < 2	Moderately polluted
Class 3	2 < Igeo < 3	Moderately to heavily polluted
Class 4	3 < Igeo < 4	Heavily polluted
Class 5	4 < Igeo < 5	Heavily to extremely polluted
Class 6	5 < Igeo < 6	Extremely polluted

3.12 Calculation of enrichment factor (EF)

The EF determines the number of elements that accumulate compared to the background concentration (UC) of the same element. The factor calculation determines the enrichment from anthropogenic sources (Newman, 2010). Normalisation of data can be done by using simple metal/normaliser ratios to more complex regression analysis (Roychoudhury & Starke, 2006). The levels of enrichment can be classified and described according to the scale in Table 8.

$$EF = \frac{C_{HM}/C_{Al\ (Heavy\ metal)}}{C_{HM}/C_{Al\ (Background)}}$$

Where: $C_{HM(Heavy\ metal)}$ = concentration of the heavy metal/ element in the sediment samples

$C_{Al(Heavy\ metal)}$ = concentration of the Aluminium (Al) concentration in the sediment samples

$C_{HM(Background)}$ = concentration of the heavy metal/element in the upper crust

$C_{Al(Background)}$ = concentration of the Aluminium (Al) concentration in the upper crust (Table 5)

Table 8: The different classification keys of EF to classify the levels of enrichment found at different sites (Chen *et al.*, 2007)

Values for EF	Level of enrichment
EF < 3	Minor enrichment
EF =3 to 5	Moderate enrichment
EF= 5 to 10	Moderately severe enrichment
EF = 10 to 25	Severe enrichment
EF = 25 to 50	Very severe enrichment
EF > 50	Extremely severe enrichment

Chapter 4: Results

The analytical results are presented in Figures 10–19, followed by the Sediment Quality Parameters (SQP) to select those compounds that could have contributed to the contamination load at sites where crocodile mortalities occurred. The map summaries were used to visually compare and interpret the data at the different sites. Finally, the PCA of the different groups of compounds and the combination thereof are shown to identify possible differences and similarities in the contamination profiles of the sites. The instances where concentrations of compounds were below the limit of detection (LOD), half the LOD was used. This was done to avoid any zero values when employing the transformation of the results for the PCA. The Howel (2007) method was used to normalise the data for the PCA's as described in Section 3.5. Please note that all concentrations are on a dry-weight (dw) basis.

4.1 Concentrations and congener profiles of organochlorine pollutants

Overall, the highest concentration of the pesticides at all the sites was the sum of the DDT metabolites (*o,p'*–DDE; *o,p'*–DDD; *o,p'*–DDE; *p,p'*–DDE; *p,p'*–DDD and *p,p'*–DDE) (Figure 10). The highest concentration of ΣDDT, Σchlordane, ΣHCH, PeCB and HCB was recorded for 2_NkoR (Figure 10). The lowest concentrations of ΣDDT and Σchlordane were quantifiable at 7_Po, an isolated, rain-fed, reference pool (Figure 10) close to where the crocodiles died. Both heptachlor and mirex were below the LOD at all the sites. The lowest PeCB concentration was detected at 4_SabR with the lowest ΣHCH concentrations at 14_LuvR (Figure 10).

DDT is degraded to DDE and DDD in the environment and it is therefore possible to determine whether the quantified DDT in the sediments was due to recent or historic use. If a ratio is greater than 1 it implicates recent application whilst a ratio of less than 1 would indicate historic use (Figure 10; Gong *et al*., 2007). The relationship between the DDT/DDE+DDD and *p,p'*-DDT is also used to indicate whether or not the presences of DDT is of recent or historic use (Table 9). This is because of *p,p'*-DDE is formed first during the degrading process of DDT (Bouwman *et al*., 2006). A high percentage *p,p'*-DDT is therefore indicative of recent use, as the DDT that is used for malaria control, contains about 75% *p,p'*-DDT, and 15% *o,p'*-DDT (Bouwman *et al.,* 2006). Overall *p,p'*-DDT was quantified at all sites.

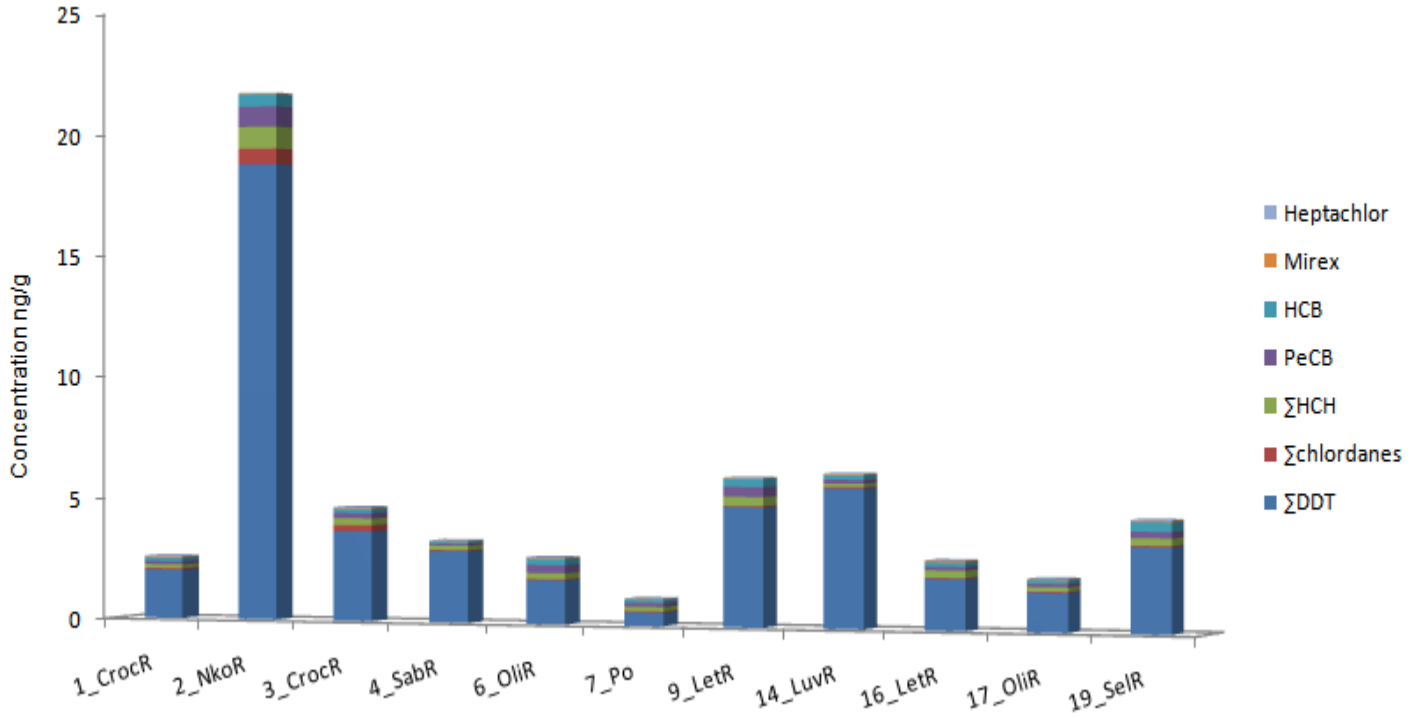


Figure 10: The concentrations (ng/g dw) of various organochlorine pesticides detected at the 11 sites. Sites 6_OliR and 9_LetR were the sites where the crocodiles died, and 7_Po was a rain-fed reference site.

Table 9: The use of DDT ratios to calculate recent or historic use (ng/g).

(ng/g)	1_CrocR	2_NkoR	3_CrocR	4_SabR	6_OliR	7_Po	9_LetR	14_LuvR	16_LetR	17_OliR	19_SelR
ΣDDT	2.00	18.78	3.68	2.91	1.78	0.54	4.83	5.62	2.06	1.57	3.42
DDT/DDE+DDD	1.76	1.21	1.16	1.62	1.14	1.14	1.33	1.63	1.20	1.26	1.21
% DDT	5.18	14.38	10.86	5.63	8.33	11.03	23.93	35.89	14.07	20.02	14.92

4.2 Concentrations and congener profiles of PAHs

Of all the compound classes analysed in this study, the highest concentration at all the sites was the ΣPAHs. One site, 19_SelR, had very high concentrations of ΣPAHs, with Nap representing the greatest contribution to ΣPAHs (Figure 11). The PAH concentrations at this site was 31 times greater than the second highest at site 2_NkoR (Figure 11). When Nap was removed from the composition graph, the relative contribution of the other PAH congeners became clearer (Figure 12).

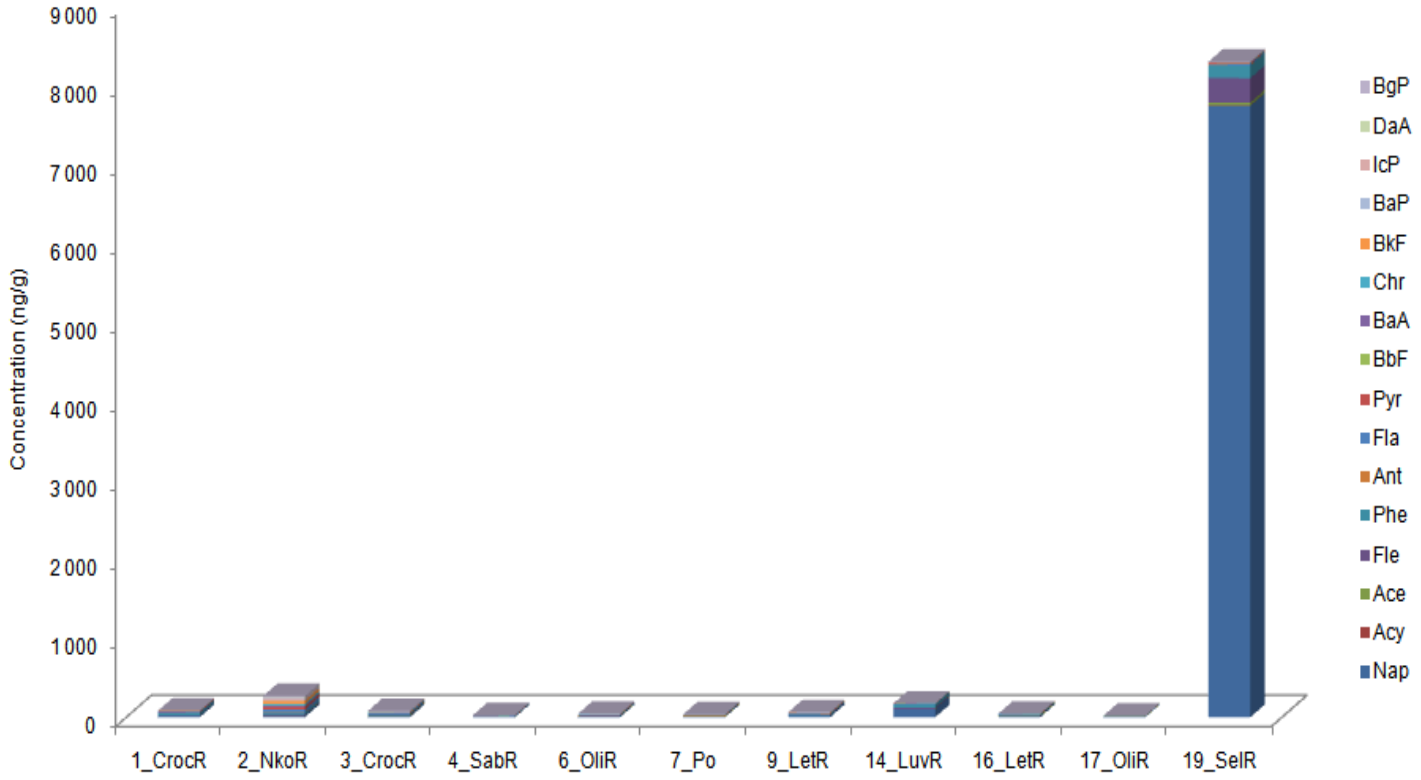


Figure 11: The contribution of the analysed 16 USEPA PAH congeners (ng/g dw), of the 11 sites. Sites 6_OliR and 9_LetR were the sites where crocodiles died, and 7_Po was a rain-fed reference site.

After removing Nap from the graph, it became clear that two other congeners; Fle and Phe also contributed significantly to 19_SelR (Figure 12). The ΣPAH congener with the lowest concentrations at all the sites was BbF, which was below the LOD at all 11 sites (Figure 12). The second highest sediment concentration of PAHs was recorded for 2_NkoR with contributing concentrations from Nap, Phe, Fla, Pyr, Chr, BkF and BgP (Figure 12). The sites where the crocodiles died, as well as the sediment from the rain-fed pool, had relatively low and comparable PAH concentrations.

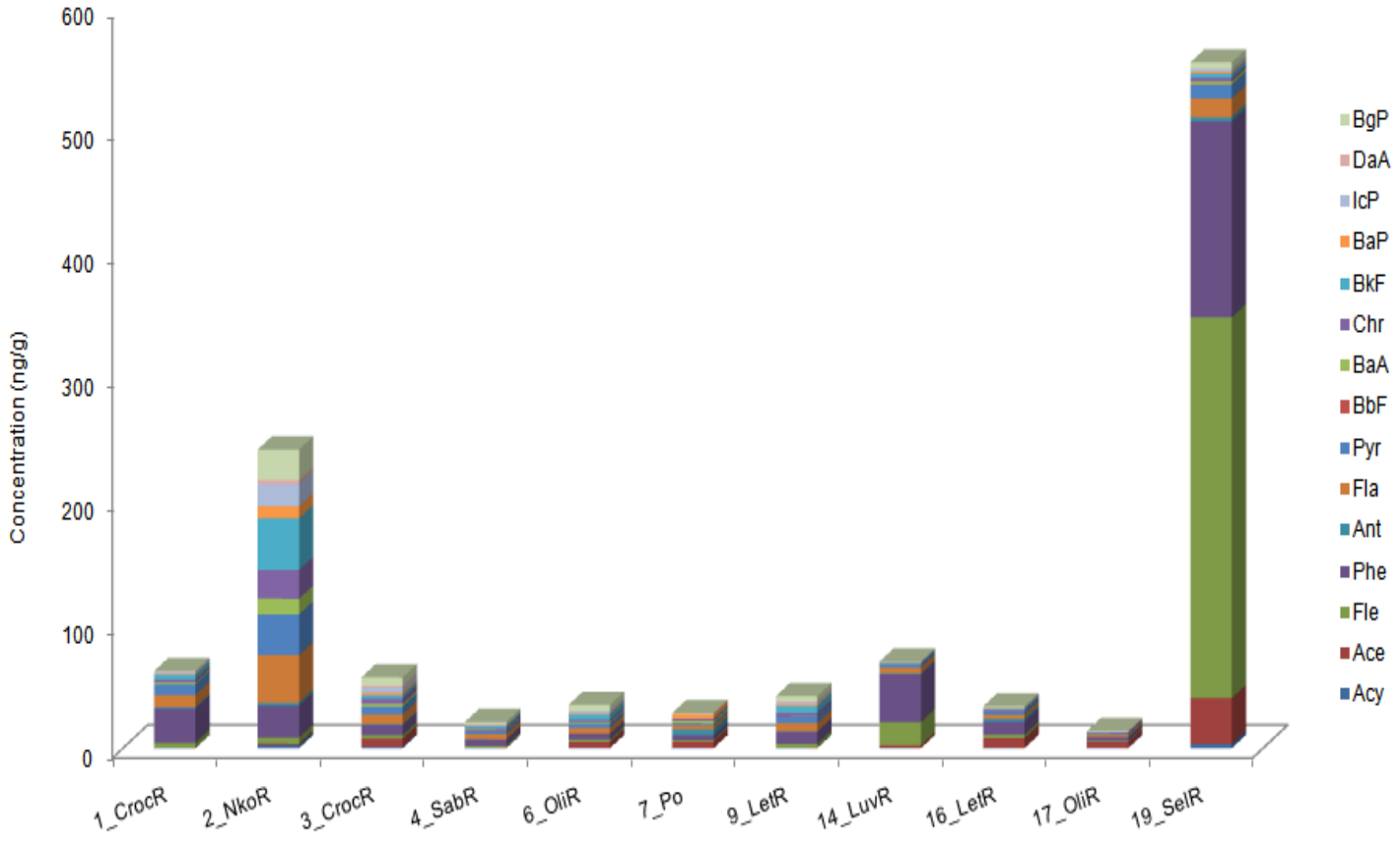


Figure 12: The composition concentration (ng/g dw) of the 16 USEPA PAHs, when Nap is excluded. Sites 6_OliR and 9_LetR were the sites where the crocodiles died, and 7_Po was a rain-fed reference site.

The low–molecular weight PAHs with 2–3 rings (Nap, Acy, Ace, Ant, Fle and Phe) contributed the most to the ΣPAHs compared to the high–molecular weight PAHs with 4–6 rings (BaP, BaA, Chr, BkF, BgP, BbF, Chr, Fla, Pyr, DaA and IcP) at all the sites, except 2_NkoR. At the following sites, 1_CrocR, 7_Po, 14_LuvR, 16_LetR, 17_OliR and 19_SelR, the low molecular weight PAHs contributed more than 70% of the ΣPAHs (Figure 13). However, at site 2_NkoR the high–molecular weight PAHs contributed 55%. The highest PAHs quantified were in the upstream localities of the Olifants River catchment, the same catchment where the mortalities occurred.

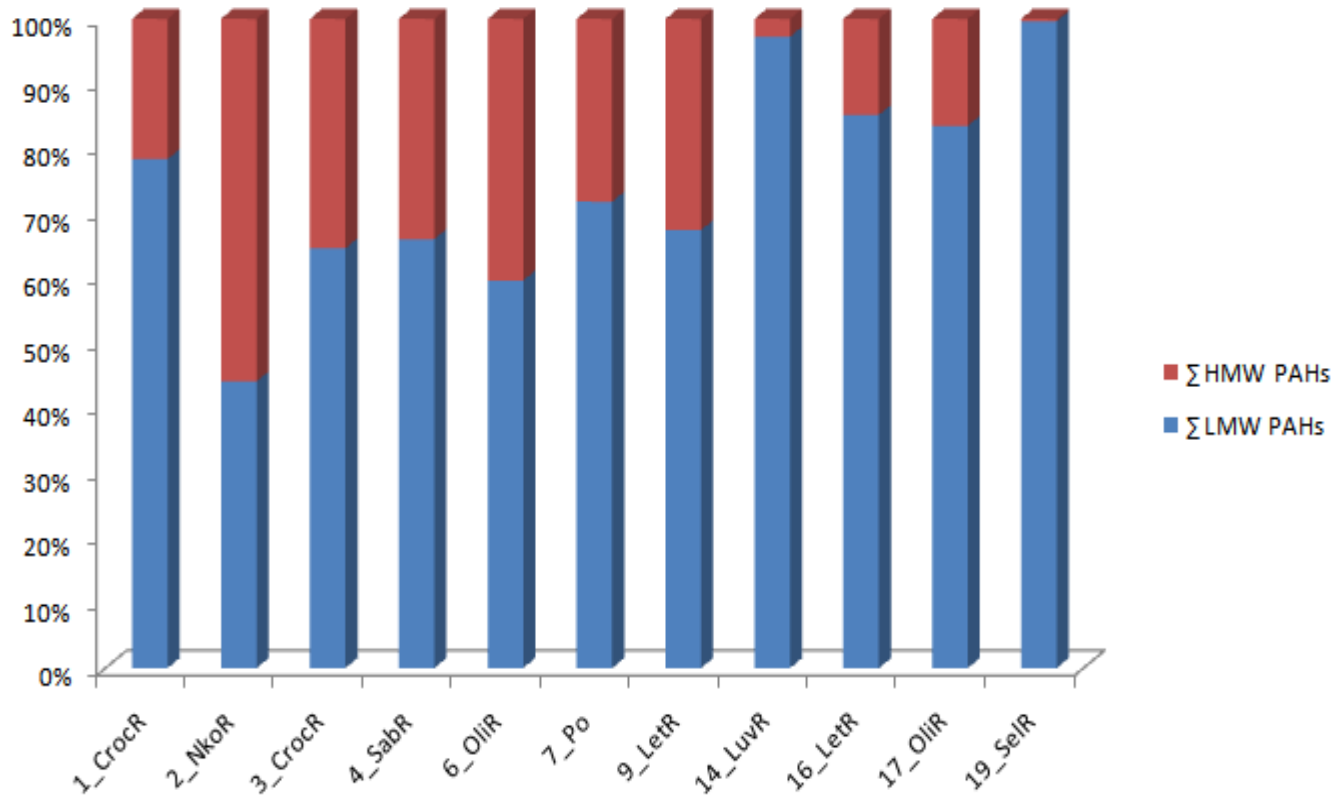


Figure 13: The percentage contribution of the high and low molecular weight PAHs at the 11 sites. Sites 6_OliR and 9_LetR were the sites where the crocodiles died, and 7_Po was a rain-fed reference site.

The ratios of the various compounds can explain information pertaining to differences in environmental input and sources. The following ratio between anthracene (Ant) and phenathrenes (Phe) was used because it can indicate whether or not the sources are pyrogenic of petrogenic. Ant is used to indicate the pyrogenic sources whereas Phe is indicative of petrogenic sources (Pies *et al*., 2008; Budzinski *et al*., 1997). The ratio between fluoranthene (Fla) and pyrene (Pyr) was used to also determine sites that associate with petrogenic and pyrogenic sources as well as the combustion of grass, wood and coal (De la Torre-Roche *et al*., 2009; Table 10). The Fla/(Fla+Pyr) and Ant/(Ant+Phe) ratios of each site was calculated and plotted in Figure 14.

Table 10: PAH diagnostic rations to distinguish between emission sources

Ratio	Range	Source	Reference
Ant/(Ant+Phe)	< 0.1	Petrogenic	Pies *et al*., 2008
	> 0.1	Pyrogenic	
Fla/(Fla+Pyr)	< 0.4	Petrogenic	De la Torre-Roche *et al*., 2009
	0.4-0.5	Pyrogenic	
	>0.5	Grass, wood and coal combustion	

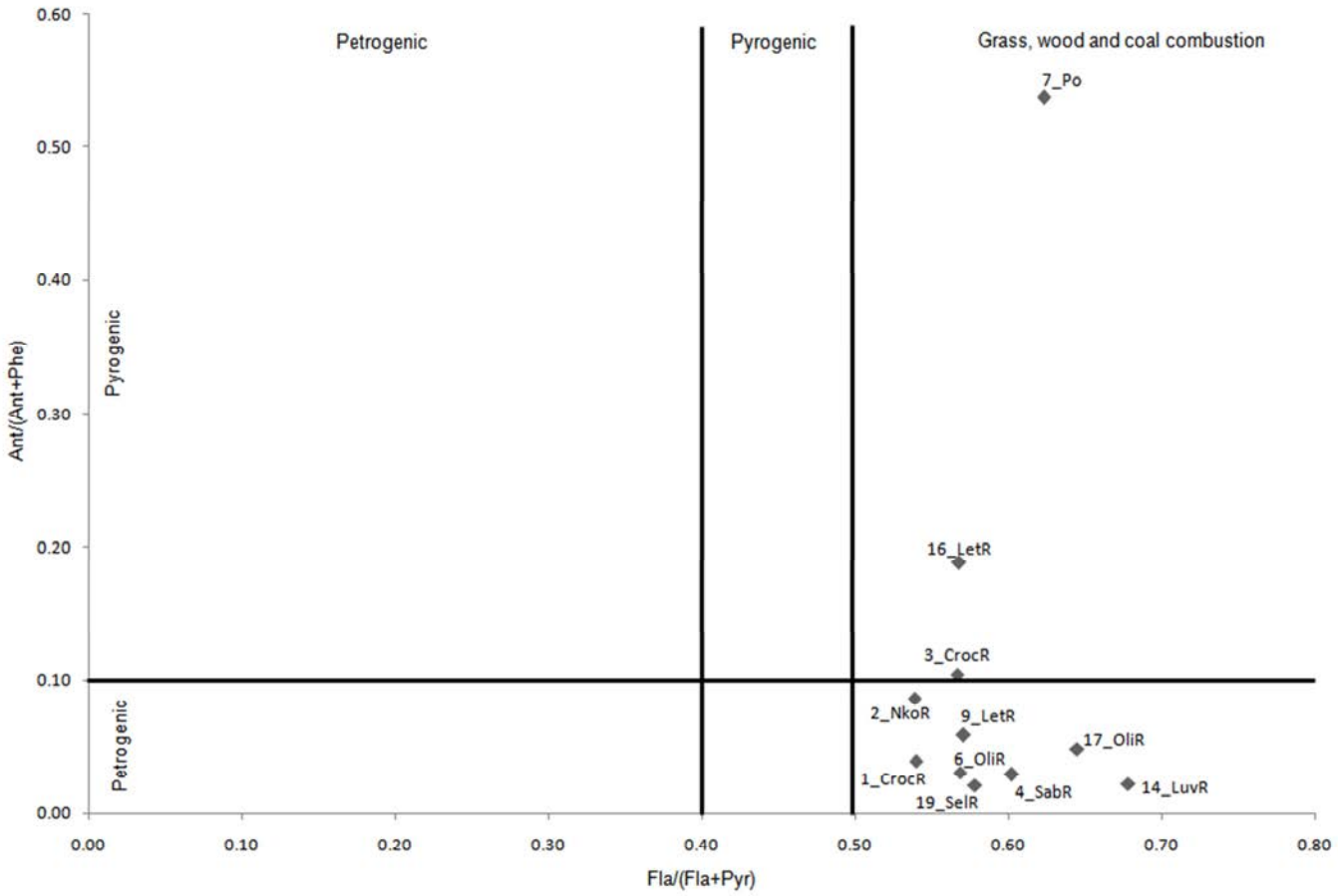


Figure 14: Cross-plot indicating the sites in relation to likely petrogenic, pyrogenic and combustion sources with the use of selected rations. Sites 6_OliR and 9_LetR were the sites where crocodiles died, and 7_Pol was a rain-fed reference site.

All sites, except 3-CrocR, 16_Letr, and 7_Po, had PAH composition indicative of petrogenic sources as indicative sources. The ordination of 7_po (rain-fed reference pool) far away from all the other sites, had a PAH composition indicative of grass, wood and coal combustion.

4.3 Concentrations and congener profiles of ΣPBDEs

Two congeners with the highest concentrations, BDE47 and BDE209 were detected at all the sites (Figure 15) whereas: BDE28, BDE66, BDE49/71, BDE77, BDE85, BE119, BDE138, BDE153, BDE154, BDE196 and BDE209 were below the LOD at all sites. Only two of the sites; 2_NkoR and 17_OliR had ΣPBDE concentrations above 1 ng/g and the lowest ΣPBDE was detected at site 3_CrocR (Figure 15). The rain-fed reference site (7_Po) had PBDE concentrations similar to the nearby sites where the crocodile mortalities occurred.

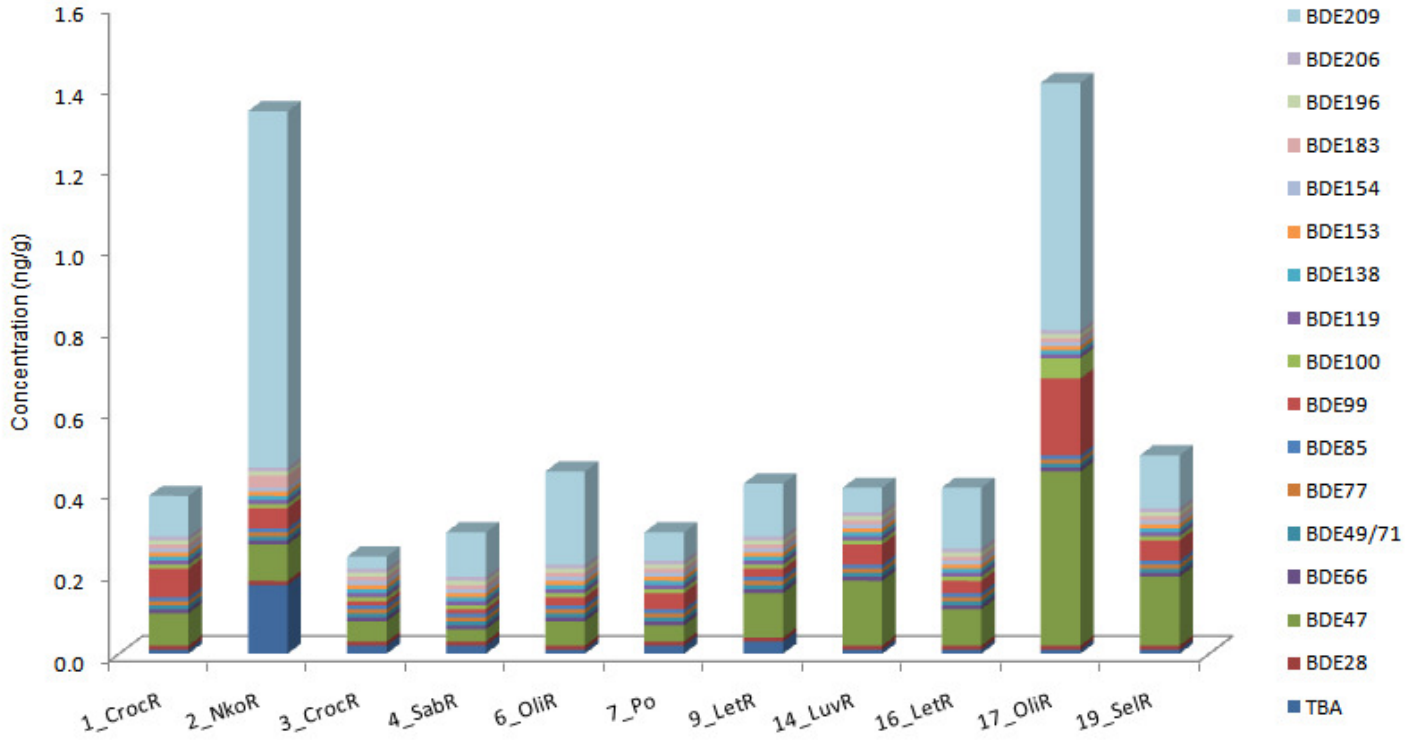


Figure 15: The composition concentration (ng/g dw) for the ΣPBDE congeners at the 11 sites. Sites 6_OliR and 9_LetR were the sites where the crocodiles died, and 7_Pol was a rain-fed reference site.

4.4 Concentrations and congener profiles of PCBs

The ΣPCBs concentration (excluding the DL–PCBs) was calculated for each locality. The highest ΣPCBs concentration was found at 17_OliR and the lowest at 4_SabR (Figure 16). Two other localities: 2_NkoR and 3_CrocR had concentrations above 1 ng/g. PCB101 was the congener with the highest concentration at all the sites. For 17_OliR the predominant PCB congeners were PCB101, PCB153 and PCB138 (Figure 16) whilst PCB122 and PCB209 were below the LOD. The

rain-fed reference site (7_Po) had PCB concentrations similar to the nearby sites where the crocodile mortalities occurred.

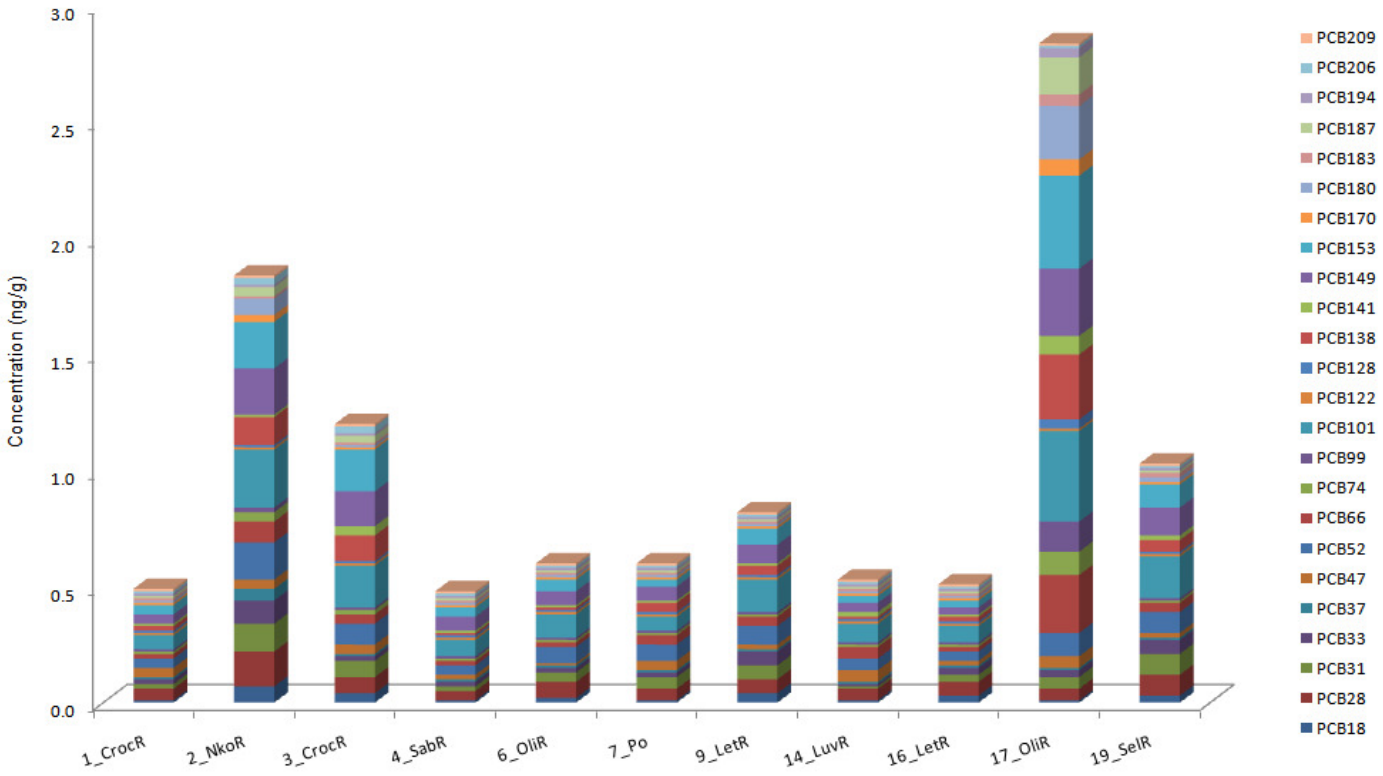


Figure 16: The composition concentration (ng/g dw) for the non-dioxin like PCB congeners at the 11 sites. Sites 6_OliR and 9_LetR were the sites where the crocodiles died, and 7_Pol was a rain-fed reference site.

4.5 Concentrations and congener profiles of dioxin-like compounds

ΣPCD/Fs and ΣDL–PCBs concentrations were converted to pg/g instead of ng/g as for the other organic analytes as they occurred at very low concentrations. The sum for each of the PCDD, PCDF and DL–PCB classes were calculated (Figure 17). The highest concentration of dioxin-like chemicals (ΣPCDD/Fs and ΣDL-PCBs) was quantified at 17_OliR with the lowest concentrations at 7_Po and 16_LetR (Figure 17). The highest ΣDL–PCBs were recorded at 17_OliR (one of the upstream localities in the catchment where the crocodiles died) and the lowest at 14_LuvR while the highest ΣPCDD/Fs were at 2_NkR and the lowest of these congeners were at 16_LetR (upstream localities of the joining catchment where the crocodiles died). The different dioxin concentrations (ΣPCDDs, ΣPCDFs and ΣDL–PCBs) contributed almost equally at 1_CrocR, 2_NkoR, 3_CrocR and 4_Sab with concentrations ranging between 15–30 pg/g. This was not true for the rest of the sites where the

ΣDL–PCBs were predominant. The area where the crocodiles died (6_OliR) had similar concentrations of DL–PCB compared to the rain-fed reference site (7_Po), but had higher concentrations of TCDD/F.

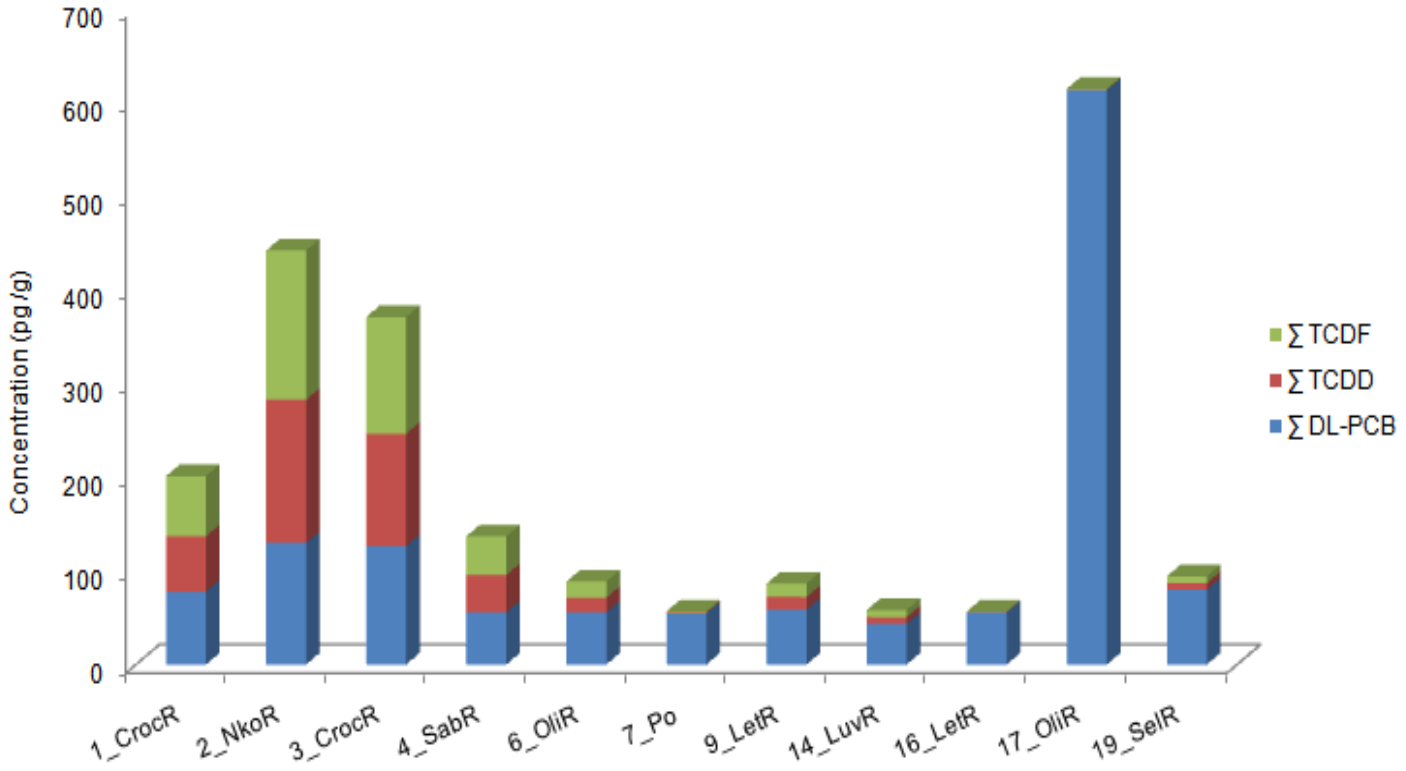


Figure 17: The concentrations (pg/g dw) of dioxin-like chemicals (ΣPCDD, ΣPCDFs and DL-PCBs) quantified in the KNP. Sites 6_OliR and 9_LetR were the sites where the crocodiles died, and 7_Pol was a rain-fed reference site.

4.6 Calculation of the Toxic Equivalent TEQ

In order to be able to compare the dioxin-like compound concentrations to sediment guideline concentrations, they had to be expressed in terms of their toxicity. The concentration of each congener was multiplied by it corresponding mammalian TEF values (Van den Berg *et al*., 2006) and the sum of the TEQ at each site is summarised in Table 11.

The TEQ indicated that ΣDL–PCBs toxicity was lower in comparison to the ΣPCDD/Fs. ΣDL–PCBs was the highest at 2_NkoR with overall low toxicity at the rest of the sites (3_CroR, 4_SabR, 6_OliR, 7_Po, 14_LuvR and 16_LetR). ΣPCDFs was the highest also at 2_NkoR with the lowest at 7_Po. The highest toxicity was calculated for ΣPCDD at 1_CrocR and the lowest at 7_Po. When calculating

the ΣDioxin TEQs, the highest was at 1_CrocR, 2_NkoR and 3_CrocR with the lowest at 7_Po (Table 11).

Table 11: The TEQ values for each of the sites based on the mammalian TEF values. Sites 6_OliR and 9_LetR were the sites where the crocodiles died, and 7_Pol was a rain-fed reference site

pg/TEQ/g	ΣDL PCBs	Σ PCDF	ΣPCDD	ΣDioxin
1_CrocR	0.04	0.3	2.31	2.65
2_NkoR	0.13	0.42	1.47	2.02
3_CrocR	0.01	0.14	1.76	1.91
4_SabR	0.01	0.04	0.37	0.42
6_OliR	0.01	0.05	0.28	0.34
7_Po	0.01	0.03	0.12	0.16
9_LetR	0.02	0.07	0.23	0.31
14_LuvR	0.01	0.04	0.15	0.19
16_LetR	0.01	0.04	0.16	0.21
17_OliR	0.04	0.05	0.19	0.28
19_SelR	0.02	0.05	0.28	0.34

4.7 Concentrations and profiles of elements

High concentrations of Fe and Al were quantified at all the sampled localities. The highest concentrations were at 6_OliR (Fe: 67 500 µg/g and Al: 50 000 µg/g) the lowest concentrations (Fe: 6 500 µg/g and Al: 1 650 µg/g) at 11_LimR (Figure 18). The Al concentration was used as the normalising factor for the SQP to determine the relative toxicity at the different sites and that of the elements. When excluding the Fe and Al concentrations from the graph it is possible to see the profile of the other elements at the different sites (Figure 19).

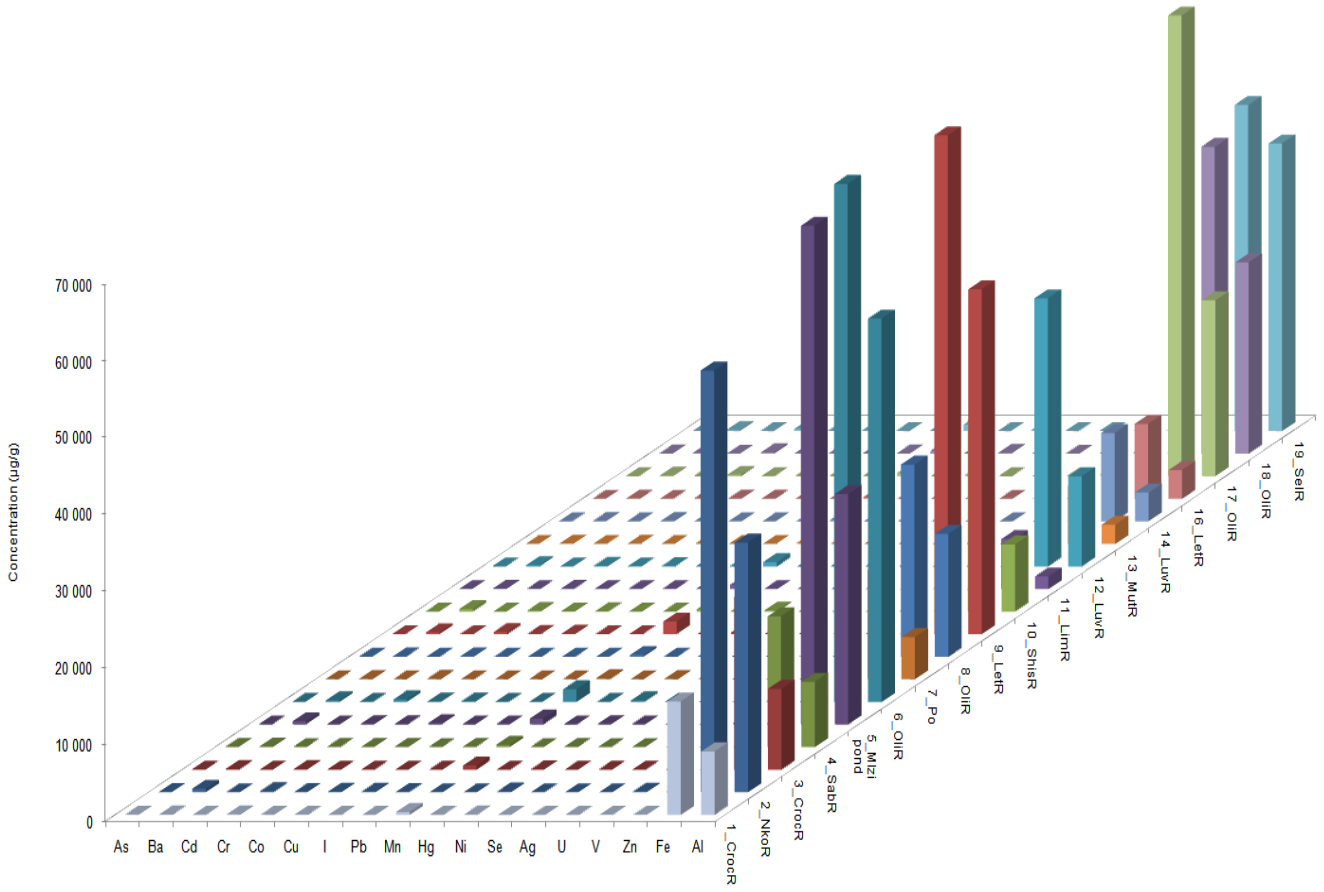


Figure 18: The concentrations of the elements (µg/g dw) from 18 sites. Sites 6_OliR and 9_LetR were the sites where the crocodiles died, and 7_Pol was a rain-fed reference site.

The element with the second highest concentration was Mn at 6_OliR and 9_LetR, and the lowest concentration was quantified at 13_MutR (Figure 19). The only sites that had concentrations below the LOD was for Cd at 1_CrocR, 3_CrocR, 4_SabR. High element concentrations were also found at the 5_Mlzi pond and 19_SelR (Figure 19).

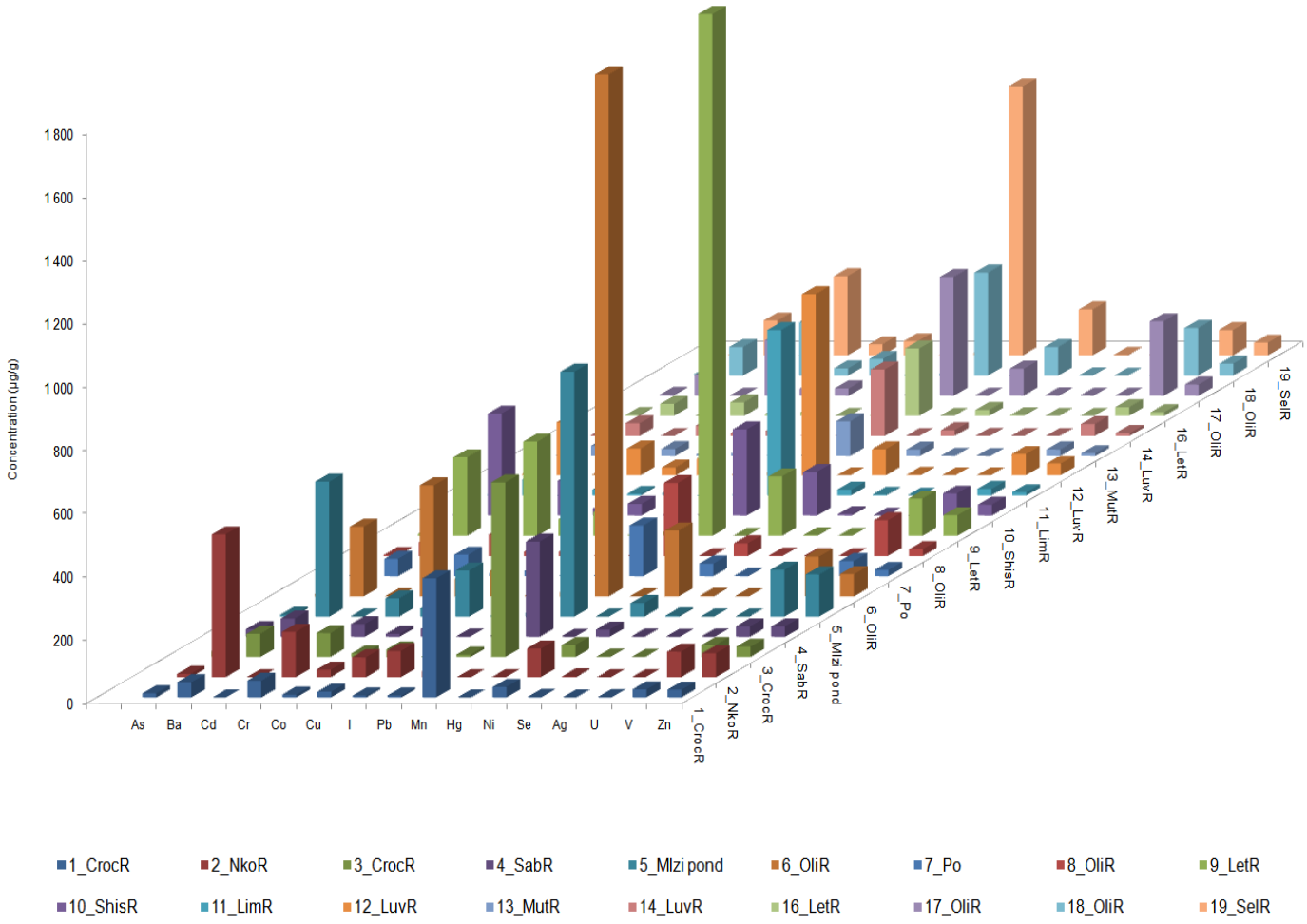


Figure 19: The concentrations of the elements at the 18 sites without Fe and Al (µg/g dw) concentrations. Sites 6_OliR and 9_LetR were the sites where the crocodiles died, and 7_ Po was a rain-fed reference site.

4.8 Sediment quality parameters (SQPs)

As mentioned before, the SQPs will be used as an additional assessment of the contamination of the different elements within the sites. The SQPs consists of the Contamination factor (CF), Geoaccumulation index (Igeo), Pollution load index (PLI) and Enrichment factor (EF).

4.8.1 Contamination factor (CF)

The CF was calculated for all the sites and the relevant guideline classification was used to interpret the level of contamination within the sites as well as which element contributed to contamination at the individual sites. The elements with the decreasing concentrations across all the sites were Se>As>Ni>Cr>Cu>Mn (Table 12).

Selenium was the one element which occurred at concentrations that classified as “very high contamination” concentrations for all of the sites (Table 12). Arsenic had the second most sites that classified as contaminated, with classifications of “moderate to very high contamination” at all the sites except 14_NkoR. Nickel had high contamination at 2_NkoR, 6_OliR, 9_LetR, 10_ShisR, 12_LevR, 17_OliR, 18_OliR and 19_SelR (Table 12). The following heavy metals classified as “moderate to very high” at different sites: Chromium (elemental chromium specifically as the guidelines did not distinguish between Cr(VI) and Cr(III)) indicated contamination at 2_NkoR, 6_OliR, 9_LetR, 10_ShisR, 17_OliR, 18_OliR and 19_SelR. Copper was classified as high at 2_NkoR, 5_Mlzi Pond, 6_OliR, 9_LetR, 12_LevR, 18_OliR and 19_SelR, whereas Mn only had high contamination at 2_NkoR, 6_OliR and 9_LetR. The Ag, Ba and U showed low contamination at all of the sites (Table 12).

The 2_NkoR had 13 elements in the “moderate contamination” and higher categories followed by 12 elements for 5_Mlzi pond (Mlondozi pond). This site had only two elements, Cu and Se, in the “very higThe geology of the KNP largely consists h contamination” category, while 2_NkoR had Mn, Se and I. The two sites, 6_OliR and 9_LetR, where the crocodiles were dying, had 11 elements in the “moderate” to “very high” contamination categories, three of which were heavy metals namely Cr, Ni and Se in the ”very high contamination” category (Table 12). The locality upstream from the area where the deaths occurred, 8_OliR, had contamination categories mainly varying between “low to moderate”. Locality 8_OliR had a Se rating of “very high contamination” in the area where the mortalities occurred. Site 14_LuvR only had two elements regarded as having any contamination implication.

Table 12: The contamination factors (CF) for the various elements with the classification categories of contamination summarised below. Sites 6_OliR and 9_LetR were the sites where the crocodiles died, and 7_Pol was a rain-fed reference site

	1_CrocR	2_NkoR	3_CrocR	4_SabR	5_Mlzi pond	6_OliR	7_Po	8_OliR	9_LetR	10_ShisR	11_LimR	12_LevR	13_MutR	14_LuvR	16_LetR	17_OliR	18_OliR	19_SelR
Ag	0.05	0.34	0.08	0.06	0.18	0.20	0.09	0.08	0.11	0.37	0.16	0.19	0.03	0.05	0.10	0.06	0.05	0.09
As	6.25	5.00	8.38	12.00	2.88	3.63	1.38	1.50	3.25	1.63	1.15	1.25	1.03	0.79	1.18	1.38	2.25	2.38
Ba	0.07	0.67	0.11	0.09	0.64	0.33	0.08	0.06	0.37	0.49	0.08	0.25	0.05	0.06	0.06	0.10	0.14	0.17
Cd	0.12	0.93	0.12	0.12	1.40	0.59	0.29	0.34	0.98	2.45	1.30	1.69	0.12	0.12	0.29	0.74	0.25	0.44
Co	0.75	2.05	0.99	0.78	2.16	4.74	0.82	1.12	4.53	1.94	0.47	2.16	0.39	0.73	0.50	1.90	1.96	3.02
Cr	1.50	4.07	2.14	1.14	1.64	10.00	1.93	1.93	8.57	3.14	0.54	2.50	0.64	0.93	1.21	5.00	4.79	7.14
Cu	1.24	4.37	1.64	1.75	10.14	5.59	1.15	0.94	5.25	2.62	0.54	3.67	0.56	1.15	0.75	1.61	3.67	3.15
Fe	0.48	1.78	0.73	0.55	2.10	2.19	0.46	0.81	2.10	0.81	0.21	1.13	0.24	0.37	0.32	1.94	1.30	1.38
Hg	0.15	2.01	0.11	0.13	0.15	0.18	0.19	0.23	0.15	0.23	0.21	0.17	0.13	0.17	0.16	0.13	0.14	0.24
I	3.93	58.93	1.29	0.50	1.79	1.79	0.30	0.25	2.68	1.23	0.20	0.57	0.38	0.34	0.27	0.29	0.64	1.09
Mn	0.71	10.91	1.04	0.57	1.47	3.13	0.30	0.44	3.13	0.52	1.00	1.09	0.21	0.40	0.40	0.71	0.62	1.61
Ni	1.75	4.84	2.02	1.22	2.29	11.16	2.15	2.15	10.08	7.39	0.98	4.57	1.10	0.98	0.98	4.57	4.84	7.80
Pb	0.32	1.18	0.43	0.25	0.69	0.72	0.18	0.16	0.74	0.38	0.16	0.50	0.13	0.10	0.15	0.28	0.40	0.46
Se	14.46	30.12	13.55	7.23	36.15	26.51	16.27	18.98	18.98	57.23	30.12	21.99	15.36	11.75	27.41	11.75	12.65	18.07
U	0.05	0.12	0.06	0.06	0.10	0.11	0.05	0.04	0.11	0.10	0.06	0.16	0.03	0.03	0.05	0.08	0.11	0.08
V	0.47	1.51	0.71	0.61	2.78	2.36	0.90	2.12	2.22	1.32	0.39	1.32	0.40	0.71	0.52	4.43	2.83	1.51
Zn	0.46	1.44	0.63	0.63	2.55	1.35	0.38	0.41	1.25	0.67	0.21	0.72	0.18	0.18	0.22	0.67	0.72	0.77

Values for CF	Level of contamination
CF<1	Low contamination
1≤CF<3	Moderate contamination
3≤CF<6	Considerable contamination
6<CF	Very high contamination

4.8.2 Pollution load index (PLI)

The PLI indexes the pollution load at all 17 elements at each of the sites. A PLI>1 indicates pollution (Chakravarty & Patigri, 2009) and according to this, 7 of the sites were polluted (Table 13). The highest was at 2_NkoR while both 9_LetR and 6_OliR had the second highest PLI, once again sites in the Letaba River and Olifants Gorge where the crocodile mortalities occurred. The smallest PLI (PLI<0.5) was calculated for 11_LimR, 13_MutR, 14_Luv and16_LetR (Table 13).

Table 13: The pollution load index (PLI) was calculated for the 18 different sites. Sites 6_OliR and 9_LetR were the sites where the crocodiles died, and 7_Pol was a rain-fed reference site

Sites	PLI	Sites	PLI
1_CrocR	0.6	**10_ShisR**	**1.2***
2_NkoR	**2.4***	**11_LimR**	0.4
3_CrocR	0.7	**12_LevR**	**1.0***
4_SabR	0.5	**13_MutR**	0.3
5_Mlzi pond	**1.5***	**14_LuvR**	0.3
6_OliR	**1.7***	**16_LetR**	0.3
7_Po	0.5	**17_OliR**	0.8
8_OliR	0.5	**18_OliR**	0.8
9_LetR	**1.6***	**19_SelR**	**1.1***

* The numbers printed in bold are those considered to be polluted by the total mixture of the elements.

4.8.3 Geoaccumulation index (Igeo)

The Igeo uses single elements to calculate the possible pollution of that element in the sediment. The six elements that had the highest Igeo values were Se>As>Ni>Cr>Cu>Mn (Table 14). The element that had the highest Igeo at all of the sites was Se. Its Igeo classifications varied from “moderately polluted to heavily polluted” to “extremely polluted” at all of the sites (Table 14). Arsenic had Igeo classifications between “moderately polluted to heavily polluted” at 1_CrocR, 2_NkoR, 3_CrocR, 4_SabR, 6_OliR and 9_LetR. Nickel, Cr, Cu and Mn classified at “moderately to heavy polluted” at different sites. The sites where most of the heavy metals classified as high were 2_NkoR, 6_OliR, 9_LetR, 10_ShisR, 17_OliR, 18_OliR and 19_SeR. The site with the highest Igeo classifications was 2_NkoR with elements such as Cr, Mn, Ni, Cu, As, Se and I standing out.

Table 14: The Igeo for the elements at the sites with the pollution categories summarised below. Sites 6_OliR and 9_LetR were the sites where the crocodiles died, and 7_Pol was a rain-fed reference site

	1_CrocR	2_NkoR	3_CrocR	4_SabR	5_Mlzi pond	6_OliR	7_Po	8_OliR	9_LetR	10_ShisR	11_LimR	12_LevR	13_MutR	14_LuvR	16_LetR	17_OliR	18_OliR	19_SelR
Ag	-5.04	-2.14	-4.28	-4.56	-3.04	-2.91	-4.04	-4.28	-3.84	-2.03	-3.20	-2.97	-5.93	-5.04	-3.97	-4.67	-5.04	-4.12
As	2.06	1.74	2.48	3.00	0.94	1.27	-0.13	0.00	1.12	0.12	-0.38	-0.26	-0.55	-0.93	-0.35	-0.13	0.59	0.66
Ba	-4.40	-1.16	-3.79	-4.12	-1.24	-2.19	-4.19	-4.56	-2.00	-1.62	-4.33	-2.56	-4.95	-4.65	-4.74	-3.95	-3.48	-3.19
Cd	-3.61	-0.69	-3.61	-3.61	-0.10	-1.35	-2.35	-2.13	-0.61	0.71	-0.21	0.17	-3.61	-3.61	-2.35	-1.03	-2.61	-1.77
Co	-0.99	0.45	-0.60	-0.95	0.52	1.66	-0.87	-0.42	1.59	0.37	-1.66	0.52	-1.95	-1.03	-1.60	0.34	0.39	1.01
Cr	0.00	1.44	0.52	-0.39	0.13	2.74	0.36	0.36	2.52	1.07	-1.49	0.74	-1.22	-0.69	-0.31	1.74	1.67	2.25
Cu	-0.27	1.54	0.13	0.22	2.76	1.90	-0.38	-0.67	1.81	0.81	-1.47	1.29	-1.42	-0.38	-1.00	0.10	1.29	1.07
Fe	-1.65	0.25	-1.04	-1.45	0.49	0.54	-1.70	-0.89	0.49	-0.89	-2.83	-0.41	-2.63	-2.01	-2.25	0.37	-0.21	-0.13
Hg	-3.35	0.42	-3.75	-3.59	-3.31	-3.07	-2.97	-2.69	-3.31	-2.72	-2.81	-3.14	-3.53	-3.14	-3.22	-3.59	-3.39	-2.66
I	1.39	5.30	-0.22	-1.59	0.25	0.25	-2.31	-2.59	0.84	-0.28	-2.93	-1.39	-2.00	-2.14	-2.49	-2.39	-1.22	-0.46
Mn	-1.08	2.86	-0.52	-1.40	-0.03	1.06	-2.31	-1.78	1.06	-1.52	-0.59	-0.46	-2.85	-1.91	-1.90	-1.08	-1.28	0.11
Ni	0.22	1.69	0.43	-0.29	0.61	2.90	0.52	0.52	2.75	2.30	-0.61	1.61	-0.45	-0.61	-0.61	1.61	1.69	2.38
Pb	-2.21	-0.35	-1.81	-2.59	-1.12	-1.06	-3.09	-3.21	-1.03	-1.97	-3.21	-1.59	-3.49	-3.85	-3.37	-2.42	-1.92	-1.72
Se	3.27	4.33	3.18	2.27	4.59	4.14	3.44	3.66	3.66	5.25	4.33	3.87	3.36	2.97	4.19	2.97	3.08	3.59
U	-4.80	-3.64	-4.74	-4.77	-3.94	-3.77	-4.94	-5.34	-3.77	-3.91	-4.62	-3.23	-5.55	-5.69	-4.97	-4.16	-3.77	-4.19
V	-1.67	0.01	-1.08	-1.29	0.89	0.65	-0.74	0.50	0.56	-0.18	-1.96	-0.18	-1.92	-1.08	-1.53	1.56	0.92	0.01
Zn	-1.70	-0.06	-1.26	-1.26	0.76	-0.16	-2.00	-1.86	-0.26	-1.16	-2.86	-1.06	-3.08	-3.08	-2.79	-1.16	-1.06	-0.96

Igeo Classes	Values	Level of pollution
Class 0	Igeo≤0	Practically unpolluted
Class 1	0<Igeo<1	Unpolluted to moderately polluted
Class 2	1<Igeo<2	Moderately polluted
Class 3	2<Igeo<3	Moderately to heavily polluted
Class 4	3<Igeo<4	Heavy polluted
Class 5	4<Igeo<5	Heavily to extremely polluted
Class 6	5<Igeo<6	Extremely polluted

4.8.4 Enrichment factor (EF)

The level of enrichment was calculated to determine if the sediment was enriched with anthropogenic sources in relation with the natural geology of the catchment (Table 15). Elements with the highest enrichment factors were Se>As>Ni>Cr>Cu.

The two elements with the highest EF were Se and As. Selenium had an EF in the “extremely severe enrichment” classifications for all of the sites except for 6_OliR, 9_LetR, 17_OliR 18_OliR and 19_SelR which were in the “very severe enrichment” classification. Arsenic was in the “extremely severe” to “very severe enrichment” classification at 1_CrocR, 3_CrocR, 4_SabR, 11_LimR and 13_MutR with Ni at 7_Po, 10_ShisR, 11_LimR, 12_LuvR and 13_MutR (Table 15). Chromium had “very severe” to “severe” enrichment classifications at all of the sites, except for 2_NkoR, 5_Mlzi pond and 8_OliR, while Cu classified into the same enrichment categories at most of the sites excluding 6_OliR, 8_OliR, 9_LetR, 17_OliR and 19_SelR. At the sites where the crocodile mortalities occurred (6_OliR, 8_OliR and 9_LetR) there were elemental enrichment classifications that ranged from “severe” to “extremely severe enrichment” for V, Cr, Fe, Co, Ni, Cu, As and Se (Table 15).

Table 15: The EF was calculated using Al as the normalising factor and the data was assessed with the specific guidelines summarised below. Sites 6_OliR and 9_LetR were the sites where the crocodiles died, and 7_Pol was a rain-fed reference site

Enrichment Factor (Ef) – Al

	1_CrocR	2_NkoR	3_CrocR	4_SabR	5_Mlzi pond	6_OliR	7_Po	8_OliR	9_LetR	10_ShisR	11_LimR	12_LevR	13_MutR	14_LuvR	16_LetR	17_OliR	18_OliR	19_SelR
Ag	0.43	0.81	0.57	0.58	0.47	0.31	1.28	0.37	0.18	3.26	7.68	1.26	0.76	0.94	1.97	0.20	0.14	0.18
As	58.67	11.91	61.77	109.33	7.42	5.61	19.36	7.26	5.59	14.38	53.97	8.24	31.75	16.26	24.26	4.63	6.97	4.90
Ba	0.67	1.61	0.80	0.78	1.64	0.51	1.16	0.31	0.64	4.31	3.51	1.68	1.51	1.24	1.16	0.33	0.42	0.34
Cd	1.15	2.22	0.90	1.12	3.61	0.91	4.14	1.66	1.69	21.69	60.97	11.15	3.80	2.53	6.07	2.48	0.76	0.91
Co	7.08	4.88	7.31	7.07	5.56	7.34	11.53	5.42	7.79	17.17	22.25	14.20	12.02	15.13	10.24	6.39	6.08	6.23
Cr	14.08	9.70	15.80	10.41	4.24	15.49	27.15	9.33	14.75	27.82	25.14	16.48	19.91	19.18	25.08	16.83	14.82	14.75
Cu	11.65	10.41	12.12	15.93	26.17	8.66	16.25	4.57	9.03	23.21	25.44	24.20	17.33	23.83	15.52	5.42	11.37	6.50
Fe	4.48	4.24	5.37	5.01	5.43	3.38	6.50	3.92	3.62	7.16	9.88	7.47	7.52	7.69	6.52	6.54	4.01	2.84
Hg	1.38	4.79	0.82	1.14	0.39	0.28	2.70	1.12	0.26	2.02	10.06	1.12	4.01	3.50	3.32	0.42	0.44	0.49
I	36.88	140.41	9.48	4.56	4.61	2.77	4.27	1.21	4.61	10.90	9.22	3.77	11.62	7.01	5.53	0.96	1.99	2.25
Mn	6.68	26.00	7.70	5.19	3.80	4.85	4.27	2.11	5.39	4.62	46.76	7.19	6.47	8.23	8.33	2.40	1.91	3.33
Ni	16.40	11.53	14.87	11.14	5.90	17.28	30.28	10.41	17.35	65.43	46.05	30.12	34.14	20.26	20.26	15.39	14.99	16.10
Pb	3.04	2.80	3.15	2.28	1.78	1.12	2.48	0.78	1.27	3.38	7.59	3.30	4.15	2.16	3.01	0.94	1.23	0.94
Se	135.71	71.77	99.97	65.86	93.30	41.05	229.01	91.84	32.66	506.49	1413.65	144.91	475.84	242.58	566.03	39.55	39.19	37.32
U	0.51	0.29	0.41	0.50	0.25	0.17	0.69	0.18	0.19	0.89	2.86	1.05	0.99	0.60	0.99	0.28	0.34	0.17
V	4.43	3.60	5.22	5.59	7.18	3.65	12.62	10.27	3.82	11.69	18.15	8.70	12.27	14.61	10.71	14.93	8.77	3.12
Zn	4.33	3.44	4.61	5.69	6.58	2.08	5.28	2.00	2.15	5.96	9.70	4.75	5.51	3.67	4.47	2.27	2.23	1.59

Values for Enrichment	**Level of enrichment**
EF<3	Minor enrichment
EF = 3–5	Moderate enrichment
EF = 5–10	Moderately severe enrichment
EF= 10–25	Severe enrichment
Ef = 25–50	Very severe enrichment
EF>50	Extremely severe enrichment

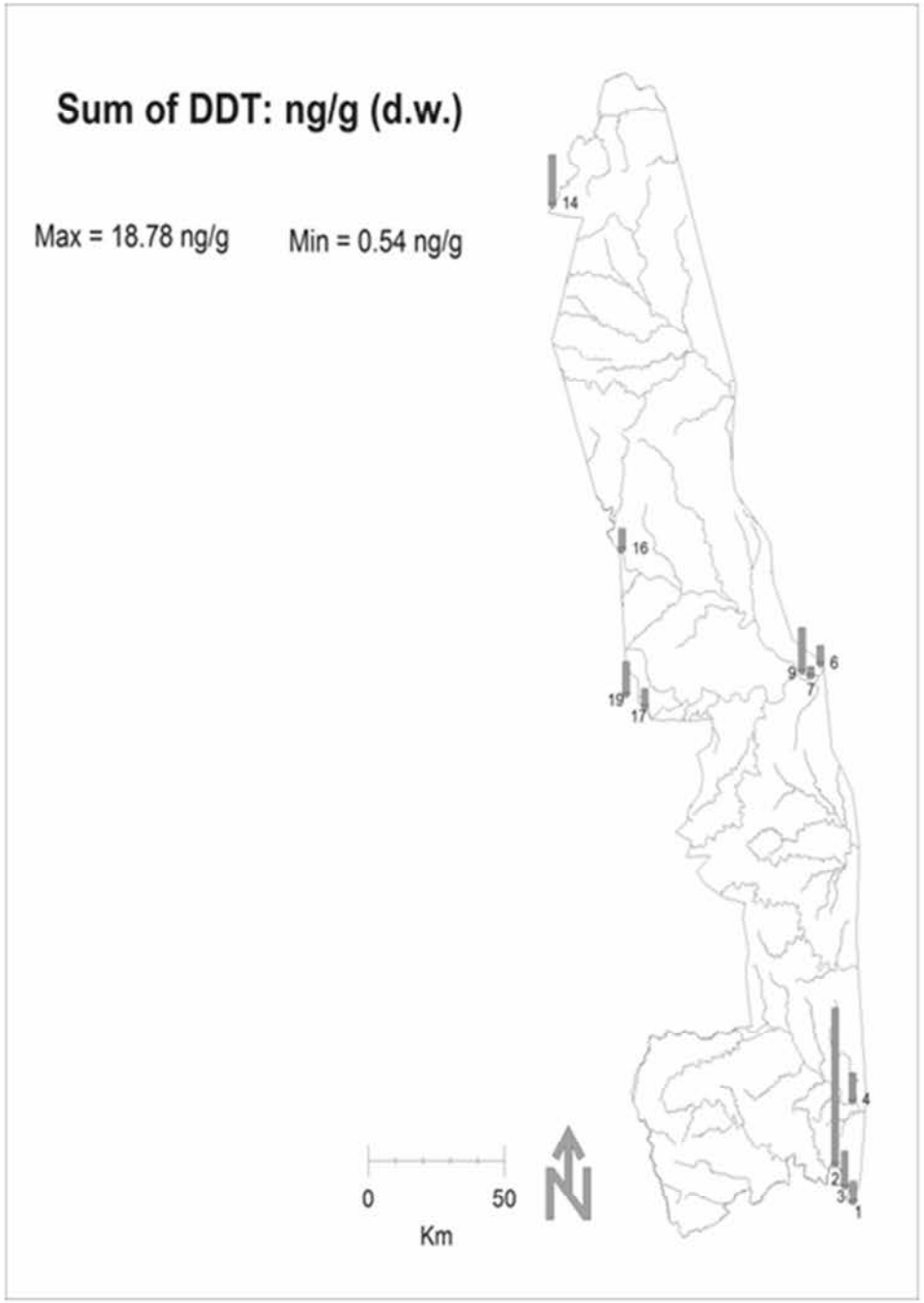
4.9 Geographical distribution

Possible trends in terms of the distribution of the different compounds were investigated with the use of distribution maps that indicate the different concentrations quantified at the different sites. There are no maps for mirex and heptachlor as both compounds were below the LOD for all samples.

4.9.1 Geographical overview of the POPs concentrations

ΣDDT and ΣChlordanes were presented together because their relative concentration patterns between the sites seemed to be similar. The highest concentration of ΣDDT was detected at 2_NkoR, 14_LuvR, 17_OliR and 19_SelR. ΣChlordanes had the highest concentration at 2_NkoR, 3_CrocR, 9_LetR, 14_LuvR and 17_OliR (Figure 20). The similarities between the distributions of the compounds were the closest at 2_NkoR, 14_LuvR and 17_OliR, and the least similar at 6_OliR and 7_Po. Both the compound classes had high concentrations at 1_CrocR, 2_NkoR, 3_CrocR and 4_SabR (Figure 20).

The highest ΣDDT concentration was at site 2_NkoR (18.78 ng/g dw) and the lowest at site 7_Po (0.54 ng/g dw), the rain-fed pond near the area (6_OliR) where the crocodile mortalities occurred (Figure 20). The highest concentration of chlordane (0.63 ng/g dw) was at 2_NkoR. The lowest concentration of chlordane, 0.03 ng/g dw, was determined for 6_OliR.



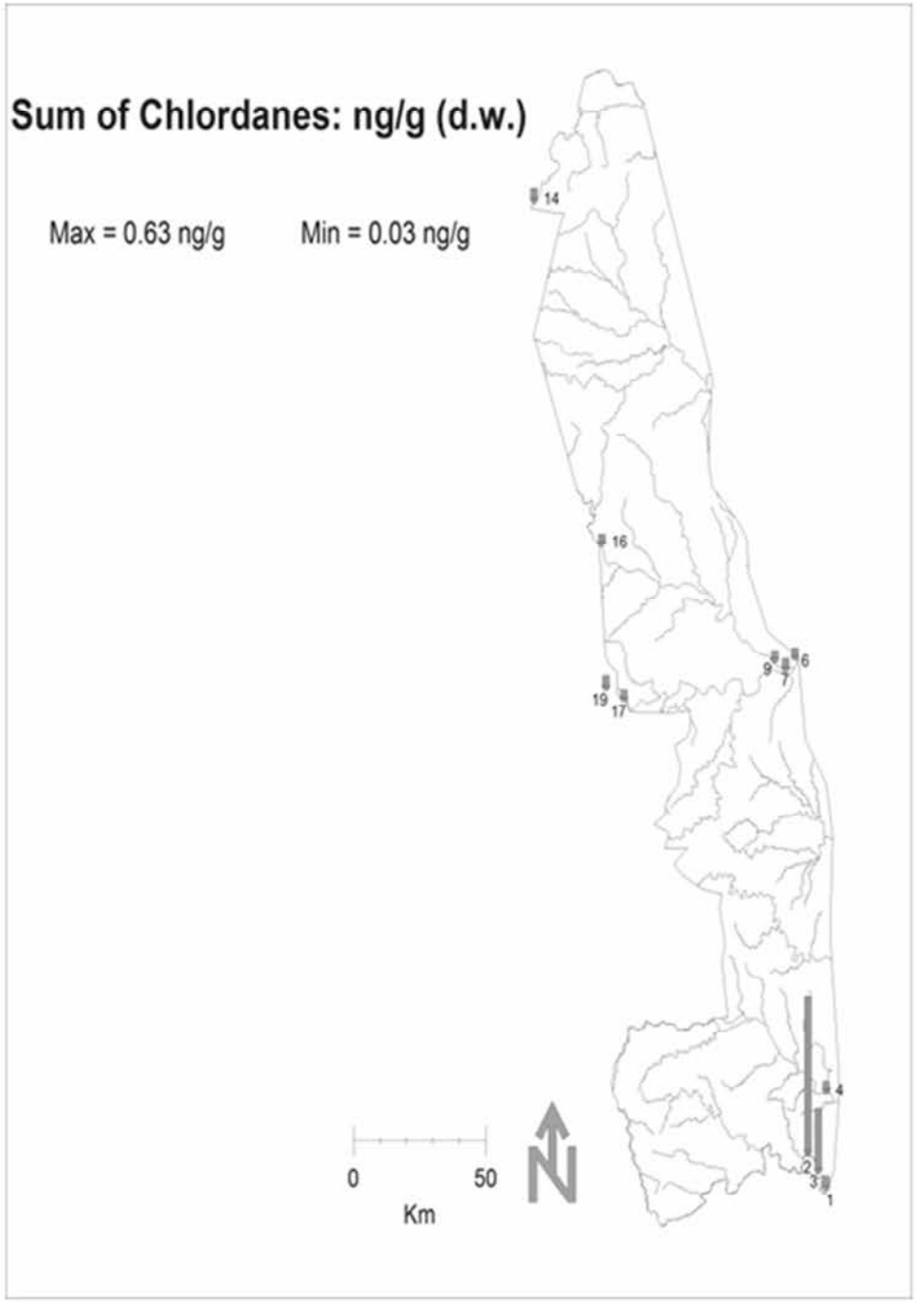


Figure 20: Maps of the sediment concentrations of DDTs and chlordanes in the KNP

The highest ΣPCDD/Fs and ΣDL–PCBs concentrations were recorded at 17_OliR, 2_NkoR and 3_CrocR (Figure 21). The highest concentration PCDD/Fs and DL–PCBs were 614.39 pg TEQ/g dw at 17_OliR; the lowest concentration was 51.04 pg TEQ/g dw at 7_Po, (Figure 21). There was a cluster of three sites (1_CrocR, 2_NkoR and 3_CrocR) in the south-eastern corner of the KNP, which also had high concentrations of PCDD/Fs and DL-PCBs. The concentrations for all three compound classes (Figure 21) at 1_CrocR, 2_NkoR and 3_CrocR were between 150–300 pg TEQ/g dw.

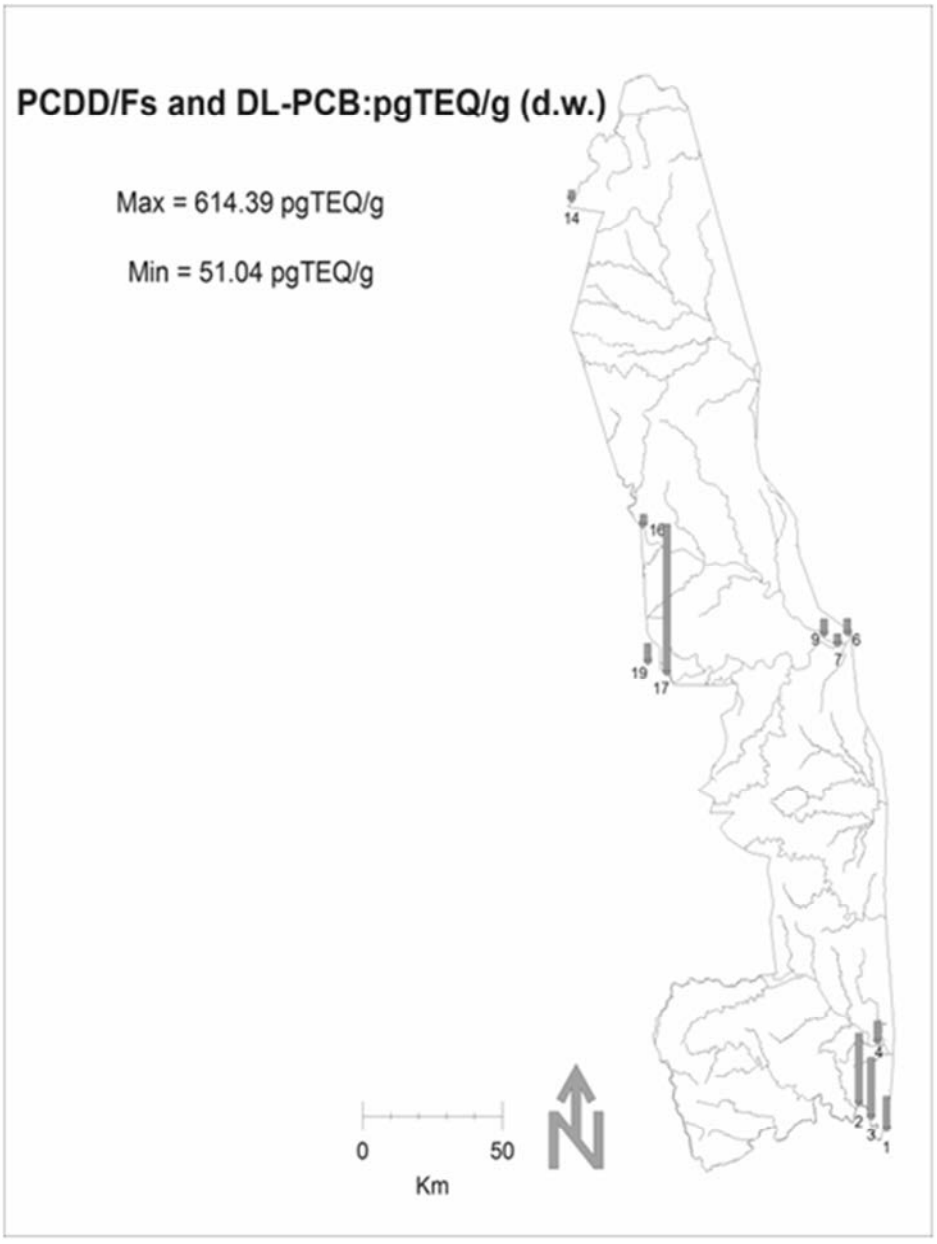


Figure 21: Map of the concentration and site distribution of ΣPCDD/Fs with ΣDL-PCB in the KNP

ΣHCH and PeCB, HCB shared their relative high concentrations at 2_NkoR, 6_OliR, 9_LetR. Additionally, HCB also had a high concentration at 19_SelR, with lower concentrations at 1_CrocR, 3_CrocR, 4_Sab_R, and 7_Po (Figure 22). The highest concentration of PeCBs was 0.84 ng/g dw at 2_NkoR, and the lowest concentration was 0.07 ng/g dw at 4_SabR (Figure 22). HCB was detected at all the sites in this region (Figure 22), with the highest concentration at 2_NkoR, 0.5 ng/g dw, and the lowest concentration of 0.07 ng/g dw at 4_SabR.

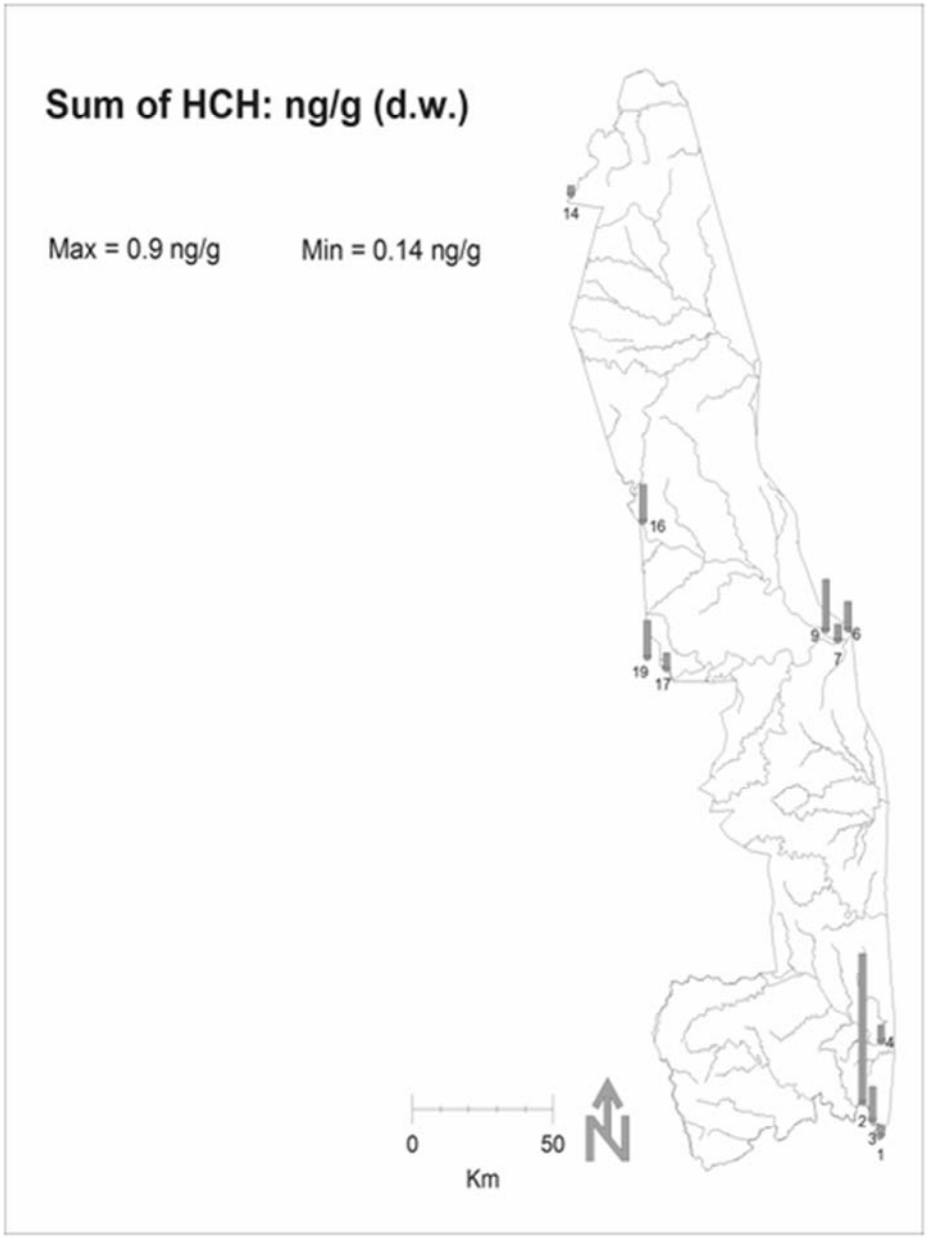
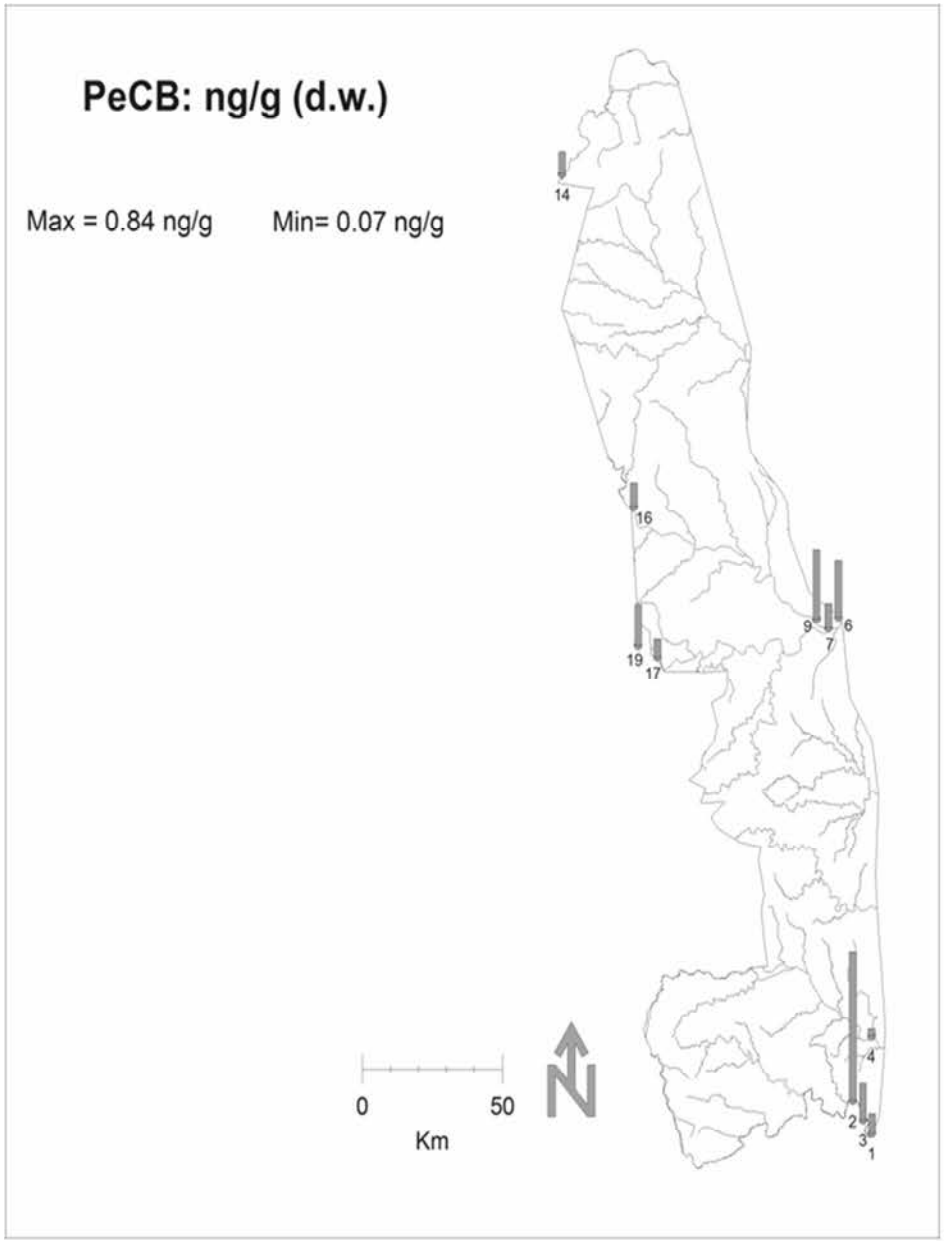


Figure 22: Maps of the sediment concentrations of ΣHCH, PeCBs and HCB in the KNP



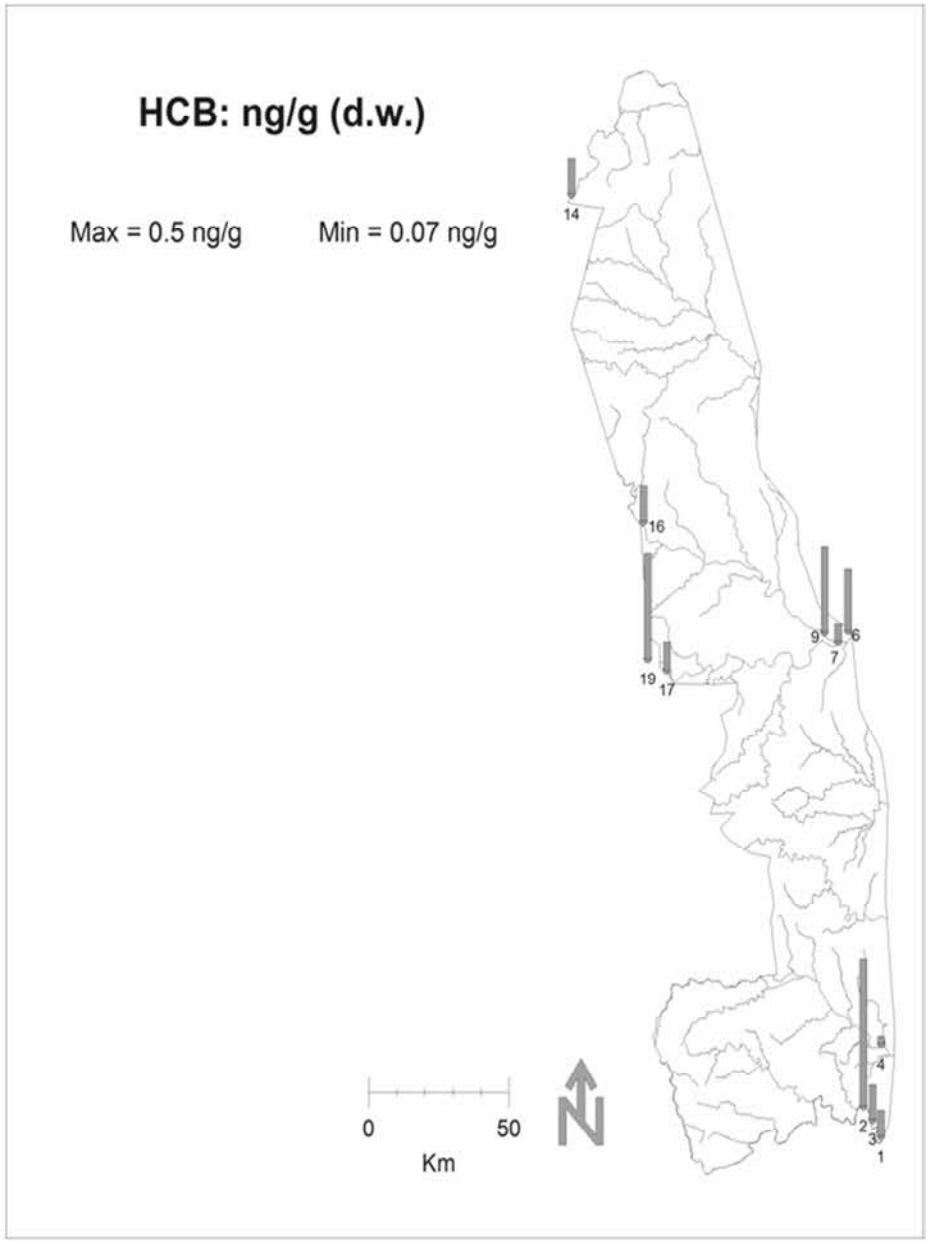


Figure 22 (Continue): Maps of the sediment concentrations of ΣHCH, PeCBs and HCB in the KNP.

The low concentrations of HCB can be expected as the registration for this compound was withdrawn in 1983. However it might still be used in the production of other chemicals (Bouwman, 2003). ΣHCH had a similar pattern with regards to the highest concentration of 0.9 ng/g dw at 2_NkoR with, and the lowest concentration at 14_LuvR, 0.14 ng/g dw (Figure 22). The concentrations of PeCB and ΣHCH increased slightly from upstream localities (17_OliR and 19_SelR) to downstream (6_OilR and 9_LetR) where the mortalities occurred. This pattern was not similar to HCB, as this compound had higher concentrations upstream at 19_SelR.

The highest concentrations of ΣPCBs and ΣPBDEs were quantified at 17_OliR, with lower concentrations towards 6_OliR and 7_Po. The sites on the far northern and western parts of the KNP were lower than the area where the crocodile mortalities occurred. The highest total PCBs was quantified at 17_OliR (3.43 ng/g dw), and the lowest concentration (0.44 ng/g dw) at 4_SabR (Figure 23). PBDEs were quantifiable at all sites (Figure 23); the highest concentration was at 17_OliR (1.34 ng/g dw), and the lowest at 3_CrocR (0.15 ng/g dw). There was a decrease in both the PCBs and PBDEs concentrations from the upstream sites (19_SelR and 17_OliR) towards the downstream sites (6_OliR) where the crocodile mortalities occurred.

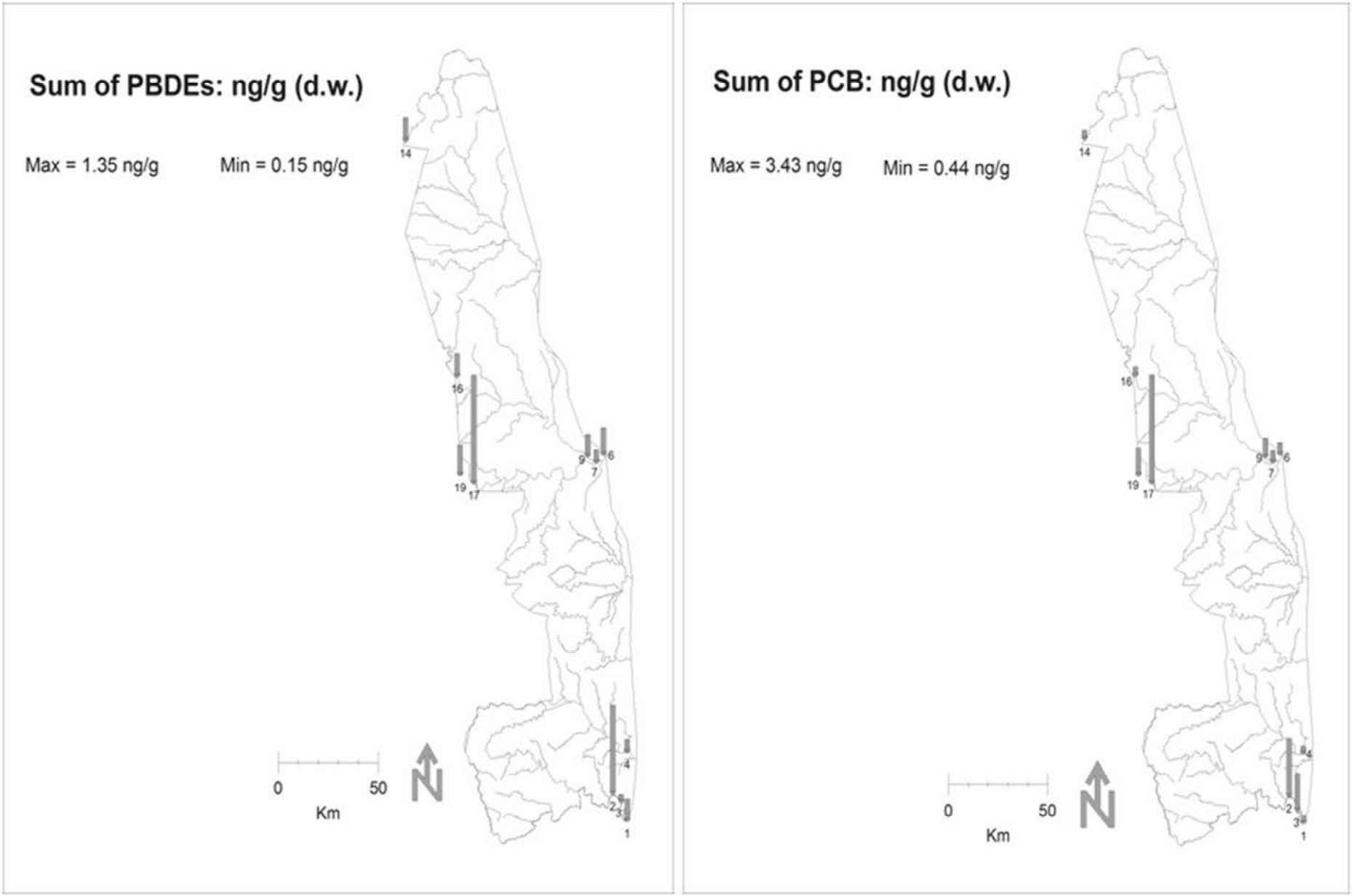


Figure 23: Maps of the sediment concentrations of ΣPCBs and ΣPBDEs in the KNP.

ΣPAHs had the highest concentrations of all the organic compound classes in this study. PAHs were also detected at all the sites (Figure 24). The LMW PAHs (Figure 24; sum concentrations of Ace, Nap, Acy, Ant, Fle and Phe) had the highest concentration at 8 249.44 ng/g dw for site 19_SelR. The HMW PAHs (Figure 24; the sum of BaP, BaA, Chr, B(a)F, B(bjk)F, BgP, IcP, Pyr and DaA) had the highest concentration at 2_NkoR (205.21 ng/g dw). The PAH congener with the highest concentration was Nap 7 737.78 ng/g at 19_SelR. The ΣPAHs (not shown) had the highest concentration (8 293.78 ng/g) of all the OMPs at 19_SelR near the highly industrialised town of Phalaborwa outside the KNP.

Heptachlor and Mirex will not be discussed in this section as both these compounds had concentrations below the LOD at all of the sites.

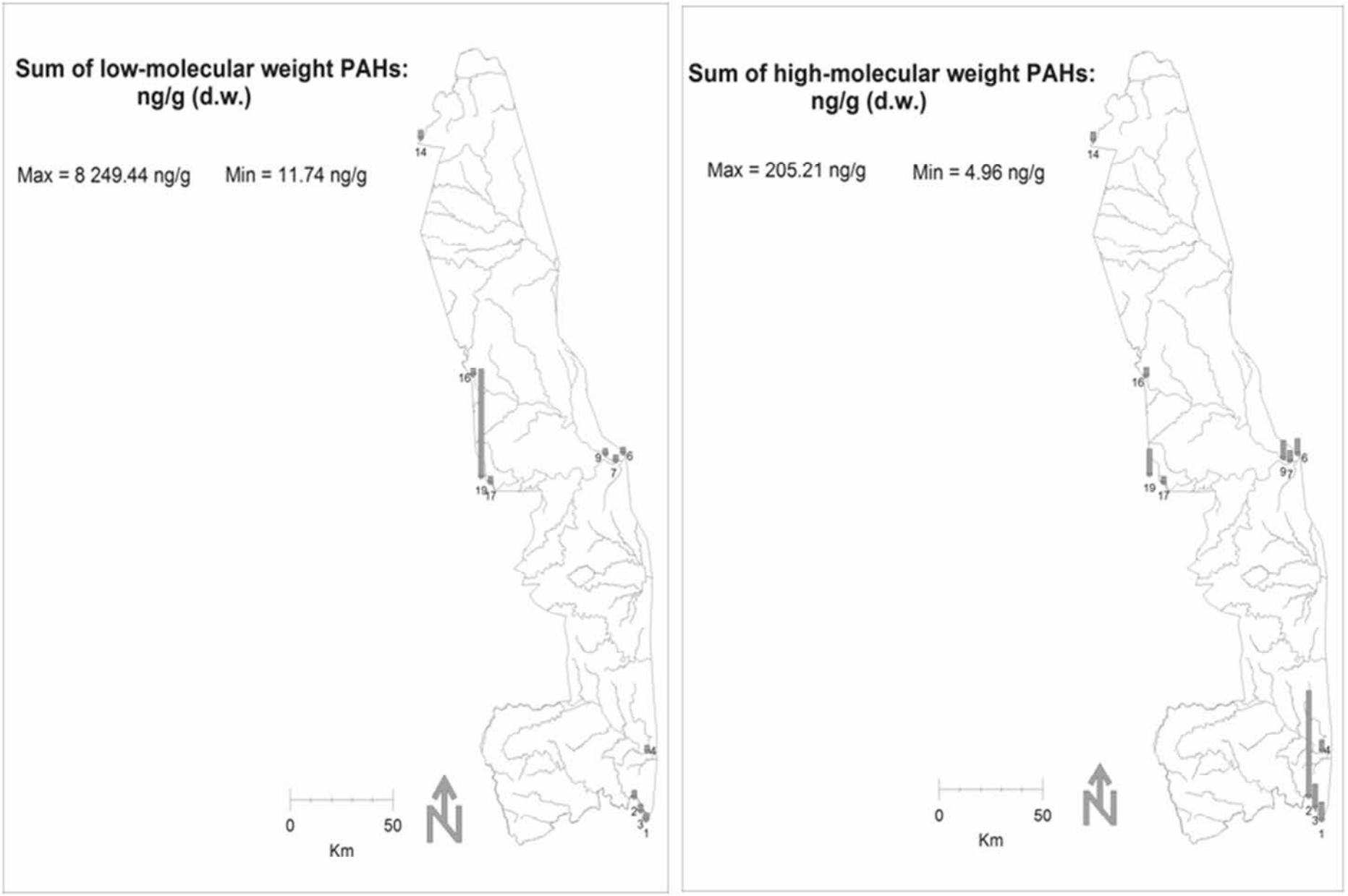


Figure 24: Maps of the sediment concentrations of ΣLMW PAHs and HMW PAHs in the KNP.

4.9.2 Geographical overview of the elemental concentrations

Both Hg and iodine had lower concentrations in the northern part of the KNP which is in contrast to the higher concentrations were quantified at 2_NkoR in the south (Figure 25). Mercury and iodine were quantified at all the sites at low concentrations (Figure 25). The highest Hg concentration was 0.1 µg/g dw and 82.5 µg/g dw for iodine, both at 2_NkoR. The rest of the localities all had Hg concentration of 0.01 µg/g dw and the lowest concentration for iodine was 0.3 µg/g dw at 11_LimR. The rain-fed reference site (7_Po) had an Hg concentration of 0.01 µg/g dw, and iodine concentration of 0.4 µg/g dw (Figure 25).

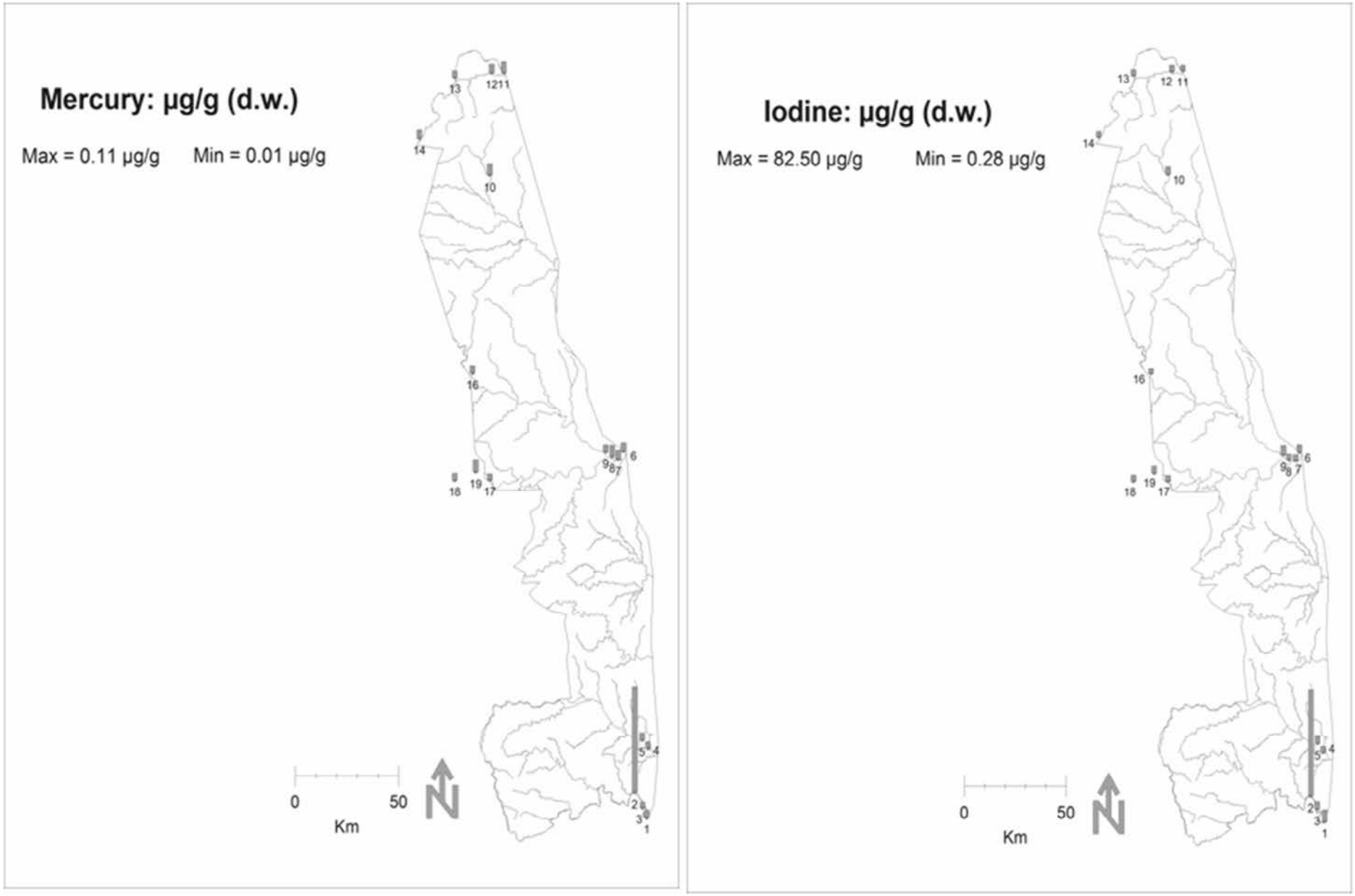


Figure 25: Maps of the sediment concentrations of Hg and I in the KNP.

Chromium, Ni, Fe and Co was grouped together in Figure 26 as all of these compounds occurred at all the sites and these four metals are next to each other on the periodic table. This means that these metals are similar in mass and therefore may explain similar distribution patterns. The highest concentrations occurred within the area where the crocodile mortalities occurred, but these concentrations were within the same order of magnitude as some of other sites such as at 10_ShisR and 12_LuvR (Figure 26).

Differences in the distribution patterns were observed for Cr, Ni, Fe and Co with higher concentrations quantified at 2_NkoR and lower concentrations at 3_CrocR and 1_CrocR. Iron had higher concentration at 5_Mlzi Pond which was not observed for Cr, Ni and Co. The highest Cr concentration was 350 µg/g dw (6_OliR) and the lowest concentration was 18.8 µg/g dw (11_LimR). The highest Ni concentration was 207.5 µg/g dw (also at 6_OliR) and the lowest concentration was 18.3 µg/g dw (14_LuvR and 11_LimR). With regards to the Fe concentrations, there were three localities (5_Mlzi pond, 6_OliR and 9_LetR) with the highest concentration of 67 500 µg/g dw, again, at 6_OliR. The lowest Fe concentration was 6 500 µg/g dw at 11_LimR. The highest concentration of Co, 55 µg/g dw was yet again 6_OliR, with the lowest concentration, 4.5 µg/g dw at 13_MutR (Figure 26).

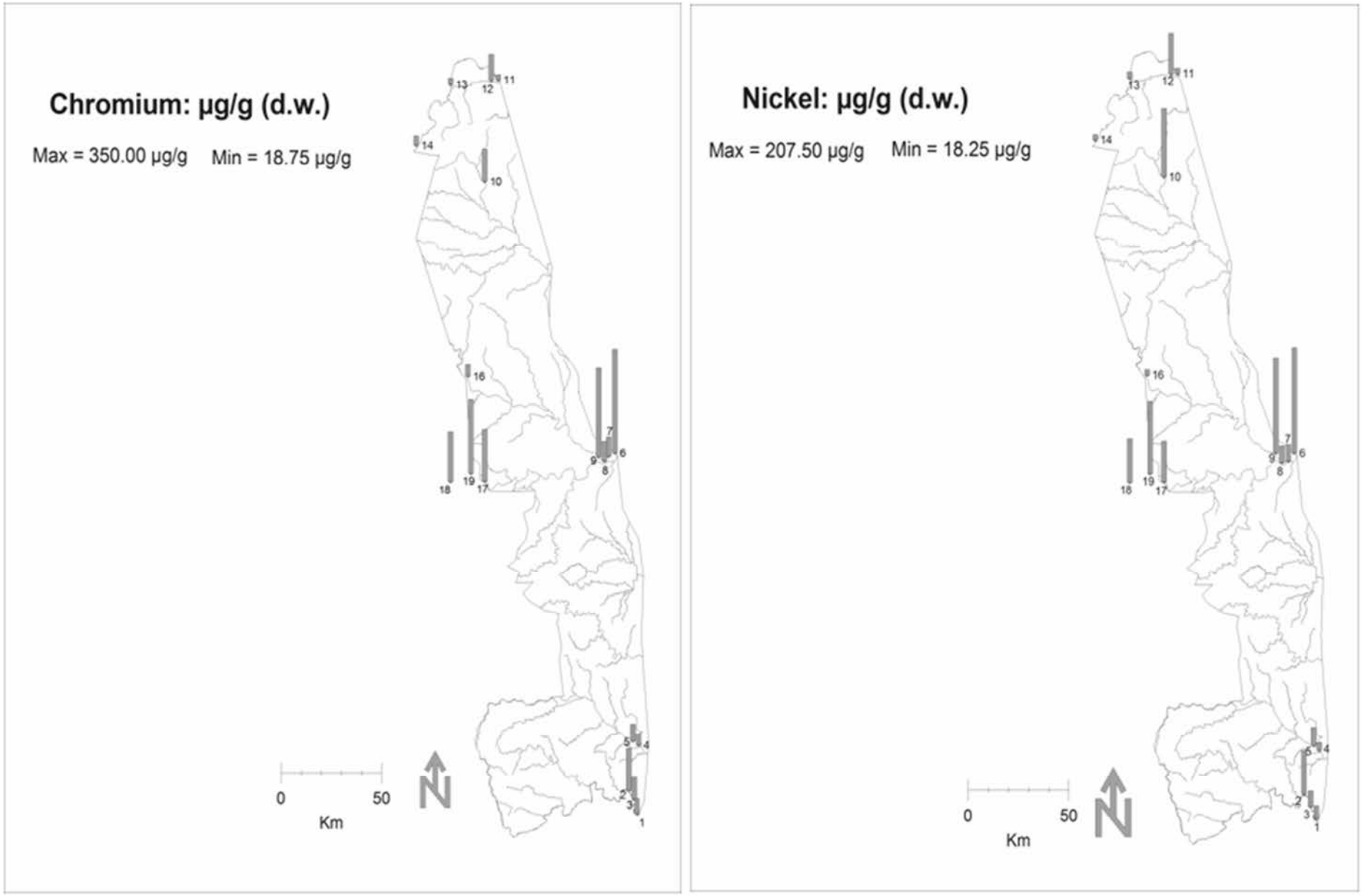
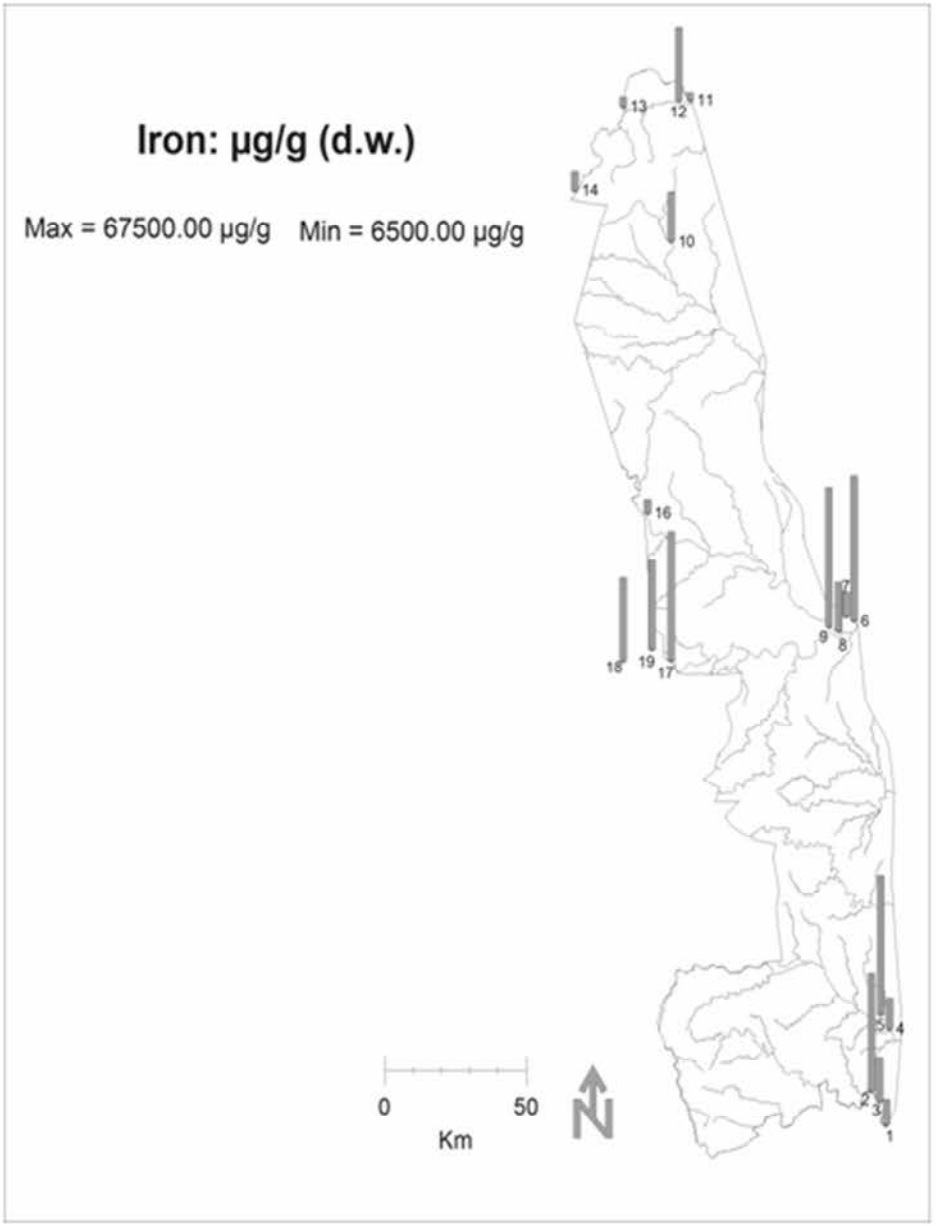


Figure 26: Maps of the sediment concentrations of Cr, Ni, Fe and Co in the KNP.



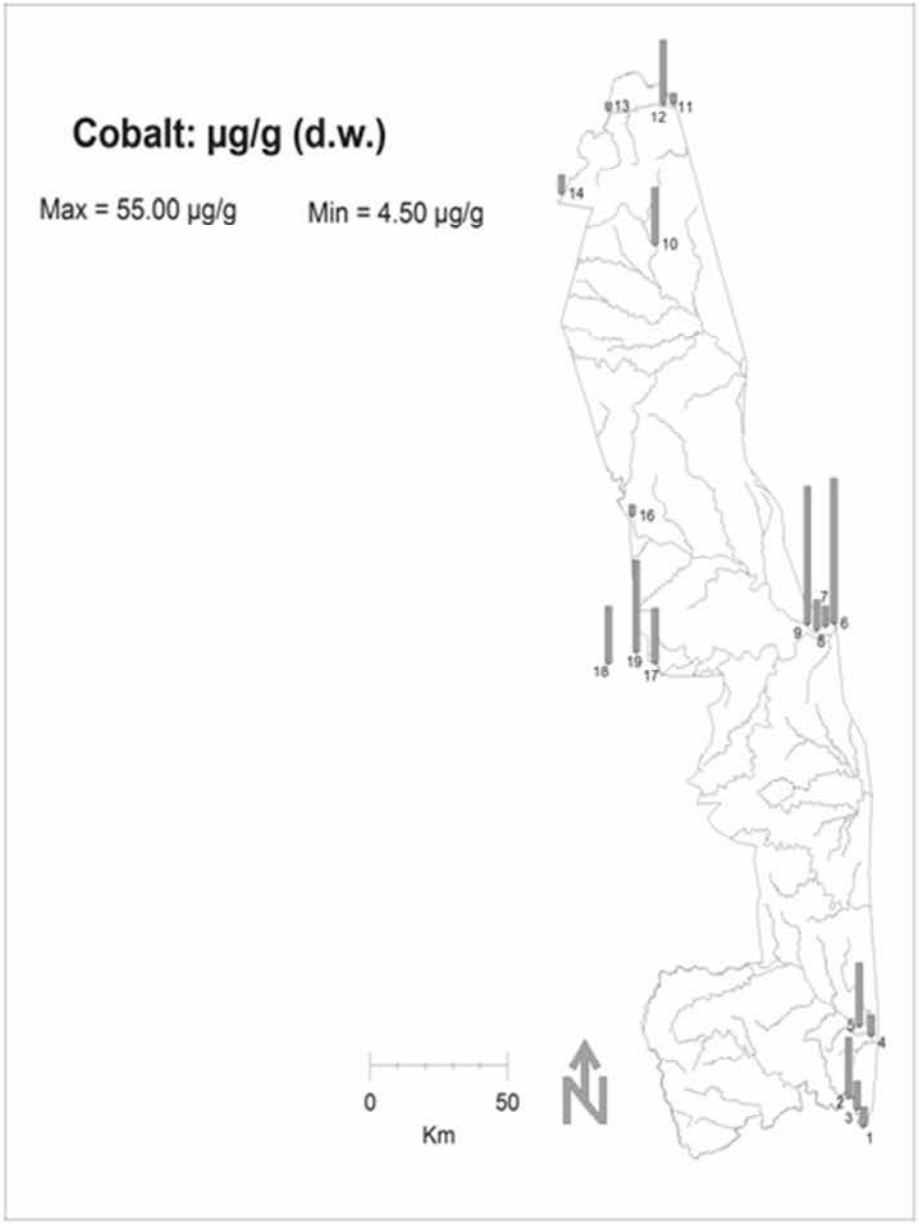


Figure 26(Continue): Maps of the sediment concentrations of Cr, Ni, Fe and Co in the KNP.

Cadmium, Ag, and Se were grouped together because of their relatively similar distribution patterns at 10_ShisR, 11_LimR and 12_LuvR in the northern part of the KNP, as well as the distribution of these compounds in the area where the crocodile mortalities occurred (Figure 27). It is possible that the higher sand shale and basalt deposit in the Limpopo areas (KNP, 2014) played a role in the distribution of the aforementioned metals. Cadmium and Ag are next to each other in the periodical table thus having similar elemental masses that may explain similar distributions. Selenium is a relatively lighter element in comparison with Cd and Ag. In contrast to Cd and Ag, Se is considered a metalloid and it is possible that the metallic characteristics of this chemical reacted the same as the aforementioned metals. Both Cd and Ag were quantified in low concentrations in all the sediment samples (Figure 27), whereas Se concentrations were higher than Cd and Ag.

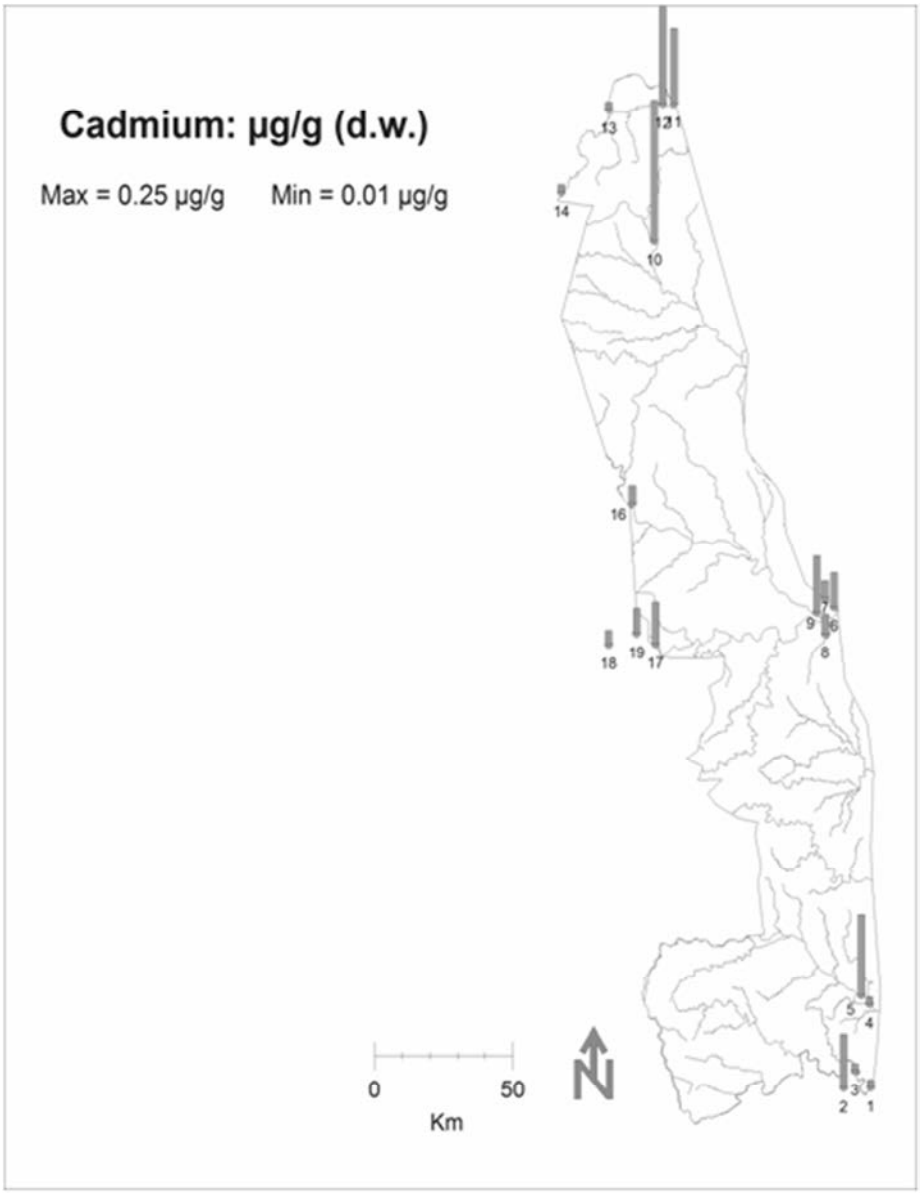
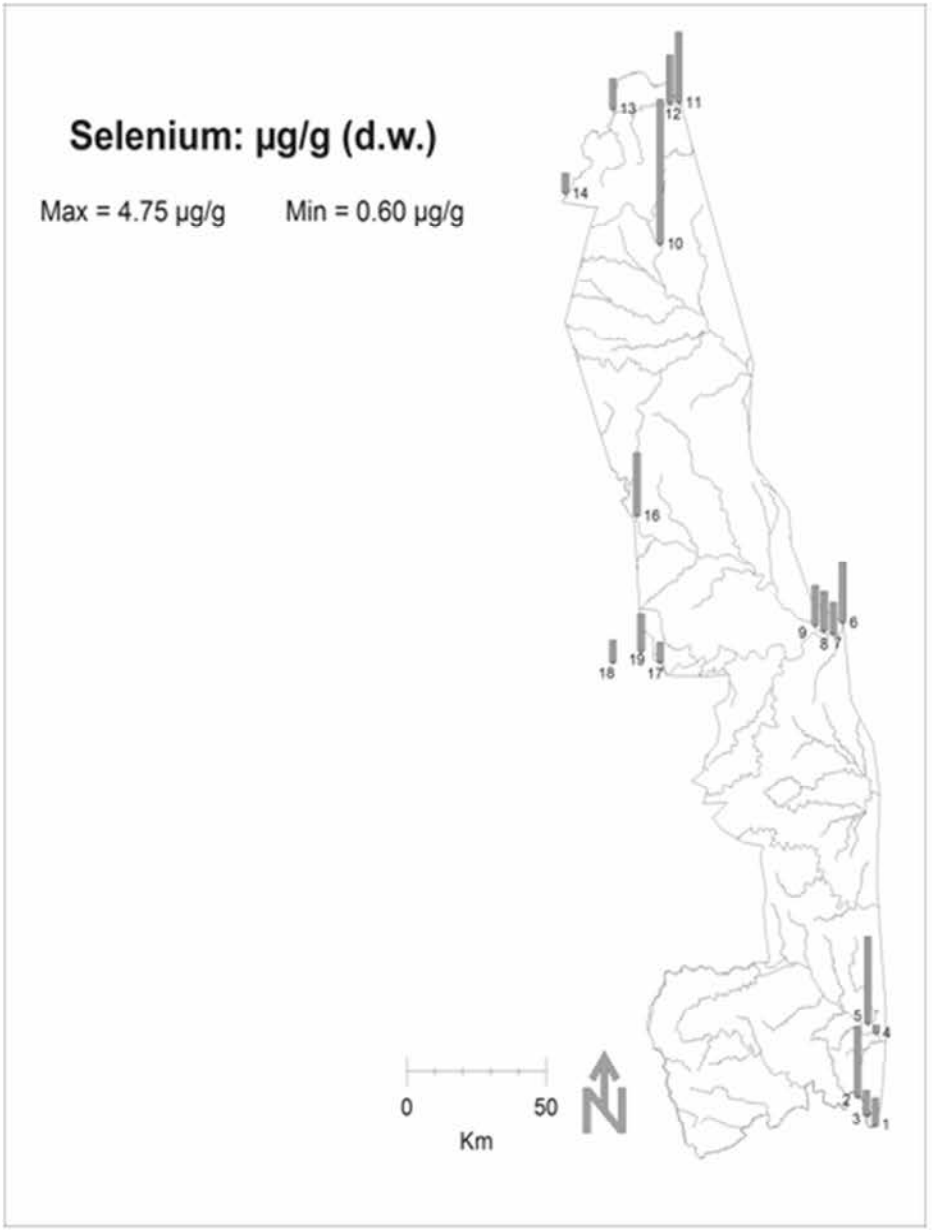


Figure 27: Maps of the sediment concentrations of Cd, Ag and Se in the KNP.



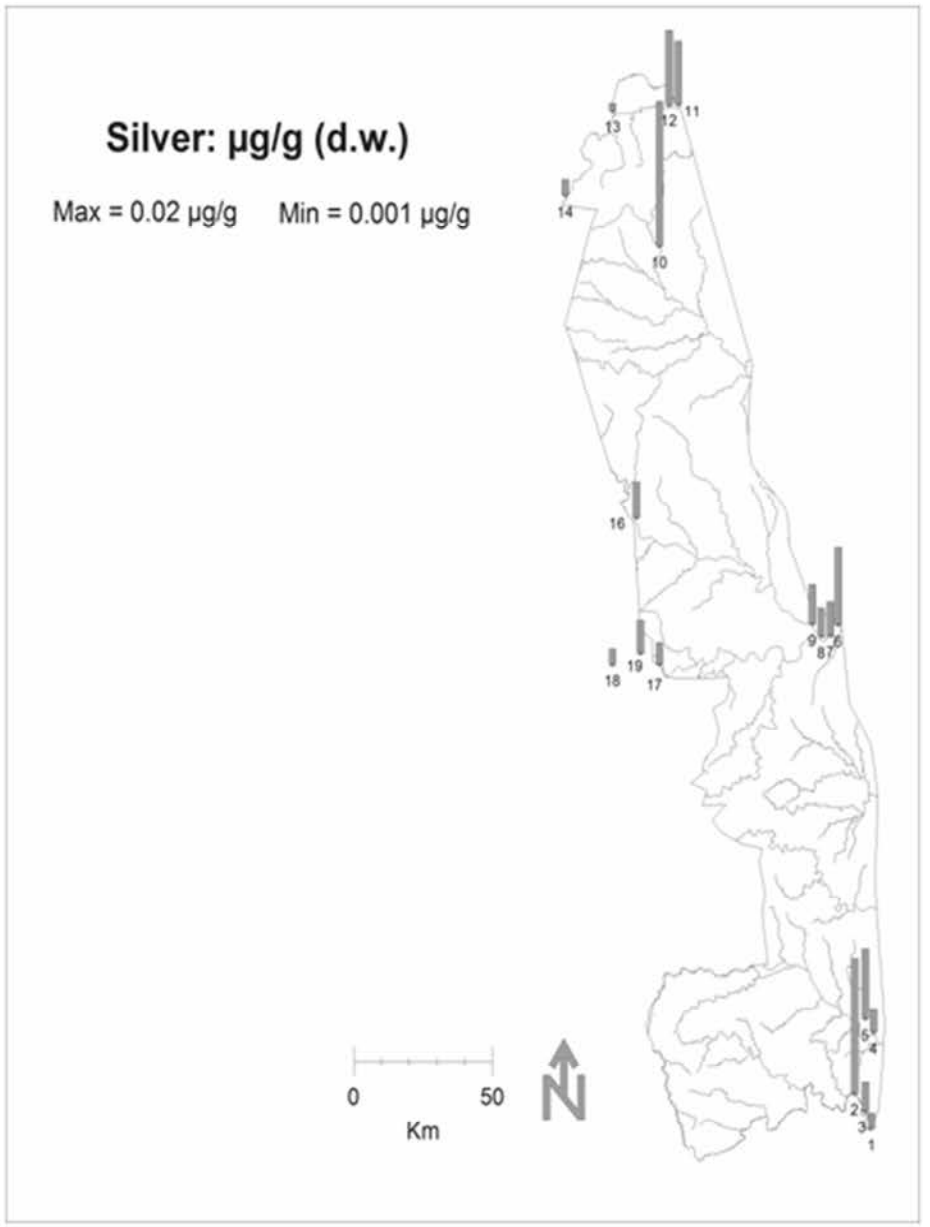


Figure 27 (Continue): Maps of the sediment concentrations of Cd, Ag and Se in the KNP.

The highest Cd concentration was 0.3 µg/g at 10_ShisR. The lowest concentrations were below the LOD (0.01 µg/g) at five of the sites (1_CrocR, 8_OliR, 16_LetR, 11_LimR, 13_MutR and 14_LuvR). The highest concentration of Ag was 0.02 µg/g dw (10_ShisR), with the lowest concentration at 0.001 µg/g dw (4_SabR). The highest quantifiable Se concentration was 4.75 µg/g dw (10_ShisR), and the lowest concentration was 0.6 µg/g dw at 4_SabR (Figure 27).

Vanadium was quantified at all the sites, with the highest concentrations upstream and downstream of the area where the crocodile mortalities occurred (Figure 28).

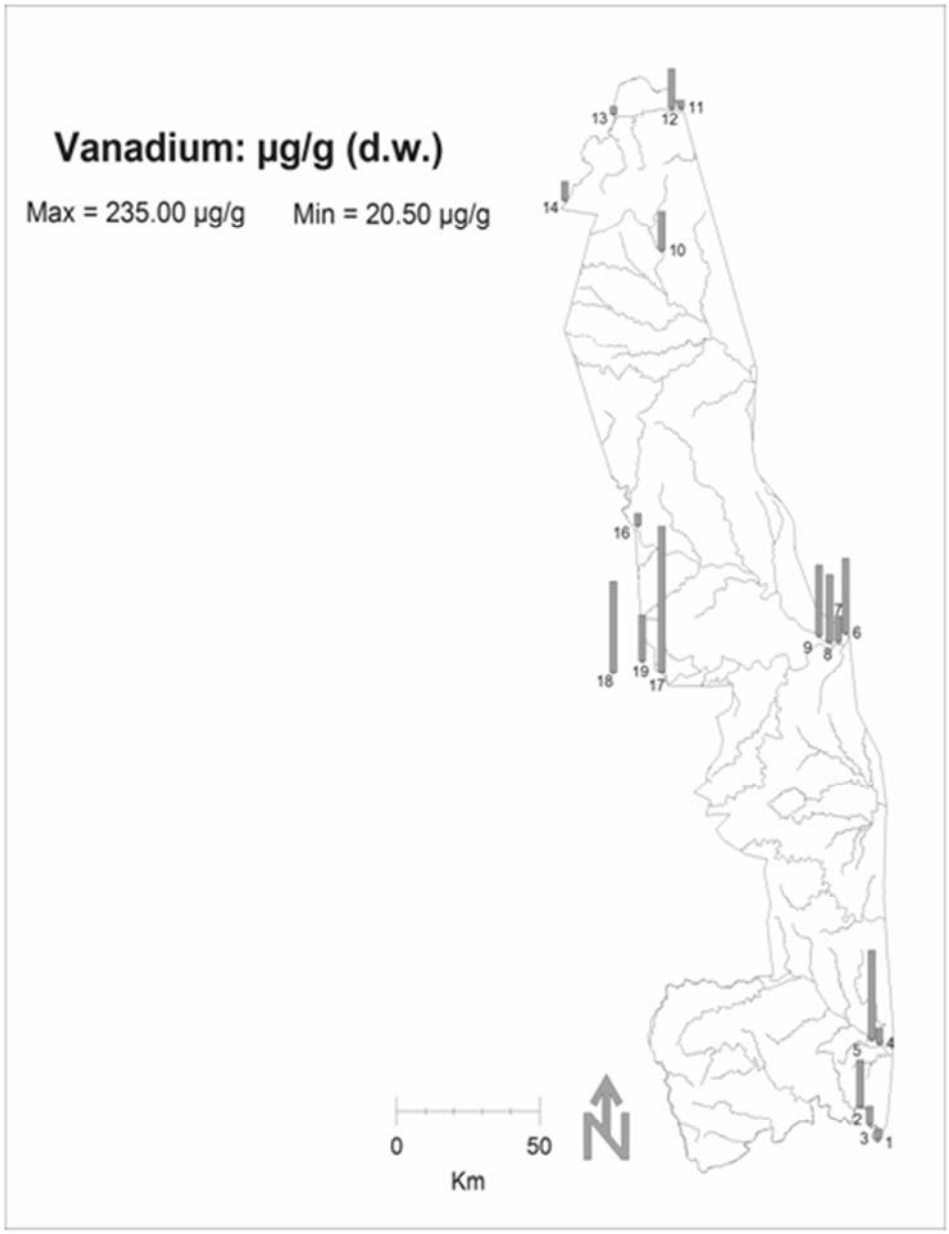


Figure 28: Maps of the sediment concentration of V in the KNP.

The highest concentration was 235 µg/g dw at 17_OliR, and the lowest was 20.5 µg/g dw at 11_LimR. This metal had a unique distribution pattern with intermediate concentrations within the area where the crocodile mortality occurred (6_OliR, 8_OliR and 9_LetR), the rivers near the Phalaborwa industrial area (17_OliR and 18_OliR) and the southern part (2_NkoR and 5_Mlzi pond) of the KNP (Figure 28). Relatively low concentrations were quantified in the far northern part (11_LimR, 12_LuvR, 13_MutR, 14_LuvR and 10_ShisR) of the KNP (Figure 28).

The Cu, Pb, U and Zn were grouped together in Figure 29 because of their similar distribution pattern in the area where the crocodiles died (6_OliR and 9_LetR). Copper and Zn have similar molecular mass as they are next to each other on the periodic table, while Pb forms part of the transition metals and U is an actinide metal. All four of these compounds were quantifiable at all of the sites (Figure 29).

Copper's highest concentration was 145 µg/g dw quantified at 5_Mlzi pond (Figure 29), and the lowest concentration was 7.8 µg/g dw at 11_LimR. The highest concentration of Pb was 20 µg/g dw at 2_NkoR, and the lowest concentration was 1.8 µg/g dw at 14_LuvR (Figure 29). The highest concentration of U was 0.4 µg/g dw at 10_ShisR, and the lowest was 0.1 µg/g dw at 13_MutR (Figure 29). The highest concentration of Zn was 132.5 µg/g dw at 5_Mlzi pond, and the lowest was 9.25 µg/g dw at both sites 13_MutR and 14_LuvR.

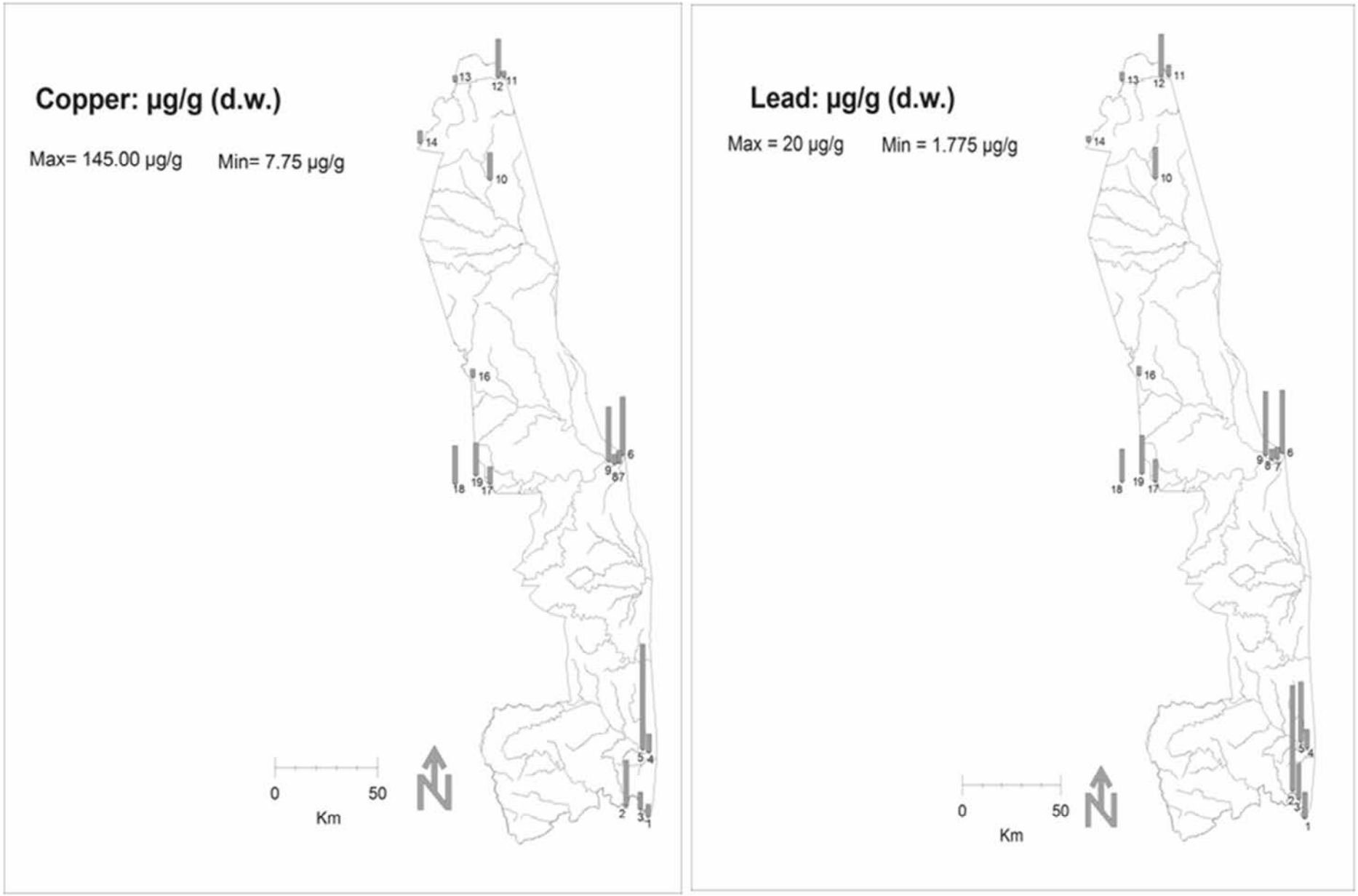
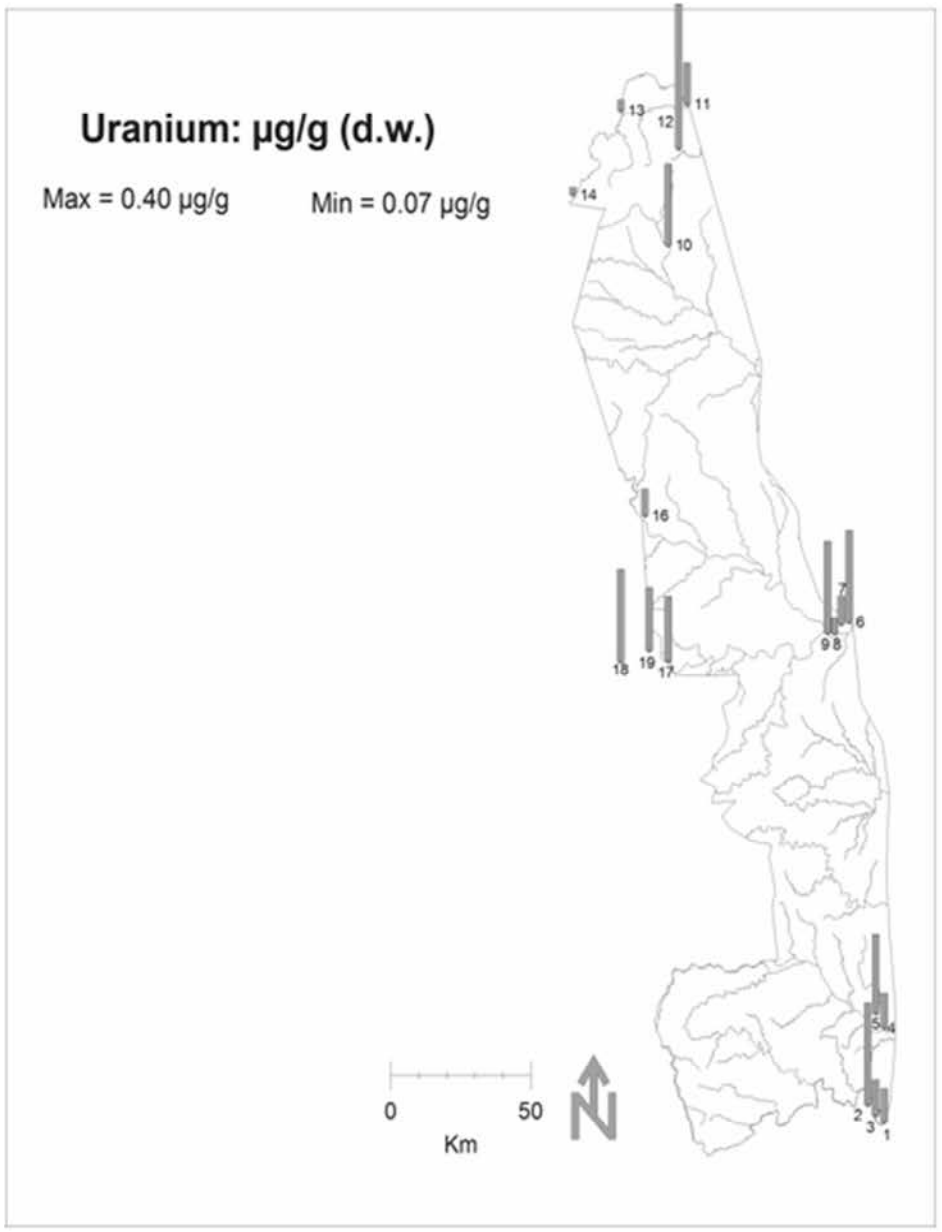


Figure 29: Maps of the sediment concentrations of Co, Pb, U and Zn in the KNP.



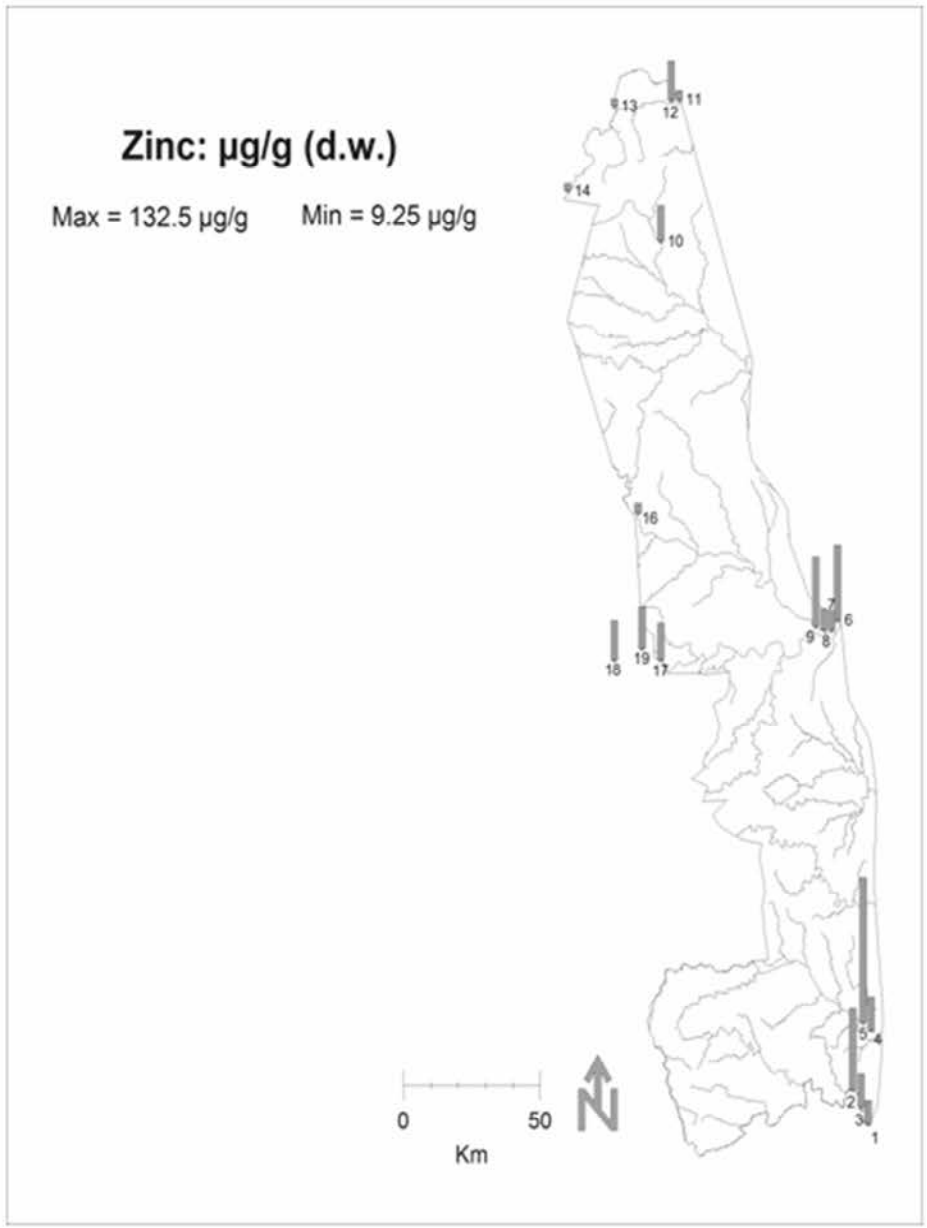


Figure 29 (Continue): Maps of the sediment concentrations of Co, Pb, U and Zn in the KNP.

The only corresponding factor that placed As, Ba, and Mn together in Figure 30 was the concentrations in the area where the crocodile deaths occurred. These concentrations increased from the upstream sites to the downstream sites (Figure 30). All three these elements had relatively high concentrations at 6_OliR and 9_LetR, with lower concentrations at 7_Po and 8_OliR. This selection of elements was quantifiable at all the localities at different concentrations. Both Mn and Ba had high concentrations quantified at 2_NkoR with the lowest concentration at 13_MutR. The highest concentration of As was 24 µg/g dw at 4_SabR, and the lowest concentration at 14_LuvR, 1.6 µg/g dw.

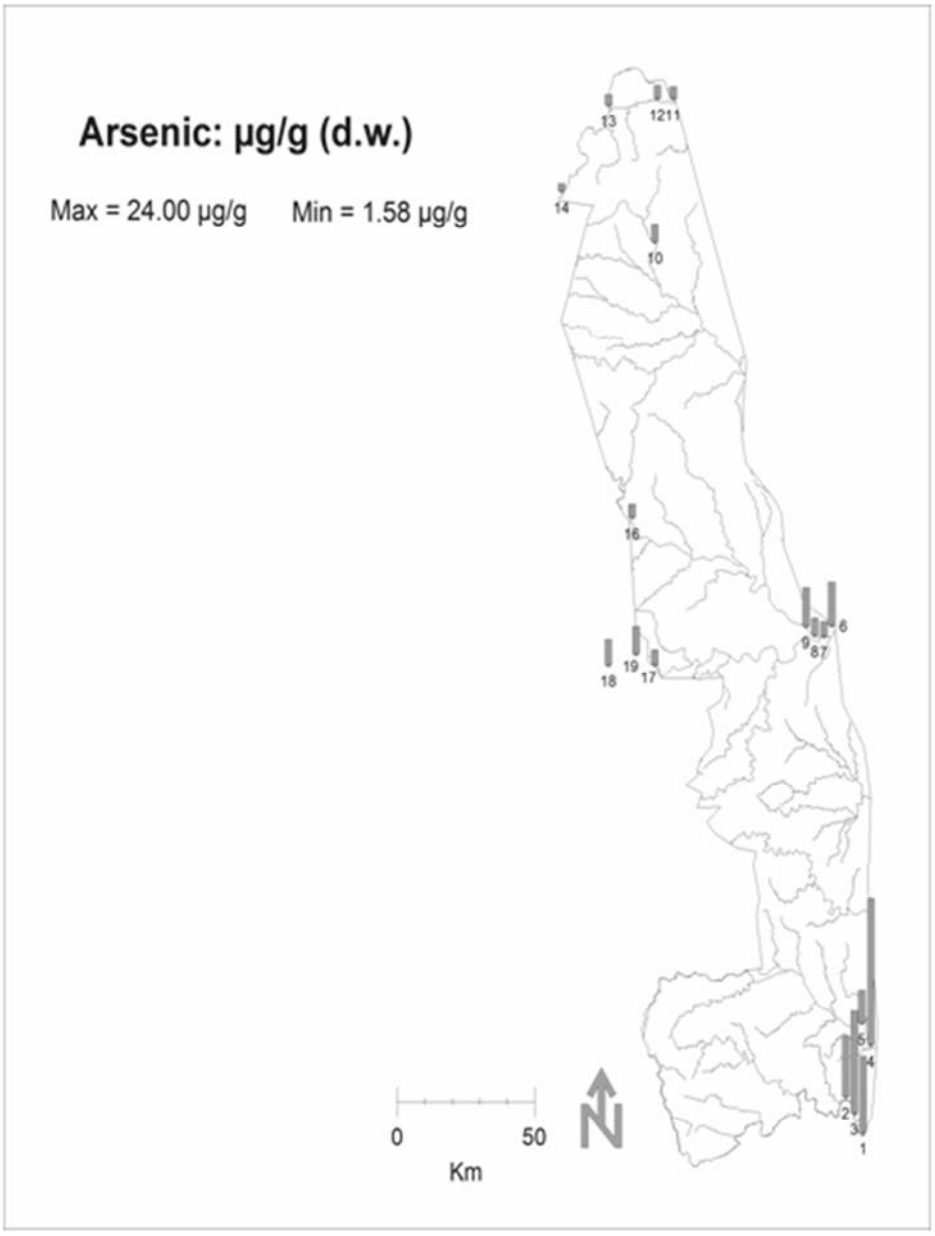
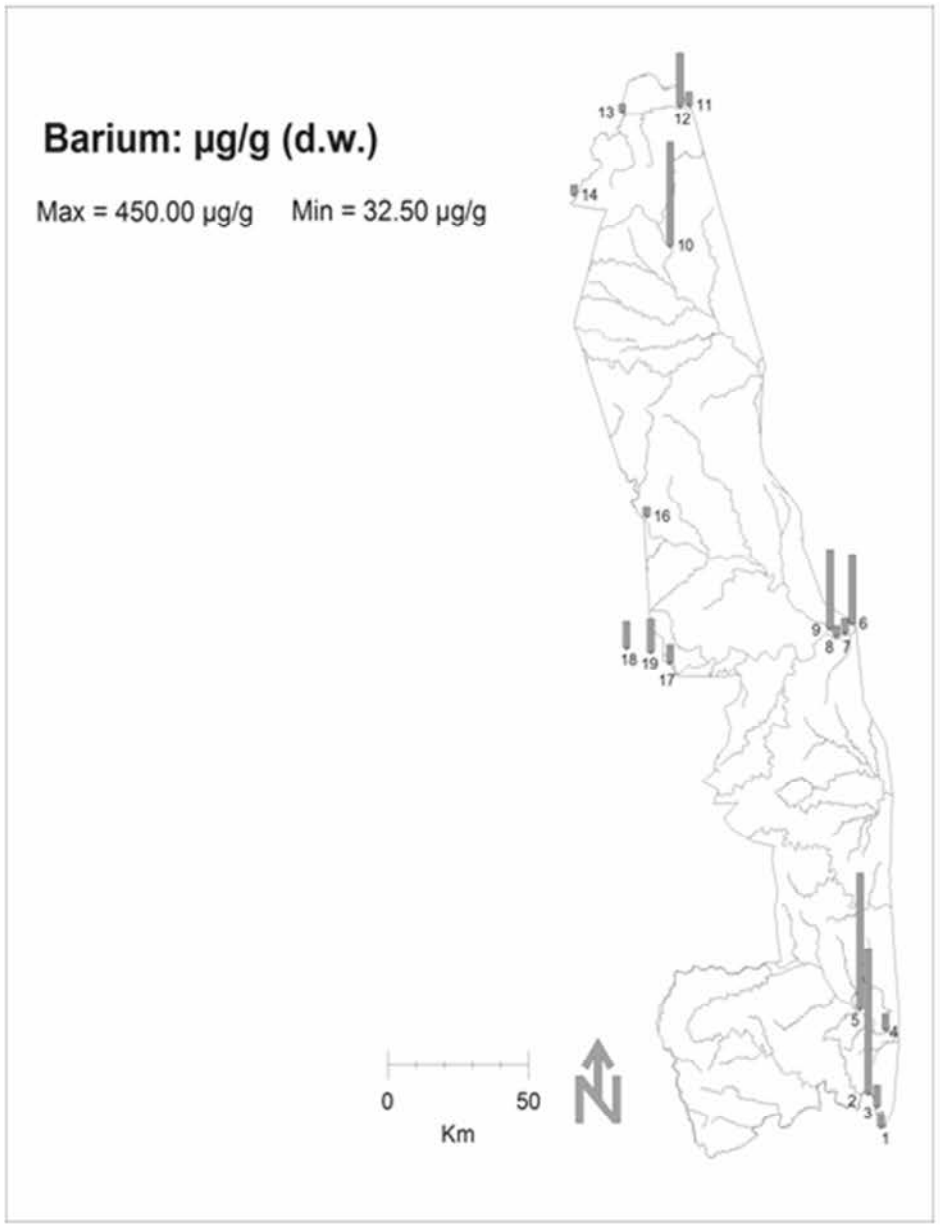


Figure 30: Maps of the sediment concentration of As, Ba and Mn in the KNP.



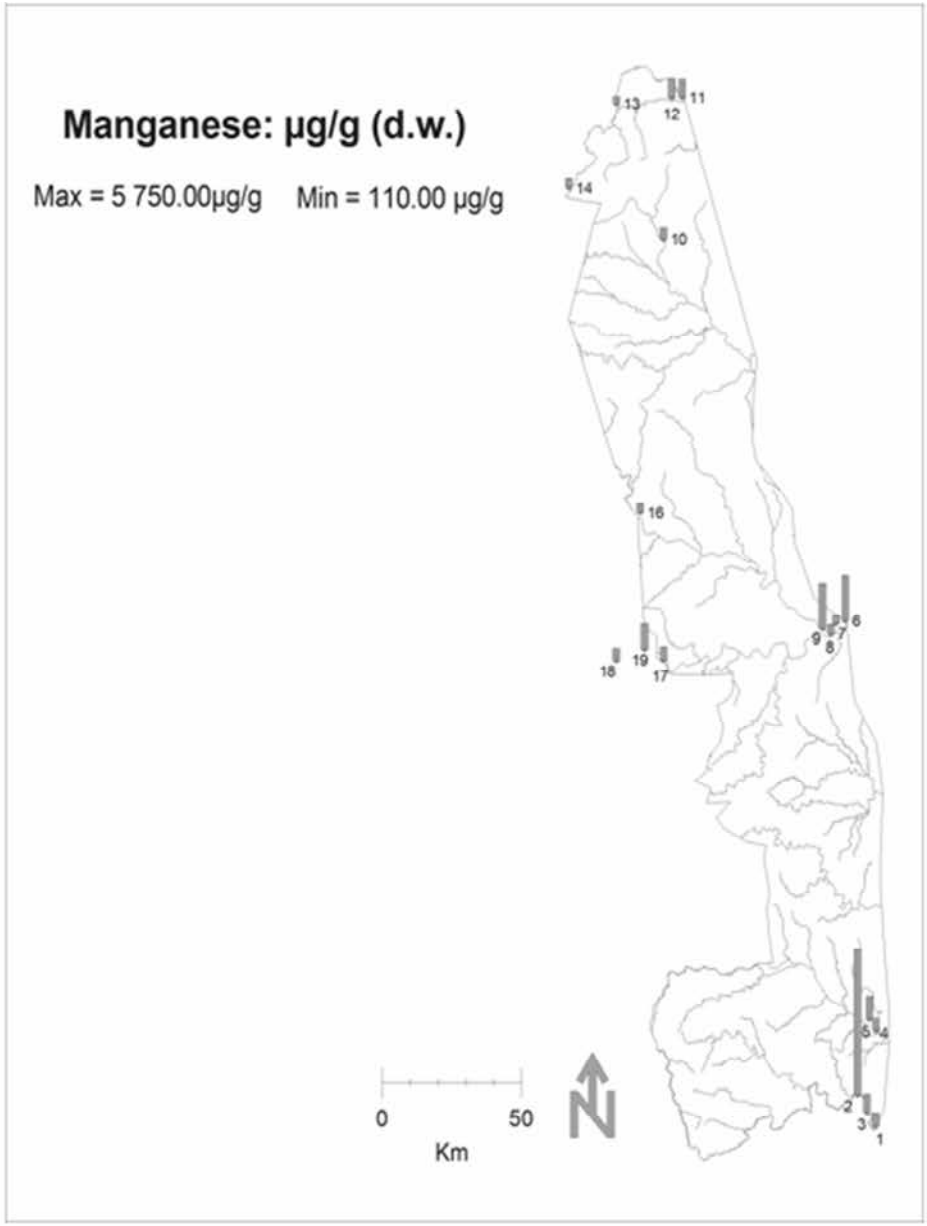


Figure 30 (continue): Maps of the sediment concentration of As, Ba and Mn in the KNP.

Barium distribution indicated the highest quantified concentration of 450 µg/g dw at 2_NkoR, and the lowest concentration at 13_MutR, 32.5 µg/g dw (Figure 30). The highest concentration of Mn was 5 750 µg/g dw 2_NkoR, and the lowest concentration was 110 µg/g dw at 13_MutR (Figure 30).

4.10 *In situ* water quality variables

The selected *in situ* water quality variables were quantified when the sediment samples were collected. The significant numbers were reported in Table 16, containing the pH-values, temperature on day of sampling, TDS and calculated EC value. The pH-values for each of the sampled sites were within the target water quality range of 6.5–9.0 (DWAF, 1996a). The average temperature for the different sampling sites was between 14–24^{0}C on the two days of sampling. This was within the target water quality range of 5–30ºC for inland waters of South Africa (DWAF, 1996a). In general, the pH of all the sites can be described as slightly alkaline (Table 16). The salinity of the water can be described according to the TDS/EC value. Non-saline inland water should have a TDS value below 450 mg/ℓ or EC value below 70 mS/m (DWAF, 1998). Most of the sites (1_CrocR, 3_CrocR, 4_SabR, 6_OliR, 8_OliR, 9_LetR, 11_LimR, 12_LuvR, 16_LetR, 17_OlR and 18_OliR) had TDS values below 450 mg/ℓ. The rest of the sites (2_NkoR, 5_Mlzi pond, 7_Po, 10_ShisR and 19_SelR) had a TDS value greater than 450 mg/ℓ (DWAF, 1998). All of the sites had values below the 70 mS/m range except for the following sites (5_Mlzi pond, 10_ShisR and 19_SelR) that were greater than 70 mS/m. The following parameters; pH, electrical conductivity (EC), total dissolved solids (TDS) and temperature were used as environmental variables in the PCAs (Figure 31–38).

Table 16: The *in situ* water quality variables

Site	Temperature (ºC)	TDS (mg/ℓ)	pH	EC (mS/m)
1_CrocR	17	31	7.3	5
2_NkoR	18	516	8.3	80
3_CrocR	16	376	8.4	58
4_SabR	15	90	7.7	14
5_Mlzi pond	14	1425	7.6	219
6_OliR	19	318	8.6	49
7_Po	20	458	8.8	70
8_OliR	20	359	8.6	55
9_LetR	21	359	8.4	55
10_ShisR	15	1400	8.7	210
11_LimR	24	307	8.4	47
12_LuvR	19	43	8.2	7
13_MutR	16	41	7.8	6
14_LuvR	17	101	8.1	16
16_LetR	20	386	8.1	59
17_OliR	19	313	8.6	42
18_OliR	17	315	8.6	48
19_SelR	21	1595	8.7	245
Mean	18	467	8.3	72
Minimum	14	313	7.32	5
Maximum	24	1595	8.7	245

4.11 Principle Component Analysis (PCA)

The PCAs were performed to compare the organic chemical and elemental fingerprint of the sites to detect similarities regarding pollutant sources. All the data and physical parameters were normalised using the log-transformation method proposed by Howel (2007). The quantified PCB concentrations were used and not the TOC normalised data in the log-transformation. The water chemistry data was used in the PCA to determine if the water chemistry parameters might have influenced the pollutant profiles in the sediment. The EC was used to calculate the TDS; therefore their vectors will most likely be very similar. PCA bi-plots of all the chemical compounds are presented in Figures 31–38.

Separate PCA's were run for the following combinations:

- All compounds (Figures 31–33): This PCA consisted of the normalised data for POPs (chlordanes, DDT and metabolites, PCBs, PBDEs, PCDFs, PCDDs, HCH, mirex, heptachlor,

HCB, PeCB), PAHs, and the selected elements. The Sites included in were; 1_CrocR, 2_NkoR, 3_CrocR, 4_SabR, 6_OliR, 7_Po, 9_LetR, 14_LuvR, 16_LetR, 17_OliR and 19_SelR.

- Unintentionally produced compounds (Figure 34): These compounds (DL-PCBs, PCDD/Fs, PeCBs and HCB) are mostly released as by-products mainly of combustion. Studying the distribution of compounds and sites in the statistical space can show relationships in sources that would not be obvious in large combination PCAs.
- PAHs only (Figure 35): The low molecular weight PAHs are mainly of petrogenic origin, and would deposit farther from the source of emission, the heavy molecular weight PAHs are mainly associated with particulate matter, and would deposit closer to their sources of emission. These characteristics may influence the PAH composition due to relative distances from possible sources.
- PCBs, DL-PCB and PBDEs (Figure 36): The PBDEs were added together with the PCBs as both these compound groups were produced intentionally and used for a wide variety of applications. The DL-PCBs are produced unintentionally; however they were also produced with intentionally produced PCBs. Combining these chemicals together in the PCA analyses may provide indications of possible sources.
- Chlorinated pesticides (Figure 37): This combination allows for a closer look at the different pesticides and its associations with the sites.
- Elements only (Figure 38): The elements were also analysed separately, because they also occur naturally and their distributions are governed by the geology of the area that might explain possible associations in ordination space.

4.12 PCA of all compounds

In Figure 31, 32 and 33 the POPs (chlordanes, DDT and metabolites, PCBs, PBDEs, PCDFs, PCDDs, DL-PCB, HCH, mirex, heptachlor, HCB, PeCB) PAHs, and the selected elements were ordinated together with the following sites: 1_CrocR, 2_NkoR, 3_CrocR, 4_SabR, 6_OliR, 7_Po, 9_LetR, 14_LuvR, 16_LetR, 17_OliR and 19_SelR. This produced a PCA where factor 1 explained 34%, factor 2, 17%, factor 3, 13% and factor 4, 9% of the variance observed in the data. It was mostly the organic compounds that loaded to factor 1, 2 and 3, while the elements featured more prominently in factor 4. The first factor consisted of a contrast between the heavier PCDD/Fs such as the hexa- and hepta PCDD/Fs, OCDD, ODCF and the HMW PAHs (eg. IcP, Chr, BkF and Pyr), *o,p'*-DDT and iodine on the negative side and the heavier PCBs, PBDEs and V on the positive side. The

water chemistry parameters also loaded on the positive side of factor 1 (Figure 31) however, the TDS and EC were loaded more to the positive side of factor 2 (Figure 31). Factor 2 consisted of non-dioxin like PCBs, *p,p'*-DDD and higher mass furans on the negative side that was in contrast to the LMW PAH (Nap, Fle, Acy, Ant & Phe) loadings, together with HCB, *o,p'*-DDE, *o,p'*-DDD and lighter weight PCBs (PCB18 and 28) on the positive side. High loadings were recorded for different masses of dioxins (TCDD/F and Hexa PCDD/Fs) on the positive side of factor 3. This was in contrast to the HMW PAHs, TDS, EC, PCBs, cChlor, tChlor, tNChlor, and cNChlor on the negative side of this factor. The localities that scored highly on the positive side of factor 3 were 14_LuvR and 1_CrocR in contrast to the high negative scores of sites 3_CrocR, 2_NkomR and 6_LetR (Figure 32). The elements loaded strongly on the negative side of factor 4. Here factor 4 indicated a contrast of the elements V, Cr, U, Fe, Co, Ni, Al, Ag, Cu, Zn, Pb, Cd, Ba and Mn together with, PeCB, HCB, γ-HCH, BDE209, PCB33 and a smaller furan on the negative side which was in contrast to HxCDD, PCB206, PCB209, PCB114, PCB47 and oChlor on the positive side (Figure 33).

The sampling sites mostly influenced by factor 1 were sites 2_NkoR, 1_CrocR 3_CrocR and 4_SabR which had high scores on the negative side with 17_OliR, 16_LetR and 7_Po scoring strongly on the positive side. On the negative side of factor 2 the only site that scored highly was 17_OliR while site 19_SelR had a high positive score (Figure 20). The fourth factor only explained 9% of the total variance in the data; however the transition metals Ba, Cd, Mn, Cu, Pb, Al, Fe, Ni, Co, Zn, Ag and V loaded highly on the negative side of factor 4. Two of the sites in the area where the crocodiles died, sites 6_OliR and 9_LetR had high scores for the negative side of factor 4. The positive side again consisted of the POPs (Hexa-PCDD, PCBs, oChlor, tNChlor, cNChlor, Hept and LMW PAHs with high scores of site 14_LuvR and 3_SabR (Figure 33).

Figure 31: PCA-biplot between factor 1 and factor 2 of the POPs, PAHs and elements including the sites sampled and the water chemistry for the different sites.

Figure 32: This PCA-biplot between factor 1 and factor 3 of POPs, PAHs and elements including the sites sampled and the water chemistry for the different sites.

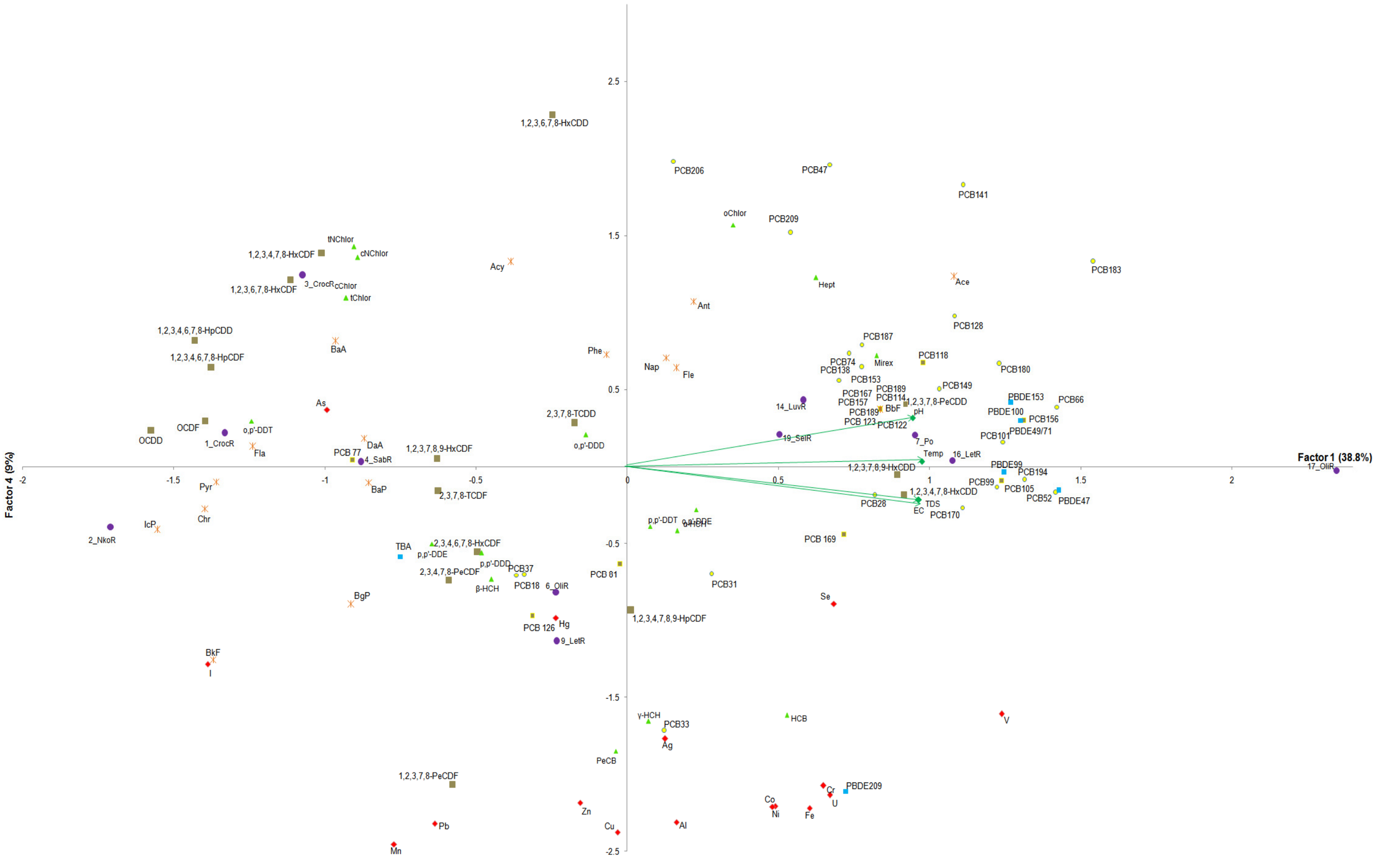


Figure 33: This PCA biplot between factor 1 and factor 4 of POPs, PAHs and elements including the sites sampled and the water chemistry for the different sites.

4.13 PCA with the unintentionally produced compounds

The following compounds (DL-PCBs, PCDD/Fs, PeCBs and HCB) were added in a PCA as they are mostly released from anthropogenic activities as by-products (Figure 34) with the variance in the data at factor 1 explaining 50% and factor 2, 21% (Figure 34). On the positive loading of factor 1 the unintentionally produced compounds consisted of the heavier dioxins and furans (HpCDD/F and OCDD/Fs) (Figure 34). The negative side was strongly loaded with the smaller to medium mass dioxins (1,2,3,4,7,8-HxCDD 1,2,3,7,8,9-HxCDD and 1,2,3,7,8-PeCDD) in combination with hexachloro-DL-PCBs (PCB 169 and 156). The contrast existed between hexachloro and heptachloro compounds. Factor 2 was loaded positively with TCDD/Fs and non-ortho substituted PCB 81. The higher mass furan; 1,2,3,7,8,9-HxCDF was also loaded highly on the positive side in contrast to the dioxins and furans; 1,2,3,6,7,8-HxCDD; 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF, 1,2,3,4,6,7,8-HpCDD, PeCB, and OCDD on the negative side. The environmental factors played a role in both Factor 1 and Factor 2 were pH, temperature, TDS and EC loaded highly on the negative side of factor 1 and factor 2 respectively (Figure 34).

Sites 17_OliR, 16_LetR and 7_Po scored highly on the negative side of factor 1, whereas sites 1_CrocR, 2_NkoR, 3_CrocR and 4_SabR scoring highly on the positive side of the same factor. In Factor 2 the sites with the highest score on the positive side were 1_CrocR and 17_OliR with the highest negative scores at site 3_CrocR (Figure 34).

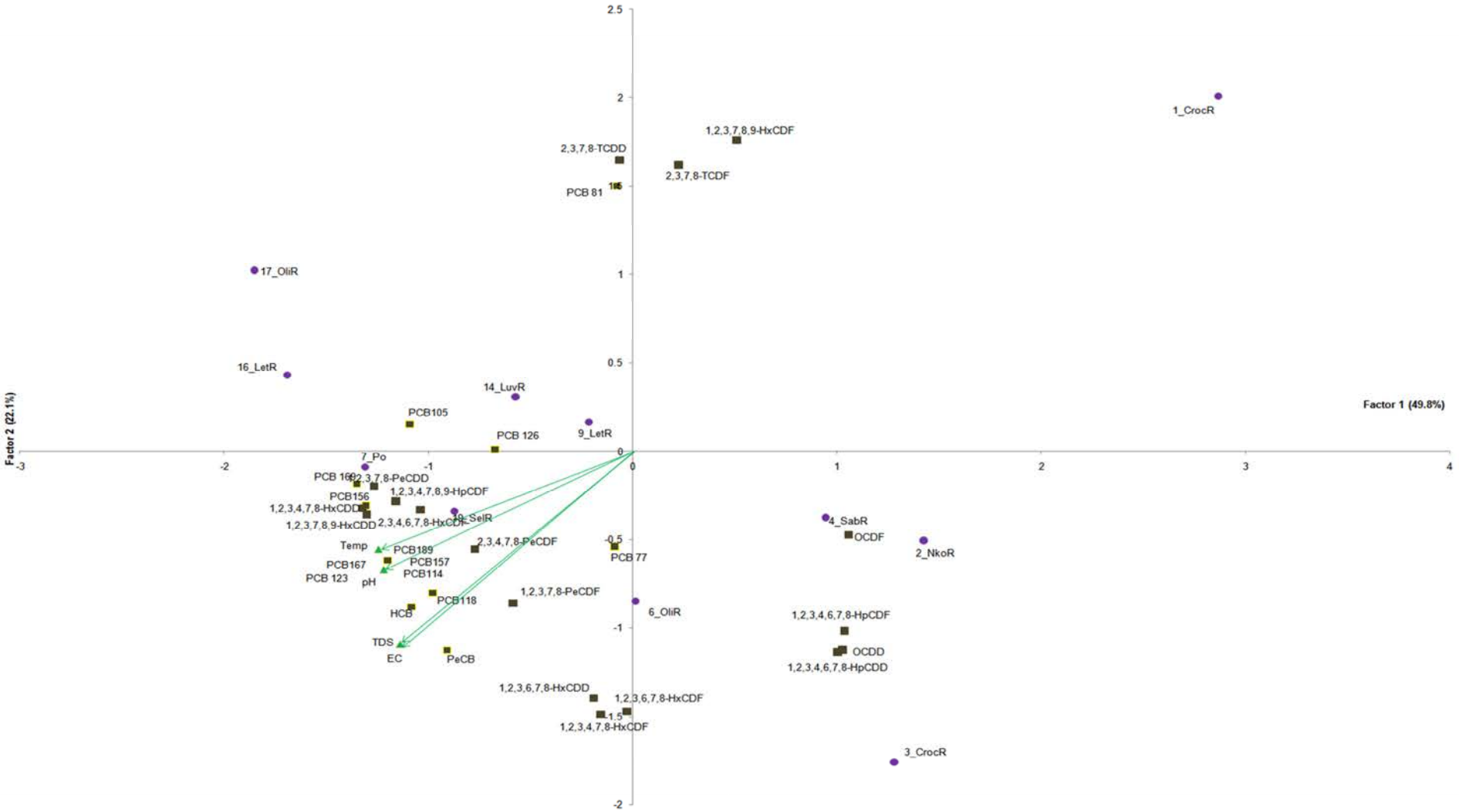


Figure 34: This PCA-biplot between factor 1 and 2 has all the unintentionally produced compounds (DL-PCBs, PCDD/Fs, PeCBs and HCB), the sites and the water chemistry information.

4.14 PCA with only the PAHs

The PAHs were loaded together as their unique characteristics will cause the PAH composition to have a distinct distribution profile. This profile was explained with factor 1 by 41% and factor 2 by 26% of the variance in the data. Factor 1, was mostly the contrast between the low weight PAHs (Ace, Nap, Acy, Ant, Fle, Phe) on the positive side and the high weight PAHs (BaP, BaA, Chr, BaF, BkF, BgP, IcP, Pyr, DaA) on the negative side (Figure 35). For factor 2 the environmental factors EC, TDS, pH and temperature loaded high on the positive side in contrast to low weight PAHs (Phe, Nap, Fle, Fla, BkF and Pyr) on the negative side.

The sampling sites with high scores on factor 1 were 19_SelR, 14_LuvR and 16_LetR on the positive side with 2_NkoR and 4_SabR on the negative side. Sites 7_Po and 17_OliR had a high positive score for factor 2 on its positive side and a negative score for sites 19_SelR and 1_CrocR (Figure 35).

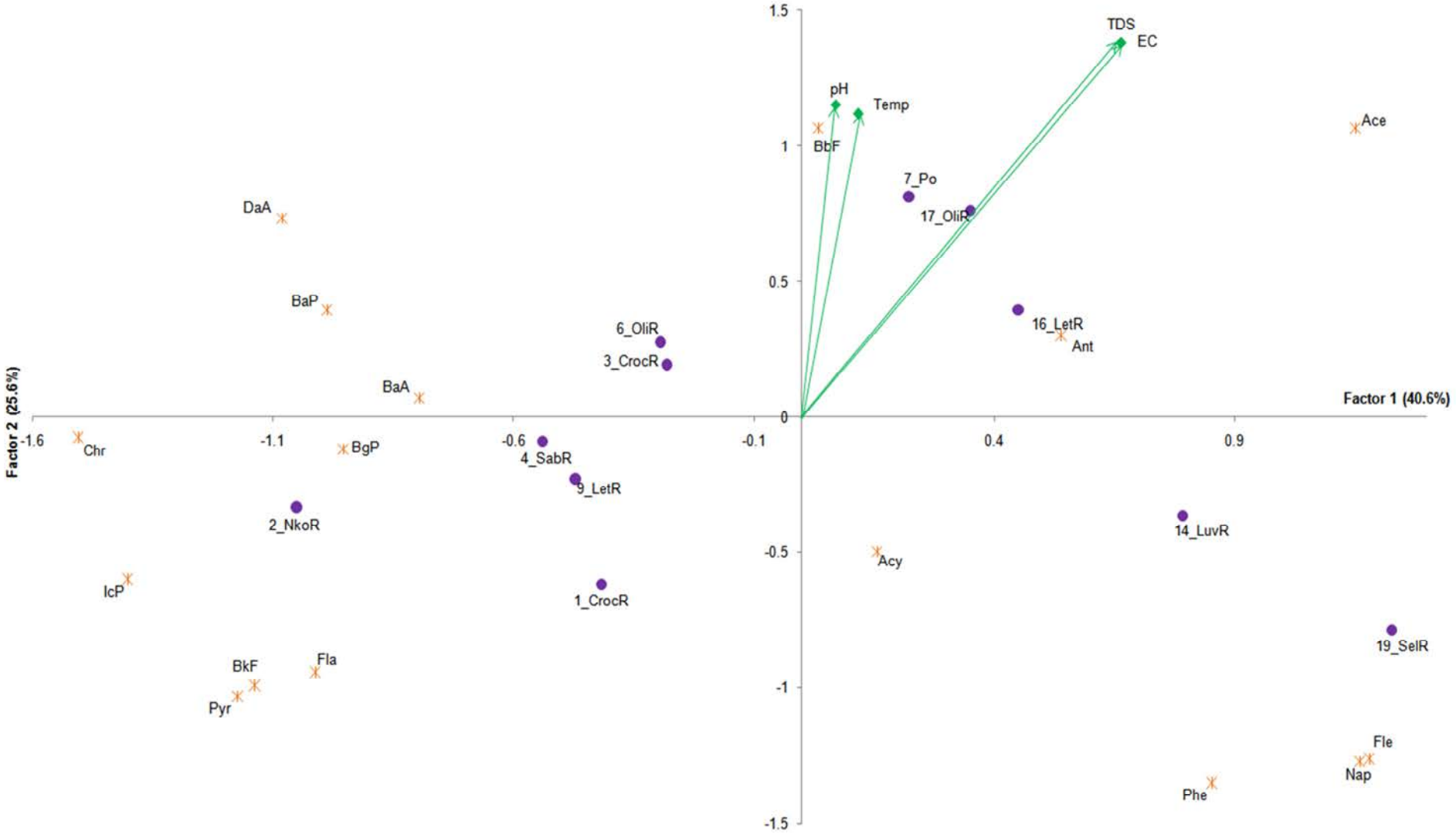


Figure 35: This PCA-biplot between factor 1 and 2 has all the PAH isomers including the sites sampled and the water chemistry information.

4.15 PCA including all the PCBs (including DL-PCBs) and PBDEs

In contrast to the DL-PCBs which are produced unintentionally; the PCBs and PBDEs are compounds both produced intentionally and used in a wide variety of applications. Combining these chemicals together in the PCA analyses may provide indications of possible sources (Figure 36). This combination profile explained 32% in factor 1 and factor 2, 25% of the variance in the data (Figure 36). The positive side of factor 1 were loaded with the higher mass non-dioxin like PCBs such as the tetra, hepta-, hexa- and penta- chlorinated isomers (PCB 66, PCB99, PCB170, PCB187, PCB180, PCB194) and DL-PCB (PCB81, PCB105 and PCB118) while the environmental factors, pH and temperature loaded on the negative side with the mono-*ortho*-chlorinated DL-PCBs, (PCB114, 123,157, 167 and 189). Factor 2 consisted of non-*otho*-chlorinated substituted DL-PCBs (PCB77, PCB169 and PCB81) on the positive side in contrast with none-DL PCBs and DL-PCBs (PCB118, PCB138, PCB153, PCB101 and PCB149) and environmental factors (EC and TDS) on the negative side (Figure 36).

17_OliR had the highest factor score on the positive side of factor 1 with 9_LetR and 6_OliR on the negative side. Factor 2 consisted of a high factor scores with 1_CrocR and on the positive side and a negative score at 3_CrocR (Figure 36)

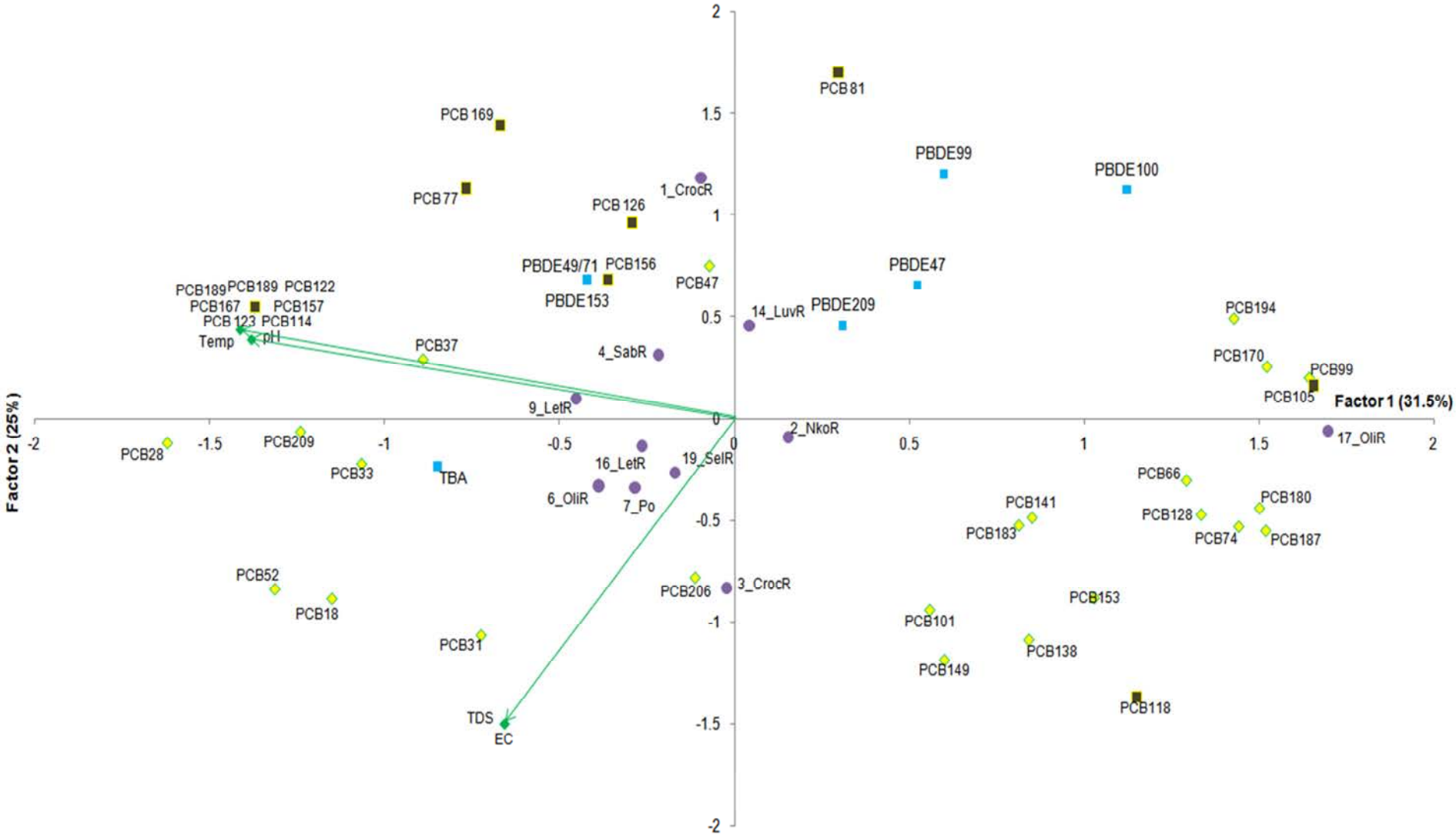


Figure 36: This PCA-biplot between factor 1 and 2 has the PCBs, DL-PCBs and PBDEs including the sites sampled and the water chemistry information.

4.16 PCA including only the chlorinated pesticides

The combination of the pesticides with the sites will most probably indicate relationships between the agricultural areas and the sites that can be influenced by their release. This combination of pesticides explained 39% of factor 1 and factor 2; 25% of the variance in the data (Figure 37). Factor 1 consisted of the contrast between the environmental factors EC, TDS, pH and temperature together with γ–HCH, mirex, heptachlor, oChlor, *o,p'*–DDD and *o,p'*-DDE on the positive side and *o,p'*-DDT, *p,p'*-DDD, *p,p'*-DDE, cChlor, tChlor, tNChlor, and cNChlor on the negative side (Figure 37) Factor 2 was mainly the loading between cChlor, tChlor, tNChlor, and cNChlor on the positive side and oChlor, mirex, heptachlor and *o,p'*-DDT on the negative side. The two environmental characteristics EC and TDS loaded higher on the positive side of factor 1 than factor 2 whereas pH and temperature loaded on the negative side of factor 2 (Figure 37).

Furthermore, site 19_SelR is on the far positive side in contrast to site 2_NkoR on the negative side. The negative side of factor 2 indicated high concentrations of *o,p'*-DDT at sites 1_CrocR, 4_SabR, and 14_LuvR that indicates active use of this compound in those areas. Opposite to the DDT was *cis*- and *trans*- chordanes and nonachlor that grouped with site 3_CrocR. Factor 3, grouped the *o,p'*-DDD, *o,p'*-DDE, *o,p'*-DDT and *p,p'*-DDT with site 14_LuvR, opposite of the HCH-isomers, chlordane-isomers, mirex and *p,p'*-DDD. The two sites in contrast with each other are sites 19_SelR and 7_Po (Figure 37).

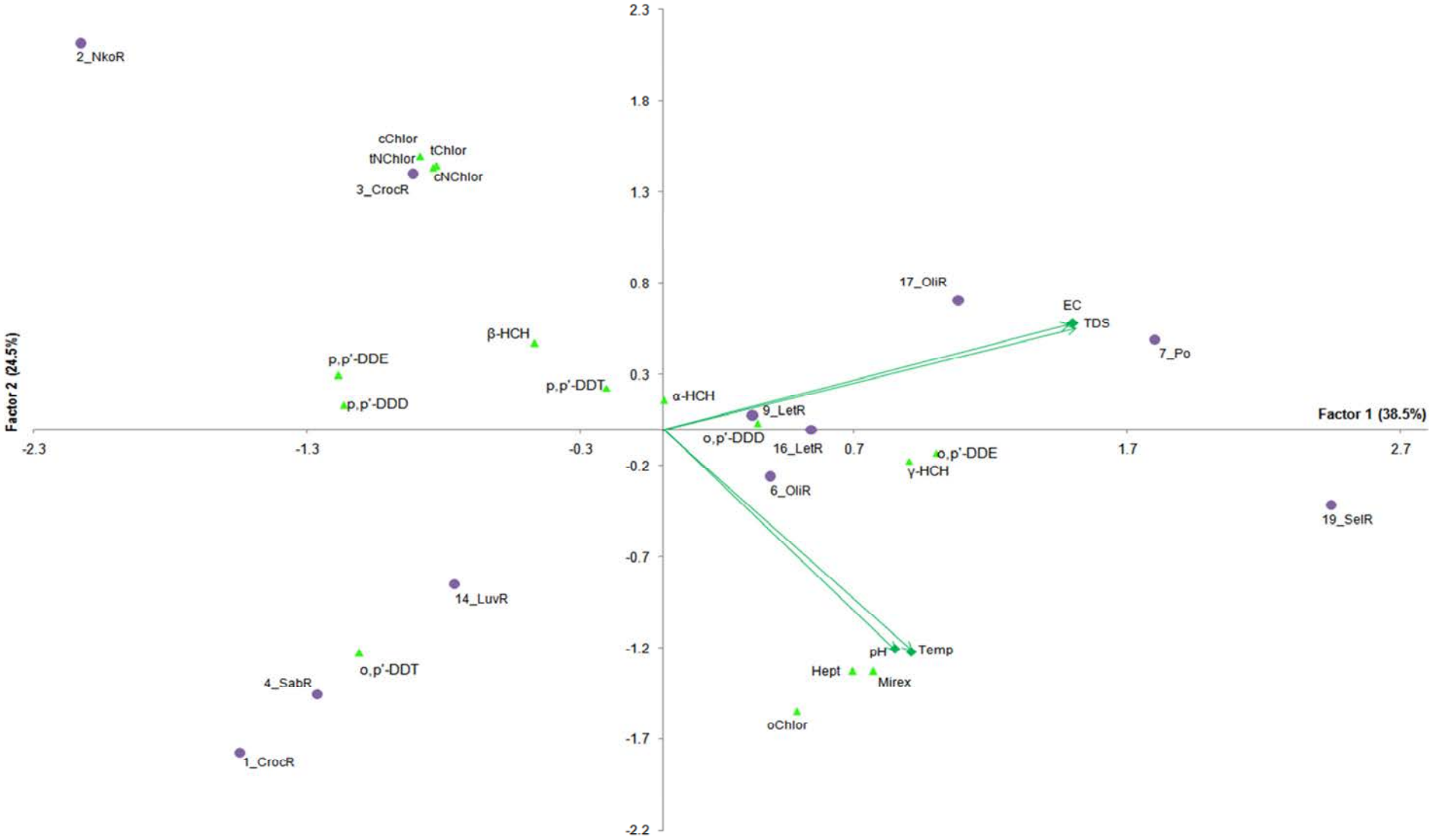


Figure 37: This PCA-biplot between factor 1 and 2 of the chlorinated pesticides, sampling sites and the water chemistry information.

4.17 PCA of the elements

The elements were loaded separately from the POP and PAHs to determine the distinct patterns. The combined PCAs also indicated that the elements did not contribute to any of their loadings. The majority of the variance in the data was explained by factor 1; 33% and Factor 2; 23% (Figure 38). In factor 1 there was a contrast between Se, Ag, Cd and the environmental factors (pH, temperature, EC and TDS) on the negative side and I, Pb, As and Mn on the positive side. Factor 2 consisted of An, Cu, Fe, So, Cr, Ni and V was highly loaded on the negative side with Hg, Ag, Se. The environmental factors (temperature and pH) had a high positive loading.

The sites that associated with the negative side of factor 1 were 11_LimR, 16_LetR, 10_ShisR, 7_Po and 8_OliR while sites, 1_CrocR, 2_NkoR, 4_SabR and 12_LuvR with high factor scores on the positive side (Figure 38). The negative side of factor 2 had three of the Olifants River sites 6_OliR, 17_OliR and 18_OliR as well as 9_LetR, and 19_SelR (Figure 38). Scores on the positive side of factor 2 consisted of 11_LimR, 16_LetR and 13_MutR.

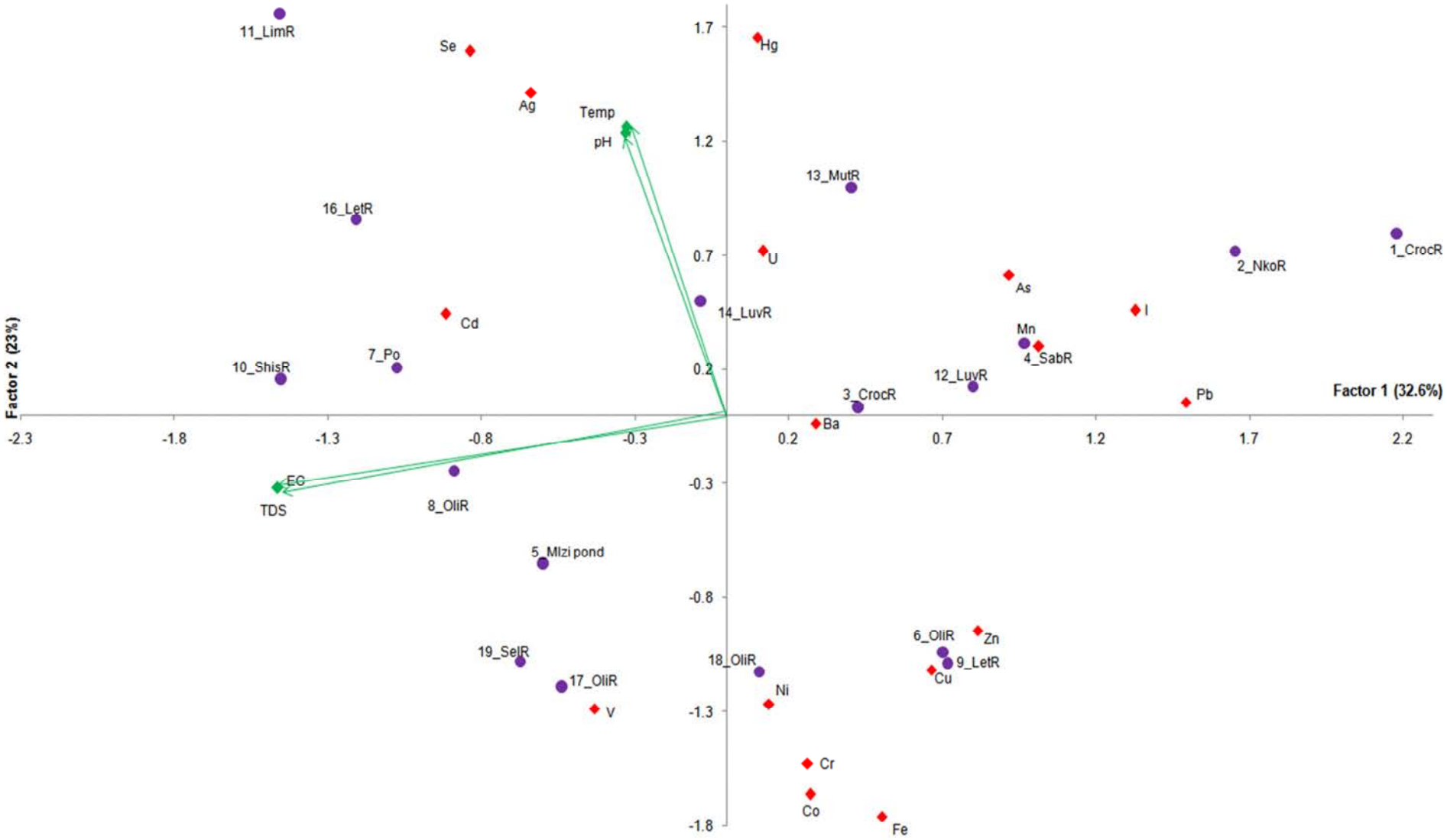


Figure 38: This PCA-biplot between factor 1 and 2 of the selected element, sampling sites and the water chemistry information

Chapter 5: Discussion

The main aim of this study was to determine whether environmental chemical contaminants may have caused or contributed to the mass mortalities of crocodiles in the KNP between 2008 until 2010. It is well documented that environmental contaminants like the compounds selected for this study, can cause toxicity in wildlife (Oberholster *et al.,* 2012; Oberholster *et al.,* 2010; Winde, 2009; SEPA, 2004; Roychoudhury & Starke, 2006; Ashton *et al*., 2001) and may possibly lead to the events that occurred in the KNP. Studies have shown that enhanced lipid peroxidation in aquatic organisms such as freshwater mussels can be caused by exposure to xenobiotics and contaminants in sediments (Doyotte *et al*., 1997). The sediment quality parameters as quantified for the present study are discussed in this chapter. The OMPs and elements are compared with selected international SQGs (Table 17 & 18). International sediment quality guidelines such as the Canadian Environmental Quality Guidelines (CCME, 2012), the Netherlands' New Dutch Target and Intervention Guideline (NDSQG, 2011; NDSQG, 2000) as well as the Australian and New-Zealand guidelines for the fresh and marine water quality (ANZECC, 2000), were consulted for comparisons. South Africa does not have sediment quality guidelines (as mentioned in Chapter 2; Section 2.3). For the sake of uniformity and to be able to compare the results to the SQGs, all concentrations were converted to ng/g (dw) for the organic compounds (PCDD/Fs were converted to pg TEQ/g dw) and µg/g (dw) for the elements.

The various sets of guideline concentrations use different terminologies. The Canadian Permissible Exposure Limits (PEL) is a specific set guideline to protect humans against the health effects of exposure to hazardous substances (CCME, 2012), whereas the Interim Sediment Quality Guidelines (ISQG) correspond with the threshold concentration effects below which adverse biological effects are not expected (CCME, 2012). Australia also has an ISQG and they define ISQG trigger guidelines which are the concentrations above which adverse effects can occur (ANZECC, 2000; Table 17). The Dutch intervention guideline is the concentration above which steps need to be taken in terms of pollution abatement and/or clean-up.

5.1 Discussion and comparison of the Persistent Organic Pollutants (POPs)

POP concentrations were converted to be expressed in terms of 1% or 10% total organic carbon (TOC) content (the amount of pollutant in 1% of the original TOC calculated to either 1% or 10% TOC

content) to be able to compare them to the selected international guideline concentrations. The guideline concentrations from Canada and Australia/New Zealand are expressed in terms of 1% TOC and for the Dutch guideline, concentrations is expressed in terms of 10% TOC (Table 17).

5.1.1 ΣDDT

DDT is a chlorinated pesticide still used in SA for vector control of malaria (Basel Convention, 2011; Stockholm Convention, 2011; Bouwman *et al*., 2006). This pesticide and its metabolites have a long biological half-life and a comparatively higher tendency to accumulate in the environment (ATSDR, 2008; Ritter *et al*., 1995). In sediment, the metabolites and their parent compound can move via diffusion and bioturbation from the subsurface to upper sediments where it may undergo re-suspension and redistribution in aquatic systems (ATSDR, 2008; Wells & Leonard, 2006).

The highest DDT guideline is the Dutch guideline at 4 000 ng/g dw (NDSQG, 2011) and the lowest is the Canadian guideline at 1.2 ng/g dw (CCME, 2012; Table 17). The area where the crocodile mortalities occurred had the lowest quantified concentrations of all the sites. Even so, the 1% normalised ΣDDT concentrations showed all of the sites exceeded the Canadian guideline (Table 17 & Figure 39). This indicates that possible adverse biological effects can be expected at all the sites that exceeded the threshold concentration. The Australian trigger limit, 1.6 ng/g dw was only exceeded at two of the sites (2_NkoR and 14_LuvR) (ANZECC, 2000; Figure 39). The Dutch guideline was not exceeded at any of the sites (Table 17).

There seems to be an increase in the concentrations of ΣDDT from 16_LetR towards 9_LetR upstream of the area where the mortalities occurred in the 9_LetR and 8_OilR area (Figures 10 & 20). The ΣDDT concentration at 6_OliR, the area where the crocodiles died, was lower than the combined concentrations of the upstream sites. It could be that the confluence of the two rivers diluted the DDTs in the Olifants Gorge.

Table 17: A comparison between the sum of the organic compound classes to three selected international guideline levels. The data was normalised to 1% TOC and 10% TOC to compare to the Canadian and Australian/New Zeeland, and the Netherlands guidelines, respectively

% TOC		ΣDDT (ng/g)	ΣPCB (ng/g)	ΣHCH (ng/g)	Heptachlor (ng/g)	ΣChlordane (ng/g)	PCDD/Fs (ngTEQ/kg)	ΣLMW PAHs (ng/g)	ΣHMW PAHs (ng/g)	ΣPAHs (ng/g)
Interim sediment quality guideline (ISQG) from Canada (CCME, 2012)										
1		1.2	4.5	-	-	4.5	21.5	-	-	-
Interim sediment quality guideline (ISQG) from Australia/New Zeeland (ANZECC, 2000)										
1		1.6	23	-	-	0.5	-	552	1 700	4 000
Intervention sediment quality guidelines from The Netherlands (NDSQG, 2011)										
10		4 000	1 000	2 000	4 000	4 000	-	-	-	40 000
					1_CrocR					
	N 0%	2	0.43	0.16	0.04	0.04	2.65	51.66	29.12	80.78
2.35	N 1%	0.85	0.18	0.07	0.02	0.01	1.13	22	12.4	34.4
	N 10%	8.52	1.81	0.66	0.17	0.15	11.27	219.97	124.01	343.98
					2_NkoR					
	N 0%	18.78	1.81	0.9	0.03	0.63	2.02	60.39	205.21	265.59
6.06	N 1%	3.1	0.3	0.15	0	0.1	0.33	9.96	33.85	43.82
	N 10%	30.98	2.99	1.49	0.05	1.05	3.34	99.63	338.54	438.16
					3_CrocR					
	N 0%	3.68	1.2	0.28	0.05	0.25	1.91	40.55	37.83	78.38
4.12	N 1%	0.89	0.29	0.07	0.01	0.06	0.46	9.84	9.18	19.02
	N 10%	8.93	2.91	0.67	0.12	0.6	4.62	98.38	91.79	190.17
					4_SabR					
	N 0%	2.91	0.4	0.18	0.03	0.03	0.42	12.19	14.05	26.24
3.91	N 1%	0.74	0.1	0.05	0.01	0.01	0.11	3.12	3.59	6.71
	N 10%	7.44	1.02	0.47	0.08	0.08	1.08	31.18	35.93	67.11
					6_OliR					
	N 0%	1.78	0.54	0.24	0.04	0.03	0.34	19.93	23.29	43.22
4.84	N 1%	0.37	0.11	0.05	0.01	0.01	0.07	4.11	4.81	8.93
	N 10%	3.68	1.1	0.5	0.08	0.05	0.71	41.15	48.11	89.26

Not normalised = N 0%; Normalised to 1% = N1%; Normalised to 10% = N10%

ΣLMW PAHs = Ace + Acy + Ant + Fle + Nap + Phe; ΣHMW PAHs = BaP + BaA + Chr + B(a)F + B(bjk)F + BgP + IcP + Pyr + DaA + Fla; ΣChlordanes = *trans*-nonachlor + *cis*-nonachlor + *trans*-chlordane + *cis*-chlordane + oxy-chlordane

Table 17 (continue): A comparison between the sum of the organic compound classes to three selected international guideline levels. The data was normalised to 1% TOC and 10% TOC to compare to the Canadian and Australian/New Zeeland, and the Netherlands guidelines, respectively

% TOC		ΣDDT (ng/g)	ΣPCB (ng/g)	ΣHCH (ng/g)	Heptachlor (ng/g)	ΣChlordane (ng/g)	PCDD/Fs (ngTEQ/kg)	ΣLMW PAHs (ng/g)	ΣHMW PAHs (ng/g)	ΣPAHs (ng/g)
Interim sediment quality guideline (ISQG) from Canada (CCME, 2012)										
1		1.2	4.5	-	-	4.5	21.5	-	-	-
Interim sediment quality guideline (ISQG) from Australia/New Zeeland (ANZECC, 2000)										
1		1.6	23	-	-	0.5	-	552	1 700	4 000
Intervention sediment quality guidelines from The Netherlands (NDSQG, 2011)										
10		4 000	1 000	2 000	4 000	4 000	-	-	-	40 000
					7_Po					
	N 0%	0.54	0.54	0.18	0.03	0.03	0.16	21.48	13.22	34.7
2.47	N 1%	0.22	0.22	0.07	0.01	0.01	0.07	8.7	5.35	14.06
	N 10%	2.19	2.17	0.73	0.12	0.12	0.66	87.02	53.55	140.57
					14_LuvR					
	N 0%	5.62	0.45	0.14	0.04	0.04	0.19	162.61	9.59	172.21
1.04	N 1%	5.41	0.43	0.14	0.04	0.03	0.18	156.4	9.22	165.62
	N 10%	54.05	4.31	1.35	0.38	0.34	1.83	1563.98	92.25	1656.23
					16_LetR					
	N 0%	2.06	0.46	0.29	0.02	0.03	0.21	33.76	9.91	43.67
1.57	N 1%	1.31	0.29	0.19	0.01	0.02	0.13	21.48	6.31	27.79
	N 10%	13.11	2.94	1.86	0.13	0.16	1.32	214.84	63.1	277.94
					17_OliR					
	N 0%	1.57	2.82	0.18	0.03	0.03	0.28	12.16	4.96	17.12
1.04	N 1%	1.51	2.71	0.17	0.03	0.02	0.27	11.69	4.76	16.45
	N 10%	15.09	27.14	1.74	0.29	0.24	2.73	116.89	47.64	164.54
					19_SelR					
	N 0%	3.42	0.96	0.3	0.05	0.04	0.34	8249.44	44.33	8293.78
2.58	N 1%	1.33	0.37	0.11	0.02	0.01	0.13	3198.43	17.19	3215.62
	N 10%	13.26	3.71	1.15	0.19	0.14	1.3	31984.28	171.89	32156.18

Not normalised = N 0%; Normalised to 1% = N1%; Normalised to 10% = N10%

ΣLMW PAHs = Ace + Acy + Ant + Fle + Nap + Phe; ΣHMW PAHs = BaP + BaA + Chr + B(a)F + B(bjk)F + BgP + IcP + Pyr + DaA + Fla; ΣChlordanes = *trans*-nonachlor + *cis*-nonachlor + *trans*-chlordane + *cis*-chlordane + oxy-chlordane

The PCA analysis indicated that DDT and its metabolites (*o,p'*-+*p,p'*-DDD and *o,p'*-+*p,p'*-DDE) had the strongest association with the following sampling sites; 14_LuvR (*p,p'*- DDT), 19_SelR (*o,p'*-DDE) and 2_NkoR (*o,p'*- DDT) (Figure 32). *p,p'*-DDE and *p,p'*-DDD associated with 6_OliR and 9_LetR on the negative side of factor 4. However, this factor only describes 9% of the variance in the data. The highest concentrations of ΣDDT were quantified at sites 2_NkoR, 14_LuvR and 9_LetR (Figure 10). Higher concentrations at these three sites could be ascribed to the fact that these rivers flow through areas where DDT is still in use (Van Dyk *et al*., 2010).

Since DDT is degraded to DDE and DDD in the environment, it was possible to determine whether the DDT in the sediments was due to recent or historic use. The ratios calculated indeed indicated recent use of DDT (Chapter 4; Section 4.10). This was expected as the KNP is downstream from areas where DDT was and still is used to combat malaria (Basel Convention, 2011; Stockholm convention, 2011; Bouwman *et al*., 2006). The highest of the *p,p'*-DDT/ΣDDT ratios were at 14_LuvR. This may have been caused by small streams that flow into the rivers outside the KNP borders where DDT is still used for malaria control (Wells & Leonard, 2006; Bouwman *et al*., 2008).

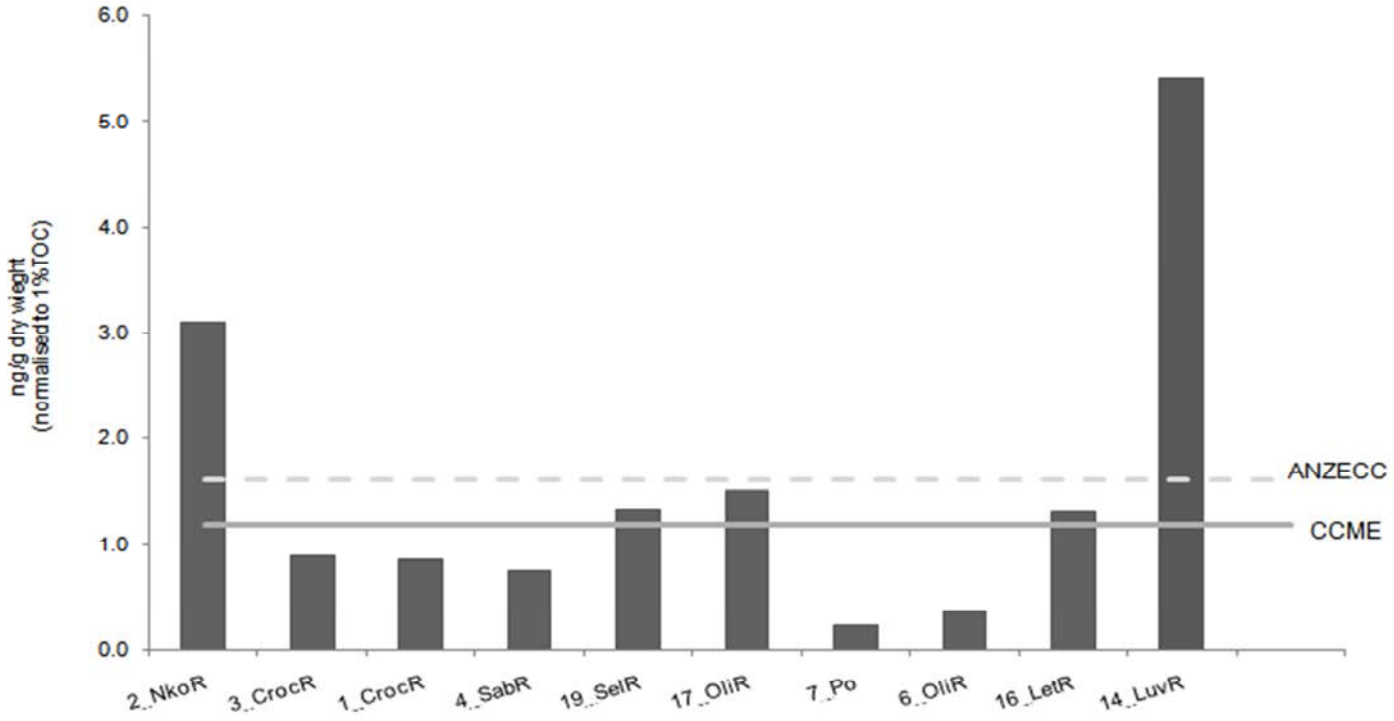


Figure 39: Comparison of the ΣDDT concentrations normalised to 1% TOC with the international SQGs.

5.1.2 ΣHCH

None of the sites exceeded the Dutch guideline for ΣHCH (NDSQG, 2011; 2 000 ng/g; Table 17). Thus, according to the Dutch guideline used to compare the ΣHCH, there is no need for precautionary steps for pollution abatement and/or clean-up at the sampled localities. The isomers of lindane (α- and β-HCH) had concentrations between 0.01 ng/g and 0.07 ng/g dw which were lower than lindane within the KNP. According to Osibanjo *et al.*, 2002, lindane (γ-HCH) was produced until the early 1980's at a site in Kempton Park in the Gauteng province. Lindane is degraded more rapidly than α-HCH under both aerobic and anaerobic conditions (Wu *et al*., 1997), indicating that there are possible sources of lindane on the outside borders of the KNP. Worldwide, the use of lindane has been restricted but it can still be found in insecticides as well as in fungicides (ATSDR, 2012; UNEP, 2011; WHO, 2011). Lindane is not registered as a fungicide in South Africa (Burger & Nel, 2008).

5.1.3 Heptachlor

According to the Dutch guideline for heptachlor, there is currently no need for abatement and/or clean-up at the sampled localities, as there were no exceedances (NDSQG, 2011; 4 000 ng/g; Table 17). The highest of these concentrations were at sites 14_LuvR, 17_OliR, and 19_SelR. This may be attributed to historic use of this chemical as it had been used in South Africa (Burger & Nel, 2008). However, the registration was withdrawn in 1976 (Bouwman, 2003; DAFF, 2011). Both sites 6_OliR and 7_Po where the mortalities occurred had concentrations of heptachlor at relatively low concentrations between 0–1 ng/g. Low concentrations of heptachlor were quantified at all of the sites.

5.1.4 ΣChlordane

The total chlordane was calculated as the sum of (cis– and trans–) chlordane and nona-chlordane. Very low chlordane concentrations were quantified in the southern part of the KNP (2_NkoR and 3_CrocR; Table 17; Figure 10). None of the normalised concentrations exceeded the guideline values (Table 17). As mentioned, these rivers flow through areas with high agricultural activities before it enters the KNP (DWA, 2009b; Mallory & Beater, 2009). The presence of chlordane and heptachlor is understandable as both these compounds were used as pesticides (Standberg *et al*.,

1998). Use of chlordane was restricted in 1993 to stem treatment in citrus orchards and vineyards, and for the treatment of structures for pest control. However, this pesticide was withdrawn in 2000 (Rother & Jacobs, 2008).

5.1.5 Mirex

There are no guidelines for mirex concentrations in the selected international guidelines (Table 17). There was overall low concentrations at all the localities (Figure 10). It is possible that the concentrations detected were from historic use or released by-products or wastes containing mirex as a flame retardant (Bouwman *et al*., 2008). This pesticide was never registered for use in South Africa. However, studies done in SA show low concentrations in eggs from a variety of different water bird species (Bouwman *et al*., 2007).

5.1.6 ΣPCBs

The site where the mortalities occurred had ΣPCBs concentrations below the selected guideline values. The highest guideline is that of the Netherlands at 1 000 ng/g. Exceedance would require pollution abatement and/or clean-up (NDSQG, 2011; Table 17). When the data was normalised to 10% TOC, all the concentrations were well below the Dutch guideline. When the data was normalised to 1% TOC to compare to the Canadian (34.1 ng/g) and Australian (23 ng/g) guideline concentrations, none of KNP sites exceeded these concentrations (Table 17 & Figure 40). The lack of exceedance indicates that the ΣPCBs concentrations quantified in the KNP are not expected to result in adverse biological effects. The ΣPCBs was highest at 17_OliR (Figure 23 & 40), probably from large coal mines and industrialised towns in the Olifants River catchment (DWAF, 2005). The Selati and Olifants rivers confluence (Ashton *et al*., 2001) is upstream of site 17_OliR and the Olifants River, and could thus contribute to the ΣPCB concentrations at 17_OliR. There may also be a dilution effect (compare site 6_OliR on the border with Mozambique, Figure 13) even though the Letaba River may also contribute PCBs (at 16_LetR; Figure 16 & 40) to the Olifants River. At the time of the sample collection, water was backing up into the Olifants Gorge due to the Massingir Dam, which might explain the dilution effect.

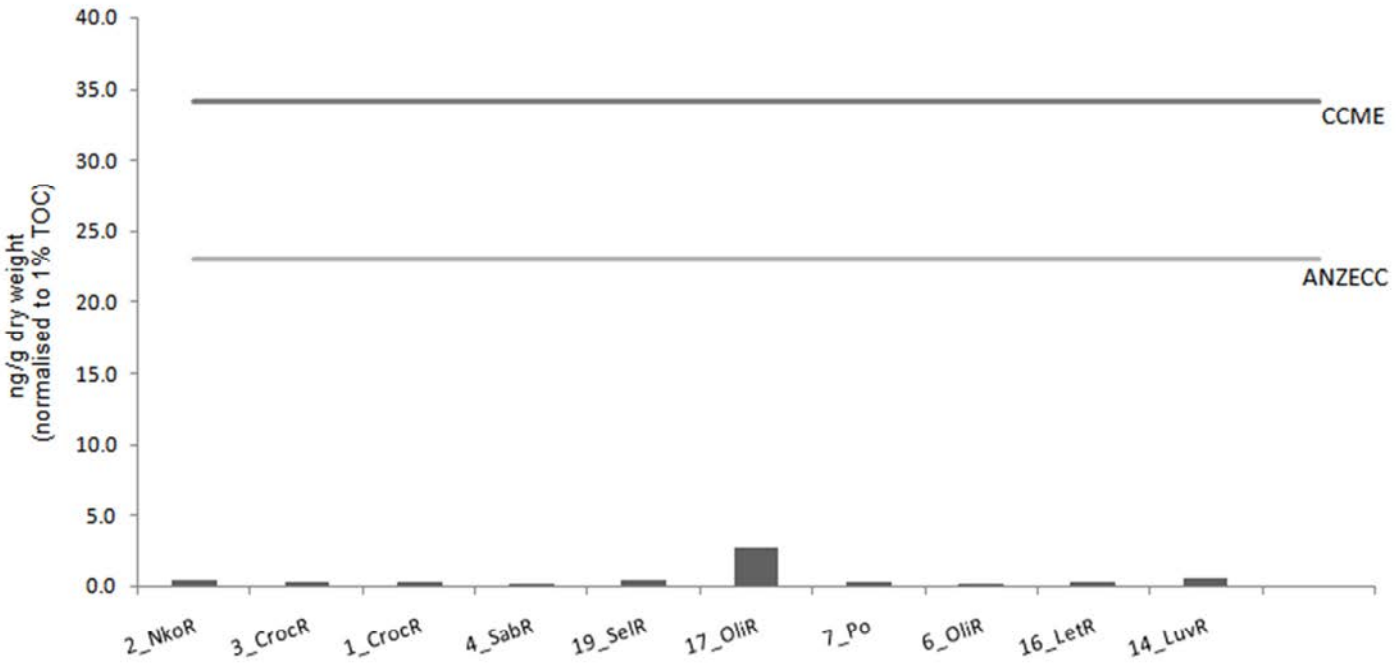


Figure 40: Comparison of the PCB concentrations normalised to 1% TOC with international SQGs.

5.1.6 ΣPBDEs

The ΣPBDE concentrations were the highest at sites 2_NkoR 17_OliR and 19_SelR (Figure 31). There are no guidelines for ΣPBDEs in the selected international guidelines (Table 17). The rest of the sites, 3_CrocR, 4_SabR, 7_Po and 6_OliR, had concentrations below the detection limit. PBDEs are extensively used as flame retardants and are added to a variety of products such as electronics and plastics because of its unique fire resistant characteristics (ATSDR, 2012; USEPA, 2010; Bouwman, 2003), but their production and use are now being phased out. The 1% normalised PBDEs data however did indicate the 14_LuvR and 16_LetR sites with relatively high concentrations that could have come from abandoned and/or active gold and coal mines upstream of these areas outside the KNP (Ashton *et al*., 2001). This compound class is commonly used to prevent fires in electronics and machinery (ATSDR, 2012; USEPA, 2010; De Wit, 2002). It is possible that materials used in these mines could contain traces of PBDEs. The majority of PBDEs contributed considerably to the negative side of factor 1 in the PCA consisting of all the analysed compounds with no distinct association with any of the sites where the crocodile mortalities occurred (Figure 15 & 23).

5.1.7 ΣPCDD/Fs and DL-PCBs

The concentrations of *Σ*PCCD/Fs were very low overall. However, the highest of these were at 1_CrocR (Figure 41). Both 6_OliR and 7_Po in the area where the mortalities occurred had low concentrations of PCDD/Fs. The quantifiable concentrations were well below the SQGs for PCDD/Fs with the threshold concentration of Canada, 21.5 ngTEQ/kg dw at which adverse biological effects maybe expected if exceeded (CCME, 2012; Table 17). The highest normalised concentrations were at 1_CrocR, 2_NkoR and 3_CrocR, which might be ascribed to the paper mills and wood processing plants near the upstream rivers outside the KNP (Figure 41). The non-normalised *Σ*PCCD/Fs concentrations indicated a contrast as the highest quantifiable concentrations were at 17_OliR, downstream of a large industrial area on the outside borders of the KNP (Figure 17 & 21; Ashton *et al*., 2001). The lowest concentrations were in the area where the crocodile mortalities occurred (7_Po and 6_OilR). These compounds are usually released because of incomplete combustion of fossil fuel, leaded gasoline, by-product of pesticides, incineration processes, as well as refinery processes and can be found in a variety of industrial, agricultural and domestic areas (ATSDR, 2012).

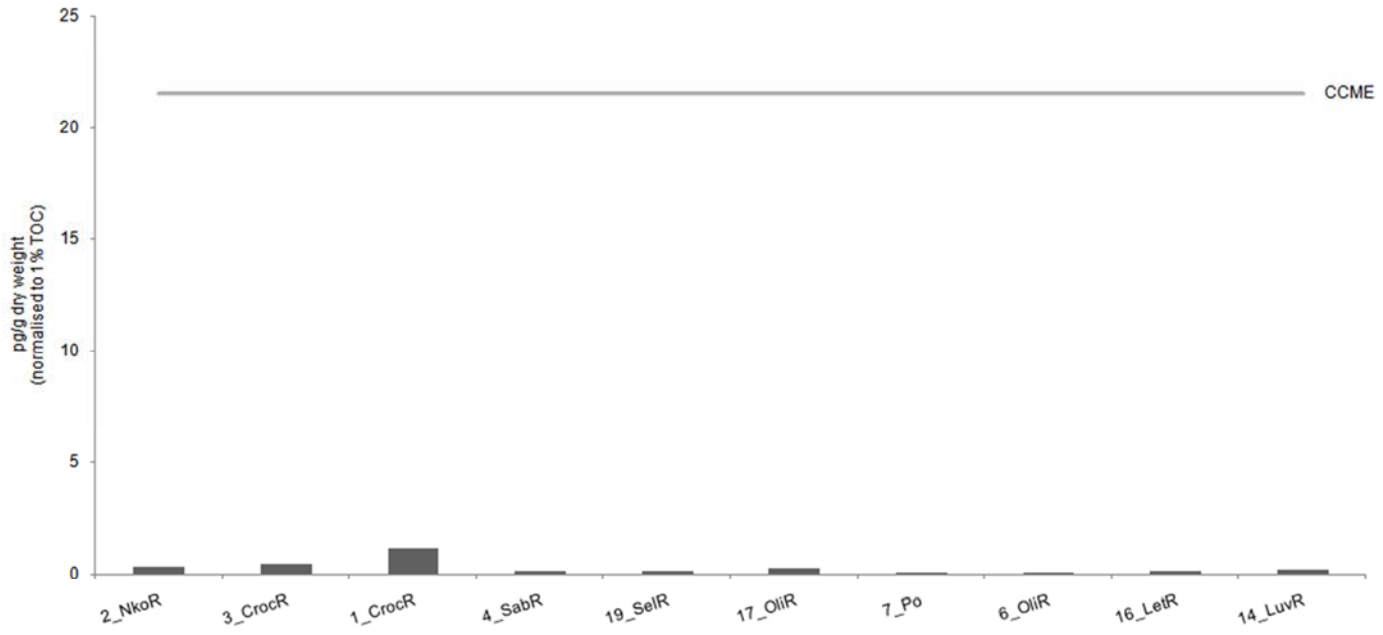


Figure 41: Comparison of PCDD/Fs concentrations normalised to 1% TOC with international SQGs.

5.1.8 PeCB

There are no guideline concentrations for PeCB in the selected international guidelines (Table 17). The highest concentrations of PeCB were quantified at sites 2_NkoR, 9_LetR, 6_OliR, 16_LetR, 17_OliR and 19_SelR (Figure 22). These sites occur in rivers that flow through areas with high agricultural and industrial industries outside the KNP borders. PeCB was used in the manufacturing of flame retardants, and agricultural and industrial products. However, this chemical is no longer produced (UNEP, 2012). It is possible that trace amounts of PeCBs are released from pesticides, herbicides and fungicides such as pentachloro nitrobenzene, atrazine and endosulfan that contain PeCB impurities (UNEP, 2012). There was no relationship between PeCB, and any of the sites in the PCA grouping that included all the analysed compounds (Figure 31). Furthermore, when a PCA was conducted with only the unintentionally produced compounds, PeCB associated with the TDS and EC on the negative side of factor 1 and 2, with no particular associations with any of the sites (Figure 34).

5.1.9 HCB

There are no guideline concentrations for HCB in the selected international guidelines (Table 17). Low concentrations of HCB were quantified at several of the sites (Figure 10), with the highest concentration quantified at 2_NkoR (Figure 10 & 22). Sites; 2_NkoR, 9_LetR, 6_OliR and 19_SelR (Table 17) had the highest concentrations of HCB in relation to the other sites. HCB loaded on the positive side of factor 2 together with the *o,p'*-DDE/DDD and lighter weight PCBs (Figure 31). In this factor, 19_SelR scored with these compounds on the positive side. These rivers are exposed to a variety of industrial and agricultural activities that may have incorporated the use of fungicides. HCB is a by-product in the production of fungicides (Basel Convention, 2011; UNEP, 2012) and traces of this contaminant can remain in the environment due to historic use (ATSDR, 2012).

5.1.10 ΣPAHs

PAHs were detected in all of the sampled sites and at various concentrations. The Australian guideline is 4 000 ng/g dw (ANZECC, 2000; Figure 42) and the Dutch guideline is ten times higher at 40 000 ng/g dw (NDSQG, 2011; Figure 43). The highest concentrations of ΣPAHs were quantified at site 19_SelR, at 32 156 ng/g dw (Figure 42; Figure 43).

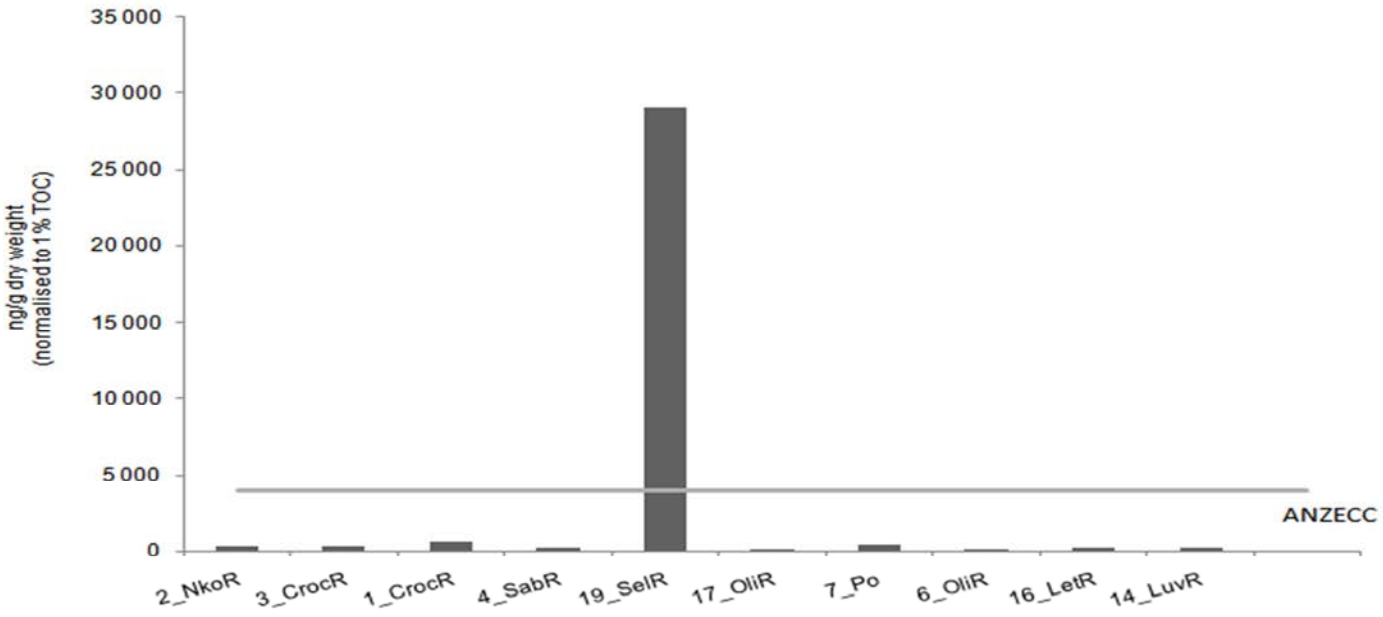


Figure 42: Comparison of total PAH concentrations normalised to 1% TOC with international SQGs.

The ΣPAHs for all the sites except 19_SelR had concentrations below the guideline for both the Australian and the Netherlands (ANZECC, 2000; NDSQG, 2011; Figures 42 & 43). There are no Canadian guideline concentrations for the ΣPAHs (Table 17).

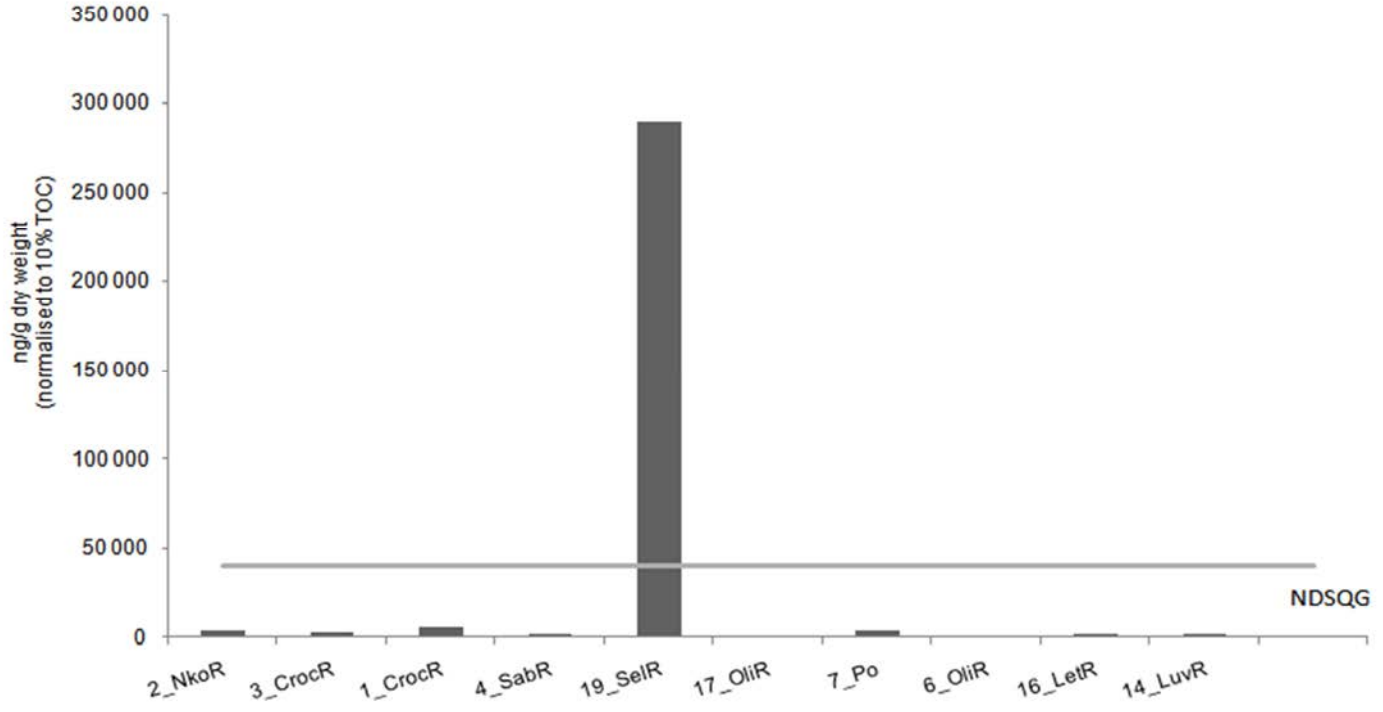


Figure 43: Comparison of total PAH concentrations normalised to 10% TOC with international SQGs.

The PAH concentrations at 19_Sel exceeded the Dutch guidelines at levels that may cause adverse environmental effects. The ΣLMW PAHs were calculated using the sum of Nap, Acy, Ace, Ant, Fle and Phe (Figure 44). The guidelines for the ΣLMW PAHs in the Australian guideline is 552 ng/g dw and for the ΣHMW PAHs it is 1 700 ng/g dw (ANZECC, 2000; Figure 44 & 45). The highest concentration of ΣLMW PAHs in the area was at 19_SelR. The concentrations at 7_Po could have been from atmospheric transport, as this locality is an isolated, rain-fed pool, not connected to any of the large rivers in that area. With regards to the ΣLMW PAHs at site 6_OliR, the main contributing compounds were Nap, Ace, Fle and Phe. The largest contributing compounds at 19_SelR were Nap, Fle and Phe (Figure 12 & 23). The only locality that exceeded the threshold guideline was 19_SelR. This river system is possibly exposed to a variety of coal, gold, copper, and platinum mines in the upstream areas outside the KNP borders (Ashton *et al*., 2001).

The ΣHMW PAHs are the sum of BaP, BaA, Chr, BkF, BgP, BbF, Chr, Fla, Pyr, DaA and IcP (Figure 45; ANZECC, 2008). All of the sites had concentrations in sediment below the guideline concentrations for HMW PAHs (Figure 45). The ΣHMW PAHs normalised data were lower in comparison with the ΣLMW PAHs. The other difference between the two PAH mass categories was that the highest concentrations ΣHMW PAHs were quantified at 2_NkoR and the lowest concentrations at 17_OliR (Figure 45). The highest contributing concentrations of the HMW PAHs were BkF, Fla and Pyr (Figure 12). The high concentrations at the Nkomati River could be because the river runs through areas where there are paper mills, informal settlements and high volumes of transport (vehicles and trains) due to the large industrial towns within the region and the nearby border post (Mallory & Beater, 2009).

ΣLMW PAHs are more volatile than their heavier counterparts, and are more readily transported through air. These molecules can return to the earth through impaction, rain, mist or dew (EC, 2001; ATSDR, 1995). The HMW PAH molecules tend to settle out near the source and accumulate in the soil or sediments (EC, 1999). This may explain why there were higher concentrations of ΣLMW PAHs compared to ΣHMW PAHs within the KNP borders (Figure 12).

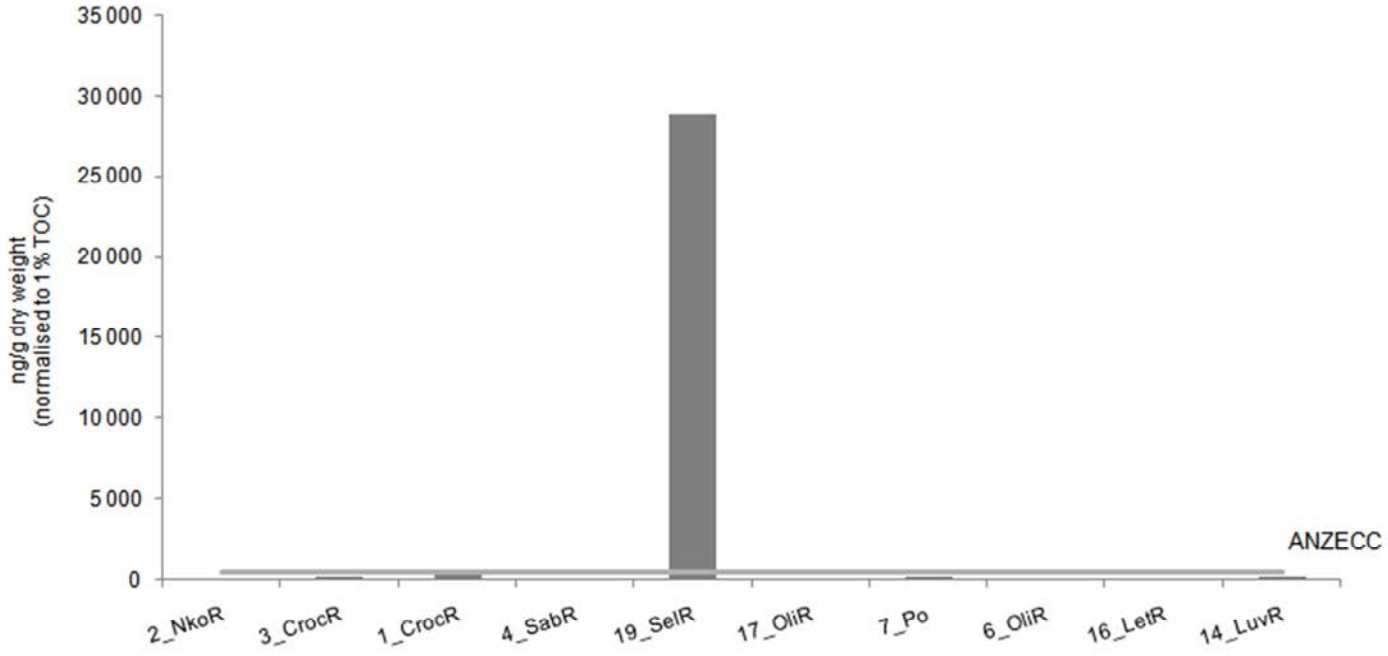


Figure 44: Comparison of ΣLMW PAHs concentrations normalised to 1% TOC with international SQGs.

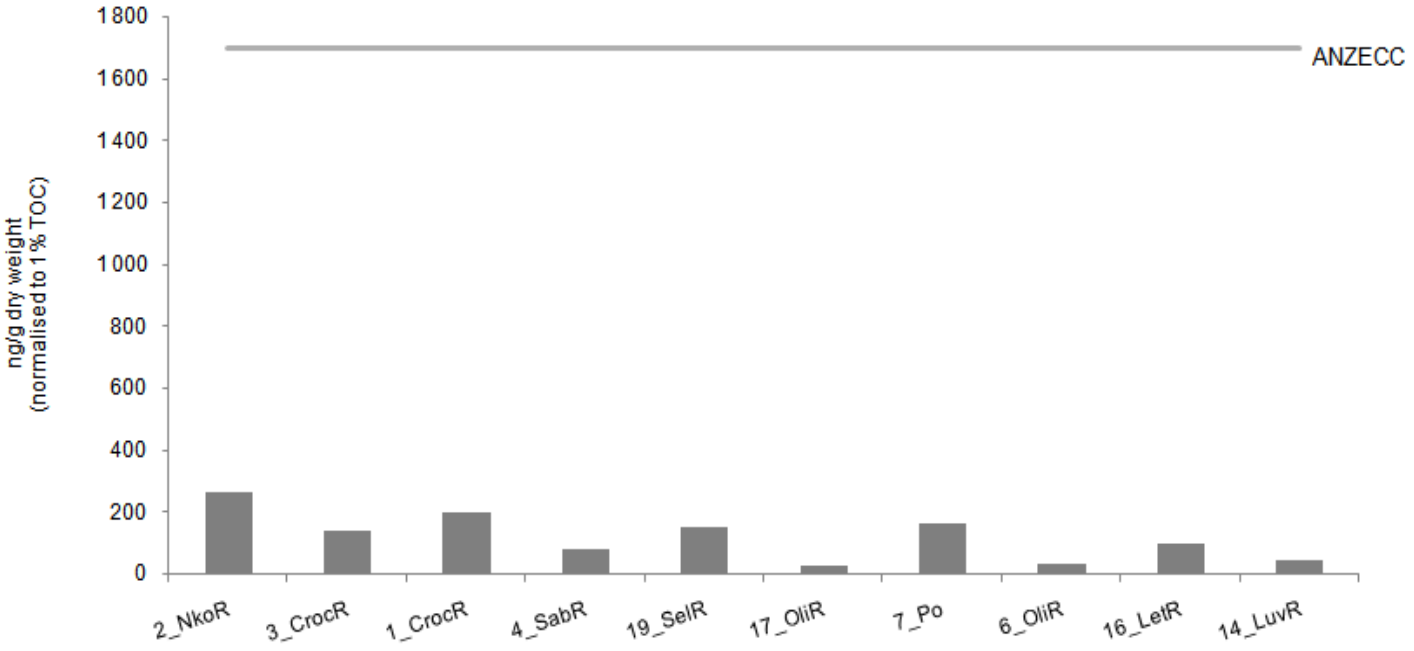


Figure 45: Comparison of ΣHMW PAHs concentrations normalised to 1% TOC with international SQGs.

There are different sources of PAH pollution which can be categorised into either pyrogenic or petrogenic. Petrogenic sources are hydrocarbon compounds that are associated with petroleum whereas pyrogenic sources are hydrocarbon compounds that form because of the combustion of

petroleum, wood and coal. With the use of isometric ratios, the two source categories can be calculated. The ratio between anthracene/phenanthrene [Ant/(Ant+Phe)] and fluoranthene/pyrene [Fla/(Fla+Pyr)] were used to determine if the PAHs were of a petrogenic or pyrogenic sources (Figure14; Yunker *et al*., 2002; Budzinski *et al*., 1997; Gschwend & Hites, 1981). Only three sites (3_CrocR, 7_Po, and 16_LetR) were identified as having a pyrogenic source by both the Ant/(Ant+Phe) and Fla/(Fla+Pyr) ratios (Figure 14). All of the other sites were determined to be of grass, wood and coal combustion by the Fla/(Fla+Pyr ratio, but petrogenic by the Ant/(Ant+Phe) ratio. This indicates that the PAH concentrations inside the KNP can either be caused by pyrogenic, petrogenic, or grass, wood and coal combustion, or combinations hereof. Long-range transport may act differentially on the different congeners, thereby moderating the eventual congener composition found at the target sites.

Long range transport probably explains why PAHs were quantified at all the sampling localities as it can be transported by both rivers and air. The different isomers also behave differently in the various matrices. It is clear however, that 7_Po can only be exposed to aerially transported pollutants and this is supported by this site's rain-fed nature (Figure 14). Both ΣLMW PAHs and ΣHMW PAHs were quantified at this locality. The high concentrations quantified at 19_SelR could have been because of the large copper mine and associated industries at Phalaborwa upstream of the sampling area.

5.2 Discussion and comparison of the elements

Although elemental speciation and pH dictate behaviour (such as mobility) of compounds between the water column and interstitial water, these parameters were not considered in the analytical method as only total elemental concentrations were determined. The larger picture of elemental distribution in sediments in the KNP was the main focus of this study and not the exact chemical behaviour of elements in which ever species they may occur in.

The sediment quality guidelines for the elements are summarised in Table 18. The same international guidelines used for the OMPs were also used for the elements. The only guideline that considers the use of a TOC was that of the Netherlands. In order to compare with the guideline, the concentrations of the elements were normalised to 10% TOC (NDSQG, 2011). Since the concentrations needed to be normalised for the Dutch guideline, the 9_LetR was not indicated on the graph as the TOC could not be determined for this site.

Arsenic, Cr, Cu, Fe, Mn, Ni and Zn had concentrations higher than some of the selected SQGs. The SQGs helped in evaluating whether or not the elements may affect the health of the ecosystems. In the event that different countries had the same guideline level, only one of these guidelines was represented on the graphs.

Not all the international guidelines had concentrations for all the elements quantified in this study. The graphs of the elements are summarised in Figures 46–61. All elemental concentrations and guideline concentrations were expressed as µg/g dw.

It should be noted from the start that all these elements have a natural background presence. The absence of comparable pre-industrial data from the sites complicates interpretation as to possible anthropogenic contamination. The use of the Igeo index is an attempt to separate the natural and anthropogenic contributions, although smaller scale natural contributions or sources cannot be ruled out. Further, comparisons were also made with reference sites, specifically 6_OliR, that was a rain-fed pool, representing local geology, with a possible airborne component of contamination.

Table 18: The international sediment quality guidelines used to compare with the quantified elements in this study

% TOC	As (ug/g)	Al (ug/g)	Ag (ug/g)	Ba (ug/g)	Cd (ug/g)	Cr (ug/g)	Co (ug/g)	Cu (ug/g)	Se (ug/g)	Pb (ug/g)	Ni (ug/g)	Hg (ug/g)	Zn (ug/g)	V (ug/g)	Fe (ug/g)	Mn (ug/g)	U (ug/g)	I (ug/g)
Interim sediment quality guideline (ISQG) from Canada (CCME, 2012)																		
N/A	5.9		-	-	0.6	37.3	-	35.7		35.0	-	0.2	123.0	-	-	-	-	-
Interim sediment quality guideline (ISQG) from Australian/New Zeeland (ANZECC, 2000)																		
N/A	20		1	-	1.5	80	-	65		50	21	0.15	200	-	-	-	-	-
Optimum sediment quality guidelines (Action Value) from The Netherlands (NDSQG, 2011)																		
10	55		15	625	12	380	240	190	100	530	210	10	720	250	-	-	-	-
1_CrocR																		
	As	Al	Ag	Ba	Cd	Cr	Co	Cu	Se	Pb	Ni	Hg	Zn	V	Fe	Mn	U	I
2.35	12.50	8 250.00	0.00	47.50	0.01	52.50	8.75	17.75	1.20	5.50	32.50	0.01	24.00	25.00	14 750.00	375.00	0.14	5.50
N 10%	53.23	35 129.75	0.01	202.26	0.05	223.55	37.26	75.58	5.11	23.42	138.39	0.04	102.20	106.45	62 807.73	1596.81	0.57	23.42
2_NkoR																		
6.06	10.00	32 500.00	0.02	450.00	0.10	142.50	23.75	62.50	2.50	20.00	90.00	0.11	75.00	80.00	55 000.00	5750.00	0.30	82.50
N 10%	16.50	53 616.47	0.03	742.38	0.16	235.09	39.18	103.11	4.12	32.99	148.48	0.19	123.73	131.98	90 735.57	9485.99	0.49	136.10
3_CrocR																		
4.12	16.75	10 500.00	0.00	72.50	0.01	75.00	11.50	23.50	1.13	7.25	37.50	0.01	32.50	37.50	22 500.00	550.00	0.14	1.80
N 10%	40.64	25 474.98	0.01	175.90	0.03	181.96	27.90	57.02	2.73	17.59	90.98	0.02	78.85	90.98	54 589.24	1334.40	0.34	4.37
4_SabR																		
3.91	24.00	8 500.00	0.00	57.50	0.01	40.00	9.00	25.00	0.60	4.25	22.75	0.01	32.50	32.50	17 000.00	300.00	0.14	0.70
N 10%	61.38	21 738.76	0.01	147.06	0.03	102.30	23.02	63.94	1.53	10.87	58.18	0.02	83.12	83.12	43 477.52	767.25	0.35	1.79
5_Mlzi pond																		
9.65	5.75	30 000.00	0.01	425.00	0.14	57.50	25.00	145.00	3.00	11.75	42.50	0.01	132.50	147.50	65 000.00	775.00	0.25	2.50
N 10%	5.96	31 072.18	0.01	440.19	0.15	59.56	25.89	150.18	3.11	12.17	44.02	0.01	137.24	152.77	67 323.06	802.70	0.25	2.59
6_OliR																		
4.84	7.25	50 000.00	0.01	220.00	0.06	350.00	55.00	80.00	2.20	12.25	207.50	0.01	70.00	125.00	67 500.00	1650.00	0.28	2.50
N 10%	14.97	103 256.81	0.02	454.33	0.12	722.80	113.58	165.21	4.54	25.30	428.52	0.02	144.56	258.14	139 396.70	3407.47	0.57	5.16

Normalised to 10% = N10%

Table 18 (continued): The international sediment quality guidelines used to compare with the quantified elements in this study

% TOC	As (ug/g)	Al (ug/g)	Ag (ug/g)	Ba (ug/g)	Cd (ug/g)	Cr (ug/g)	Co (ug/g)	Cu (ug/g)	Se (ug/g)	Pb (ug/g)	Ni (ug/g)	Hg (ug/g)	Zn (ug/g)	V (ug/g)	Fe (ug/g)	Mn (ug/g)	U (ug/g)	I (ug/g)
Interim sediment quality guideline (ISQG) from Canada (CCME, 2012)																		
N/A	5.9	-	-	-	0.6	37.3	-	35.7		35.0	-	0.2	123.0	-	-	-	-	-
Interim sediment quality guideline (ISQG) from Australian/New Zeeland (ANZECC, 2000)																		
N/A	20	-	1	-	1.5	80	-	65		50	21	0.15	200	-	-	-	-	-
Optimum sediment quality guidelines (Action Value) from The Netherlands (NDSQG, 2011)																		
10	55	-	15	625	12	380	240	190	100	530	210	10	720	250	-	-	-	-
7_Po																		
	As	Al	Ag	Ba	Cd	Cr	Co	Cu	Se	Pb	Ni	Hg	Zn	V	Fe	Mn	U	I
2.47	2.75	5 500.00	0.01	55.00	0.03	67.50	9.50	16.50	1.35	3.00	40.00	0.01	19.50	47.50	14 250.00	160.00	0.12	0.43
N 10%	11.14	22 278.91	0.02	222.79	0.12	273.42	38.48	66.84	5.47	12.15	162.03	0.04	78.99	192.41	57 722.63	648.11	0.50	1.74
8_OliR																		
1.31	3.00	16 000.00	0.00	42.50	0.04	67.50	13.00	13.50	1.58	2.75	40.00	0.01	21.50	112.50	25 000.00	230.00	0.09	0.35
N 10%	22.92	122 234.25	0.03	324.68	0.27	515.68	99.32	103.14	12.03	21.01	305.59	0.10	164.25	859.46	190 991.02	1757.12	0.71	2.67
9_LetR																		
N/A	6.50	45 000.00	0.01	250.00	0.10	300.00	52.50	75.00	1.58	12.50	187.50	0.01	65.00	117.50	65 000.00	1650.00	0.28	3.75
N10%	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
10_ShisR																		
1.39	3.25	8 750.00	0.02	325.00	0.25	110.00	22.50	37.50	4.75	6.50	137.50	0.01	35.00	70.00	25 000.00	275.00	0.25	1.73
N 10%	23.42	63 048.08	0.15	2341.79	1.80	792.60	162.12	270.21	34.23	46.84	990.76	0.09	252.19	504.38	180 137.37	1981.51	1.80	12.47
11_LimR																		
1.10	2.30	1 650.00	0.01	50.00	0.13	18.75	5.50	7.75	2.50	2.75	18.25	0.01	10.75	20.50	6500.00	525.00	0.15	0.28
N10%	2.09	1 496.56	0.01	45.35	0.12	17.01	4.99	7.03	2.27	2.49	16.55	0.01	9.75	18.59	5895.54	476.18	0.14	0.25
13_MutR																		
0.75	2.50	11 750.00	0.01	170.00	0.17	87.50	25.00	52.50	1.83	8.50	85.00	0.01	37.50	70.00	35 000.00	575.00	0.40	0.80
N 10%	3.32	15 604.39	0.01	225.77	0.23	116.20	33.20	69.72	2.42	11.29	112.88	0.01	49.80	92.96	46 481.15	763.62	0.53	1.06

Normalised to 10% = N10%

Table 18 (continued): The international sediment quality guidelines used to compare with the quantified elements in this study

% TOC	As (ug/g)	Al (ug/g)	Ag (ug/g)	Ba (ug/g)	Cd (ug/g)	Cr (ug/g)	Co (ug/g)	Cu (ug/g)	Se (ug/g)	Pb (ug/g)	Ni (ug/g)	Hg (ug/g)	Zn (ug/g)	V (ug/g)	Fe (ug/g)	Mn (ug/g)	U (ug/g)	I (ug/g)
Interim sediment quality guideline (ISQG) from Canada (CCME, 2012)																		
N/A	5.9	-	-	-	0.6	37.3	-	35.7		35.0	-	0.2	123.0	-	-	-	-	-
Interim sediment quality guideline (ISQG) from Australian/New Zeeland (ANZECC, 2000)																		
N/A	20	-	1	-	1.5	80	-	65		50	21	0.15	200	-	-	-	-	-
Optimum sediment quality guidelines (Action Value) from The Netherlands (NDSQG, 2011)																		
10	55	-	15	625	12	380	240	190	100	530	210	10	720	250	-	-	-	-
14_LuvR																		
	As	Al	Ag	Ba	Cd	Cr	Co	Cu	Se	Pb	Ni	Hg	Zn	V	Fe	Mn	U	I
1.04	1.58	3 750.00	0.00	40.00	0.01	32.50	8.50	16.50	0.98	1.78	18.25	0.01	9.25	37.50	11 500.00	210.00	0.07	0.48
N 10%	15.15	36 066.50	0.02	384.71	0.12	312.58	81.75	158.69	9.38	17.07	175.52	0.09	88.96	360.67	110 603.95	2 019.72	0.70	4.62
16_LetR																		
1.57	2.35	3 750.00	0.01	37.50	0.03	42.50	5.75	10.75	2.28	2.48	18.25	0.01	11.25	27.50	9 750.00	212.50	0.12	0.38
N 10%	14.96	23 865.48	0.03	238.65	0.19	270.48	36.59	68.41	14.48	15.75	116.15	0.06	71.60	175.01	62 050.25	1 352.38	0.76	2.42
17_OliR																		
1.04	2.75	23 000.00	0.00	65.00	0.08	175.00	22.00	23.00	0.98	4.75	85.00	0.01	35.00	235.00	60 000.00	375.00	0.21	0.40
N 10%	26.43	221 019.18	0.03	624.62	0.72	1681.67	211.41	221.02	9.37	45.65	816.81	0.07	336.33	2258.24	576 571.78	3 603.57	2.02	3.84
18_OliR																		
2.69	4.50	25 000.00	0.00	90.00	0.03	167.50	22.75	52.50	1.05	6.75	90.00	0.01	37.50	150.00	40 000.00	325.00	0.28	0.90
N 10%	16.75	930 46.90	0.01	334.97	0.09	623.41	84.67	195.40	3.91	25.12	334.97	0.03	139.57	558.28	148 875.04	1 209.61	1.02	3.35
19_SelR																		
2.58	4.75	37 500.00	0.00	110.00	0.05	250.00	35.00	45.00	1.50	7.75	145.00	0.01	40.00	80.00	42 500.00	850.00	0.21	1.53
N 10%	18.42	145 392.94	0.02	426.49	0.17	969.29	135.70	174.47	5.82	30.05	562.19	0.05	155.09	310.17	164 778.67	3 295.57	0.79	5.93

Normalised to 10% = N10%

5.2.1 Arsenic (As)

The reference sites (5_Mlzi pond, 7_Po and 10_ShisR) had As concentrations below the lowest SQG (Figure 46). The highest concentrations were in the western part of the KNP at 3_CrocR and 4_SabR. Six of these sites had concentrations higher than the Canadian guideline of 5.9 µg/g dw (CCME, 2012) with only one site higher than the Australian guideline of 20 µg/g dw (ANZECC, 2000; Table 18 & Figure 46). Only one locality, 4_SabR, exceeded the Netherlands value of 55 µg/g dw (NDSQG, 2011) after the data was normalised to 10% TOC (Table 18 & Figure 47). The overall highest concentration was recorded at 4_SabR (Figure 30; Figure 46).

The CF for As classified as "very high" for 1_CrocR, 3_CrocR and 4_SabR (Table 12) and the Igeo for 1_CrocR and 3_CrocR classified as "moderate to heavily polluted", and for 4_SabR "heavily polluted" (Table 14). Extremely high enrichment values were calculated for 1_CrocR, 3_CrocR, 4_SabR and 11_LimR (Table 15). The CF for the area where the crocodile mortalities occurred, 6_OliR, 7_Po, 9_LetR and 8_OliR classified as "moderate to considerable" contamination (Table 12) which corresponded with an exceedance of the Canadian guideline (Figure 47). The guideline exceedances recorded at the sites may indicate possible adverse biological effects (ANZECC, 2000). With lLocality 4_SabR hadat concentrations above the Dutch intervention guideline, at which means that steps need to be taken in terms of pollution abatement and/or clean-up (NDSQG, 2011). The Igeo index of the same sites classified at "unpolluted to moderately" polluted (Table 14). These SQP applied to non-normalised data indicates that As was at "moderate to considerable" concentrations at similar sites in the KNP.

The As quantified in the western part of the KNP could be from paper or sugar mills as well as base metal processing plants within the Komati River catchment (DWA, 2009b). Arsenic is used in the processing ores and minerals and is also present in pesticides and wood preservatives (Pershagen, 1981). Once As is released into an environment it spreads easily through water and air and can accumulate in living organisms (Lenntech 2011; Ayres, 1992). It is possible that some of the industries and farms within the Sabie River catchment could be the sources of the As in the area. It seems the As load increased at the downstream sites in the Olifants River (the area where the mortalities occurred: 17_OliR (2.75 µg/g dw)<18_OliR (4.5 µg/g dw)<19_SelR (4.75 µg/g dw)<8_OliR (3 µg/g dw)<9_LetR (3 µg/g dw)<6_OliR (7.25 µg/g dw; Figure 30 & Table 18). The As in the Olifants

River catchment might also be partially derived from the antimony and gold mines outside Phalaborwa as As is commonly used in minerals processes (Mbendi, 2014)

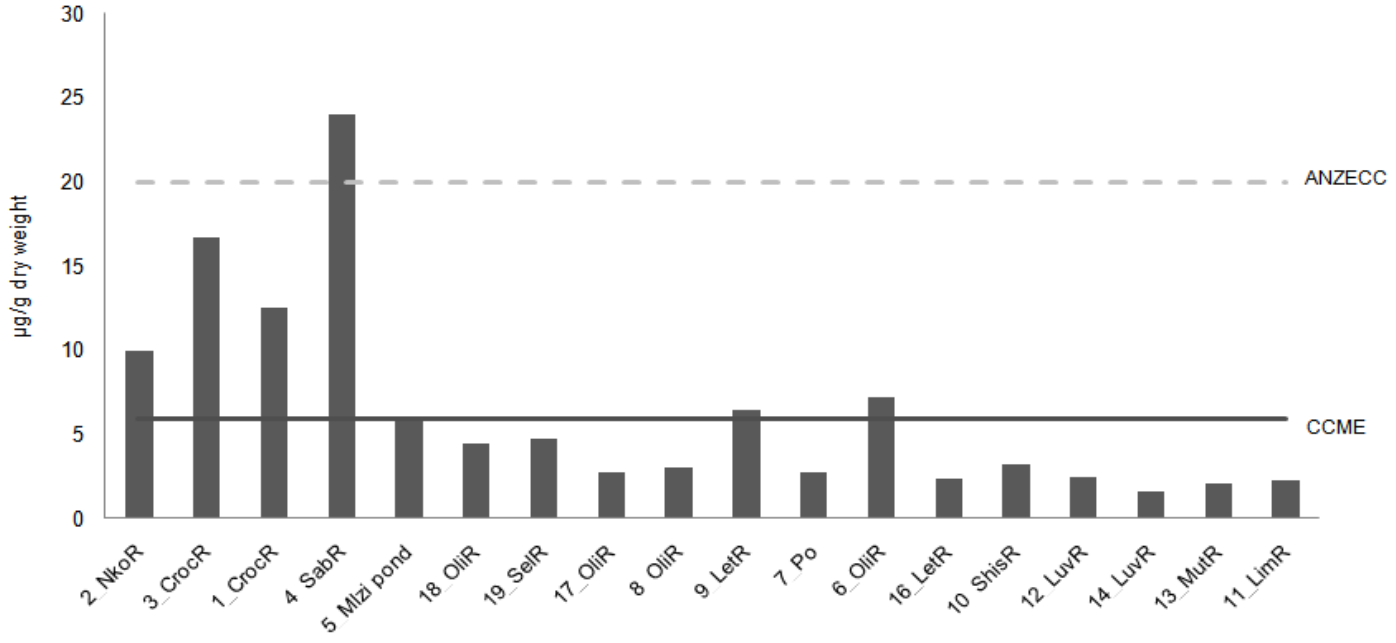


Figure 46: The arsenic concentrations compared to the Australian/New Zealand and Canadian SQGs.

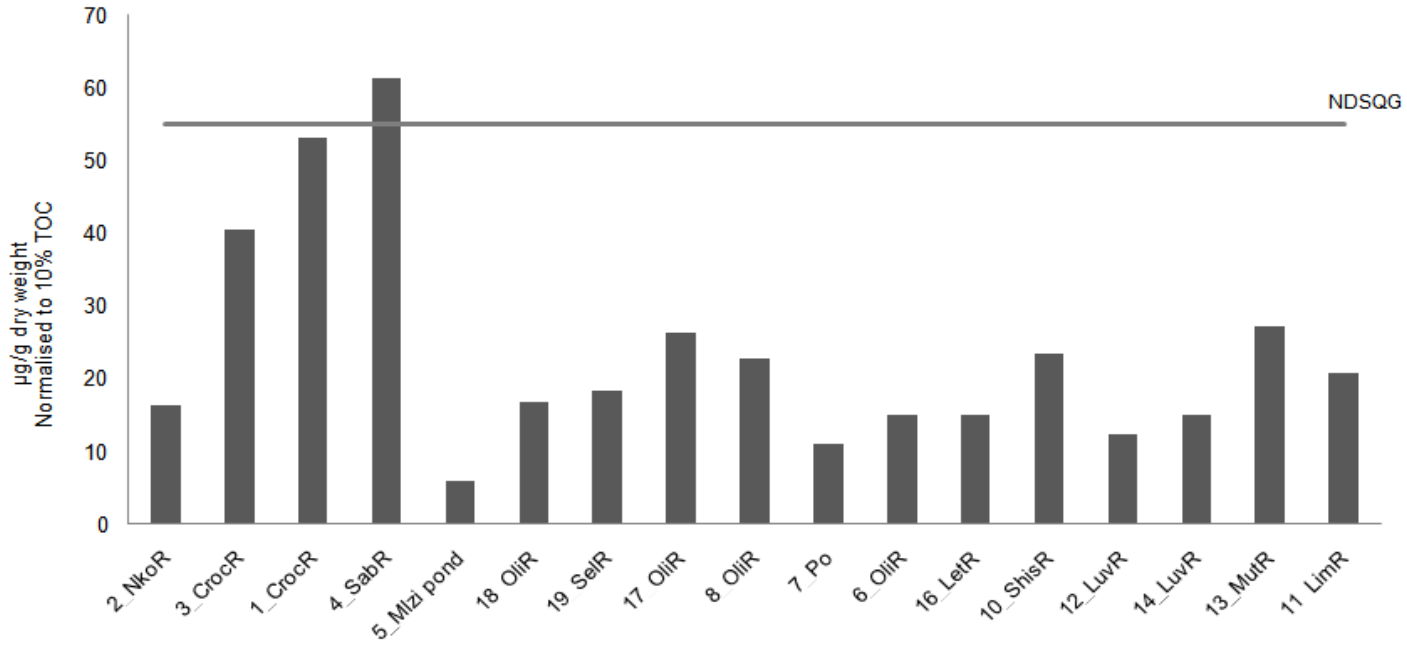


Figure 47: Arsenic concentrations, normalised to 10% TOC, compared with the Netherlands' SQG. No TOC value was available for 9_LetR.

5.2.2 Silver (Ag)

As mentioned in the results very low levels of Ag were quantified (Figure 27). Only the Netherlands had a guideline for Ag at 15 µg/g dw (NDSQG, 2011); all of the sampled sites were well below the guideline concentration (Table 18), and no additional steps in reducing the Ag in the areas are currently necessary (NDSQG, 2011). Silver had the lowest contribution to the total element concentrations (Figure 19). The SQP corresponded with the guidelines and none of the used indices indicated that Ag was unlikely to have had a possible effect on the crocodiles because CF and Igeo showed that the concentrations of Ag were in the low contamination or practically unpolluted categories (Tables 12, 14 & 15). Silver is used in the electrical industry, in paints, circuits and computer parts, and in personal care products. It is also found in long-life batteries, cutlery, jewellery, and mirrors (Lenntech, 2001).

5.2.3 Barium (Ba)

Again, it was only the SQG form the Netherlands that had a guideline concentration for Ba, 625 µg/g (NDSQG, 2011). The following localities had normalised concentrations higher and equal to the SQG value for Ba; 2_NkomR, 17_OliR, 10_ShisR and 12_LuvR (Figure 48). According to the guidelines steps may to be considered to reduce or control the Ba load in the aforementioned areas (NDSQG, 2011). The sites where the crocodile mortalities occurred were below the guideline concentration. According to the non-normalised data, the Ba was the most abundant element at 2_NkoR and the second most abundant at 5_Mlzi pond (Figures 19 & 30).

The EF classified sites 10_ShisR and 11_LimR as “moderately severe” enriched (Table 13), while the CF and Igeo indicated that the various sites in the KNP pointed towards “low contamination to practically unpolluted” levels (Table 12 & 14). The area where the crocodile mortalities occurred were in the “low contamination/practically unpolluted” category and corresponded with non-exceedance of the guideline concentrations (Table 12; 14 & 15). The natural geology at 10_ShisR and 11_LimR differs markedly from the rest of the KNP, which may explain the higher Ba concentrations at these two sites (KNP, 2014). The possible sources of Ba can be from the large copper and coal mining activities upstream of the KNP. This element enters the atmosphere during the mining and refining processes, and also during coal and oil combustion (Lenntech, 2011). It is known that Ba is one of the ten most abundant elements in the earth’s crust (Railsback, 2008) and can account for the

concentrations at the reference sampling sites 5_Mlzi pond and 10_ShisR, because these are dried up riverbeds. This can account for the different sites indicated between the non-normalised and normalised data (Figures 19 & 48).

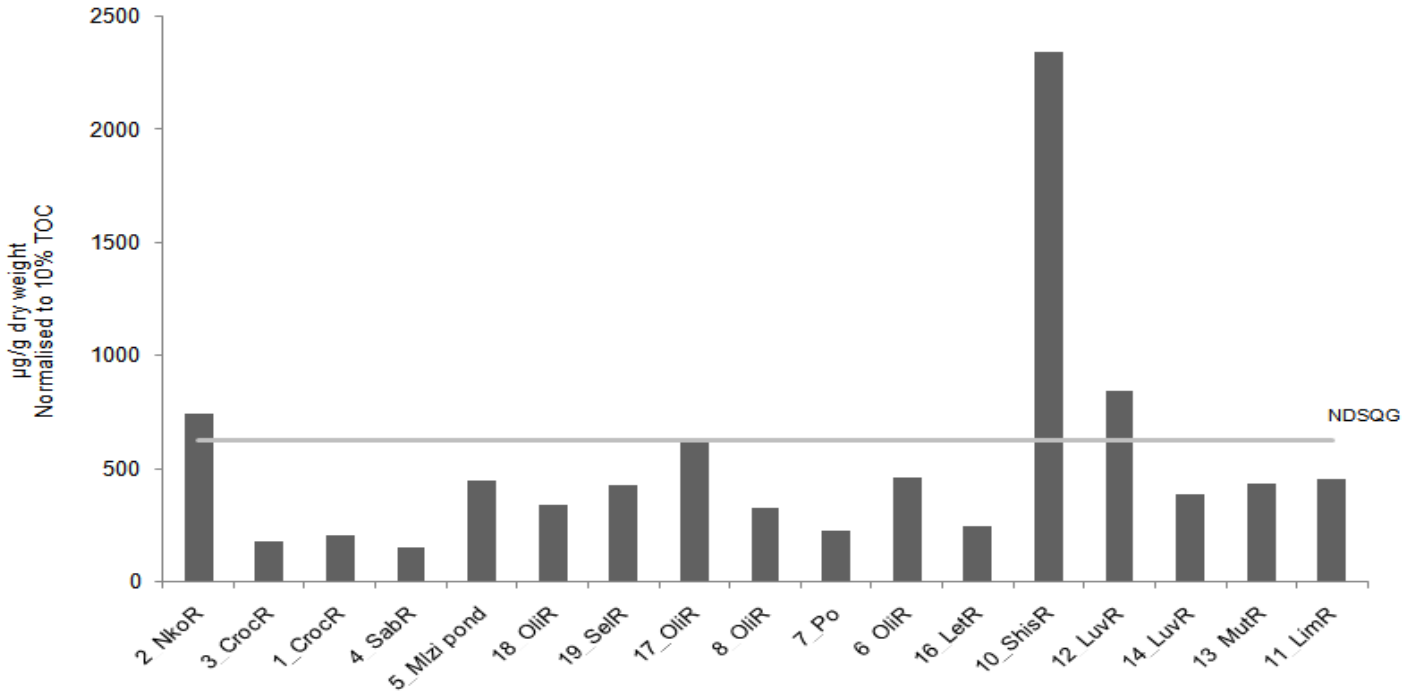


Figure 48: Barium concentrations, normalised to 10% TOC, compared with the Netherlands' SQG. No TOC value was available for 9_LetR.

5.2.4 Cadmium (Cd)

The concentrations of Cd were below any of the listed SQG (normalised and non-normalised) and could point to its natural occurrence in sediment (Railsback, 2008; Figure 49; Figure 50), as Cd use had been restricted because of its detrimental effects on humans and animals (Chedrese, 2004, Hilscherova *et al*., 2000; Ayres, 1992).

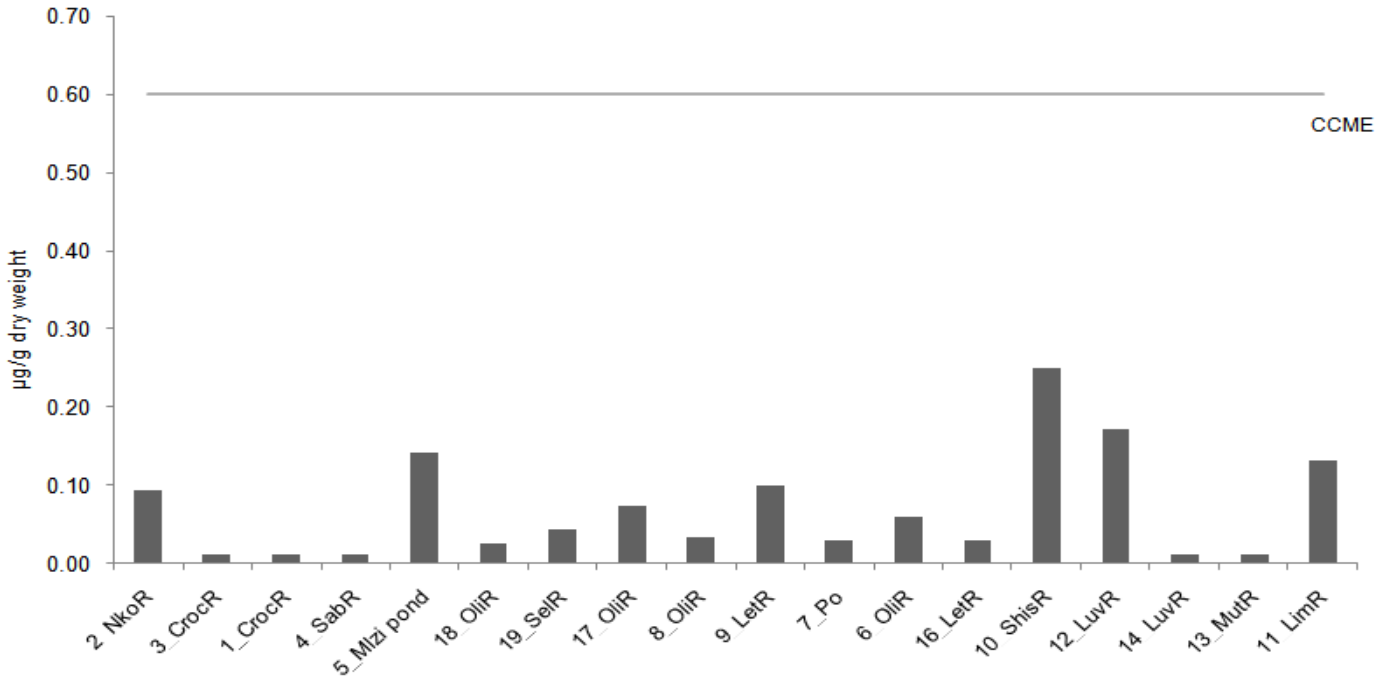


Figure 49: The Cadmium concentrations compared with the Canadian SQG.

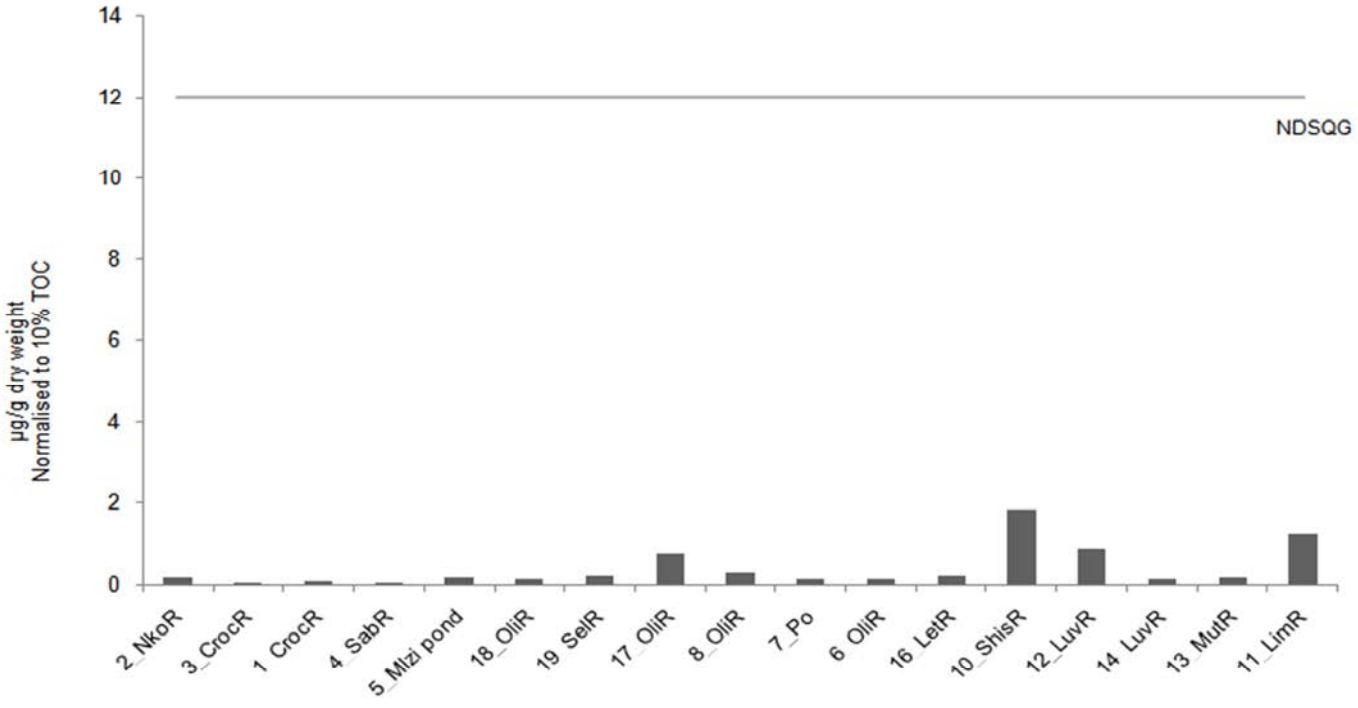


Figure 50: Cadmium concentrations, normalised to 10% TOC, compared with the Netherlands' SQG value. No TOC value was available for 9_LetR.

The CF classified Cd in a “moderate contaminant” range at sites 10_ShisR, 11_LimR and 12_LevR (Table 12). Only sites 10_ShisR and 12_LevR were classified in an Igeo index of “unpolluted to moderately polluted” range (Table 14). The EF classifications were the second highest for Cd at 10_ShisR and 12_LevR in the “severe enrichment range” and the highest was at 11_LimR in the “extremely severe enrichment” range (Table 15). Chemicals such as Cd are released into the atmosphere mainly by industries and can then travel to non-emission areas where it can settle out and bio-concentrate in the environment (WHO, 2007). This can account for the slightly higher concentrations quantified at 10_ShisR and 12_LuvR in relation to the other sites (Figure 49).

5.2.5 Cobalt (Co)

There was only one SQG for Co in the selected guidelines; 250 µg/g dw from the Netherlands (Figure 51; NDSQG; 2011). All of the normalised concentrations were below the SQG value and no interventions are necessary (NDSQG, 2011). It seems that normalised data of Co showed a load increase at the Olifants river sites situated downstream, 18_OliR (84.7 µg/g dw) <19_SelR (135.7 µg/g dw) <17_OliR (211.4 µg/g dw; Figure 26 & Table 18). After inspecting the non-normalised data, it indicated that the highest concentrations were at 6_OliR and 9_LetR (Table 18).

The SQP indicated that the highest contamination was within the Olifants River catchment. CF for Co varied from “low contamination” to “considerable contamination” within the localities where the crocodile mortalities occurred (6_OliR & 9_LetR) having the highest CF together with 19_SelR (Table 12). The same sites that were indicated by the CF were also shown by the Igeo calculations at “moderately to heavily polluted ranges” (Table 14). The EF indicated that the northern part (10_ShisR; 11_LimR; 12_LevR; 13_MutR; 14_LevR & 16_LetR) of the KNP had “severe enrichment levels” (Table 15). The reference pools (5_Mlz pond & 7_Po) had lower concentrations of Co compared to the other sites. This may indicate that the higher concentrations quantified in the rivers may have been from upstream sources outside the KNP. Cobalt can be released into the environment as a by-product form nickel and copper mines; it is also used in the production of petroleum and chemicals (Lenntech 2011; Hilscherova *et al*., 2000). The coal, copper, platinum, and nickel mines in the Mpumalanga province, could have possibly contributed to the higher concentrations of Co at sites 17_OliR, 18_OilR and 19_SelR (Mbendi, 2014; Van Vuuren, 2010; Van Vuuren, 2009; Asthon *et al*., 2001).

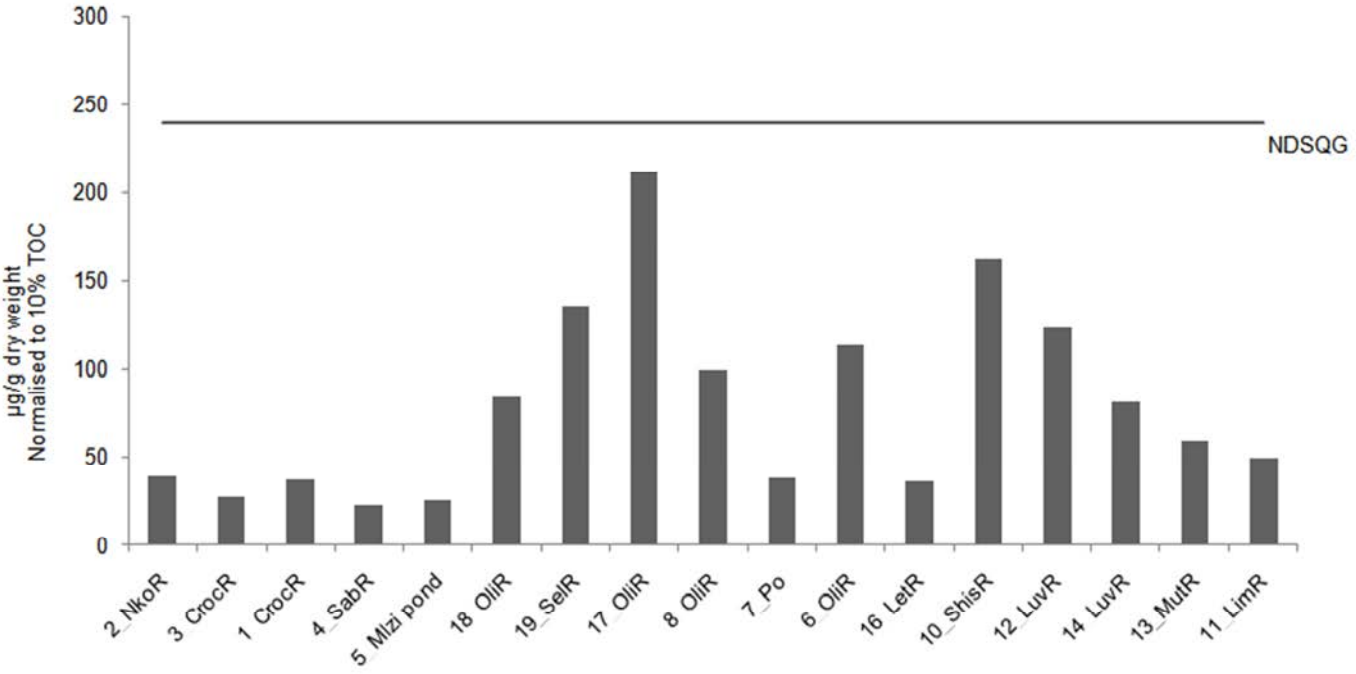


Figure 51: Cobalt concentrations, normalised to 10% TOC, compared with the Netherlands' SQG value. No TOC value was available or 9_LetR.

5.2.6 Chromium (Cr)

Chromium was quantified at all of the localities (Figures 19 & 52) and all of the sites exceeded the Canadian guideline of 37.3 µg/g except for 14_LuvR, 13_MutR and 11_LimR (Figure 52; CCME 2012). The Australian guideline was exceeded at 2_NkoR, 18_OliR, 19_SelR, 17_OliR, 39_LetR, 6_OliR, 10_ShisR and 12_LuvR (Figure 52; ANZECC, 2000). The sites that exceeded the Dutch guideline of 280 µg/g dw were 18_OliR, 19_SelR, 17_OliR, 8_OliR, 6_OliR, 10_ShisR, 12_LuvR, 14_LuvR and 13_MutR (Figure 53; NDSQG, 2011). Of all the normalised data, the highest value was at 17_OliR (Figure 53). In terms of the exceedance of the international guidelines the Cr concentrations measured may cause acute effects (ANZECC, 2000).

The CF for Cr classified localities; 6_OliR, 9_LetR and 19_SelR in the “very high contamination” category with 2_NkoR, 10_ShisR, 17_OliR and 18_OliR in the “considerable contamination” category (Table 12). This corresponds with the high concentrations quantified at the different sites (Figure 19). Igeo ranges corresponded with the CF, classifying similar sites 6_OliR, 9_LetR and 19_SelR in the “moderately to heavily polluted” category and 2_NkoR, 10_ShisR, 17_OliR and 18_OliR in the “moderately polluted” category. The EF on the other hand classified sites 7_Po, 10_ShisR, 11_LimR,

and 16_LetR in the “very severe enrichment” category with the rest of the sites (1_CrocR, 2_NkoR, 3_CrocR, 4_SabR, 6_OliR, 9_LetR, 12_LevR13_MutR, 14_LuvR, 17_OliR, 18_OliR and 19_OliR) in the “severe enrichment” range (Table 14).

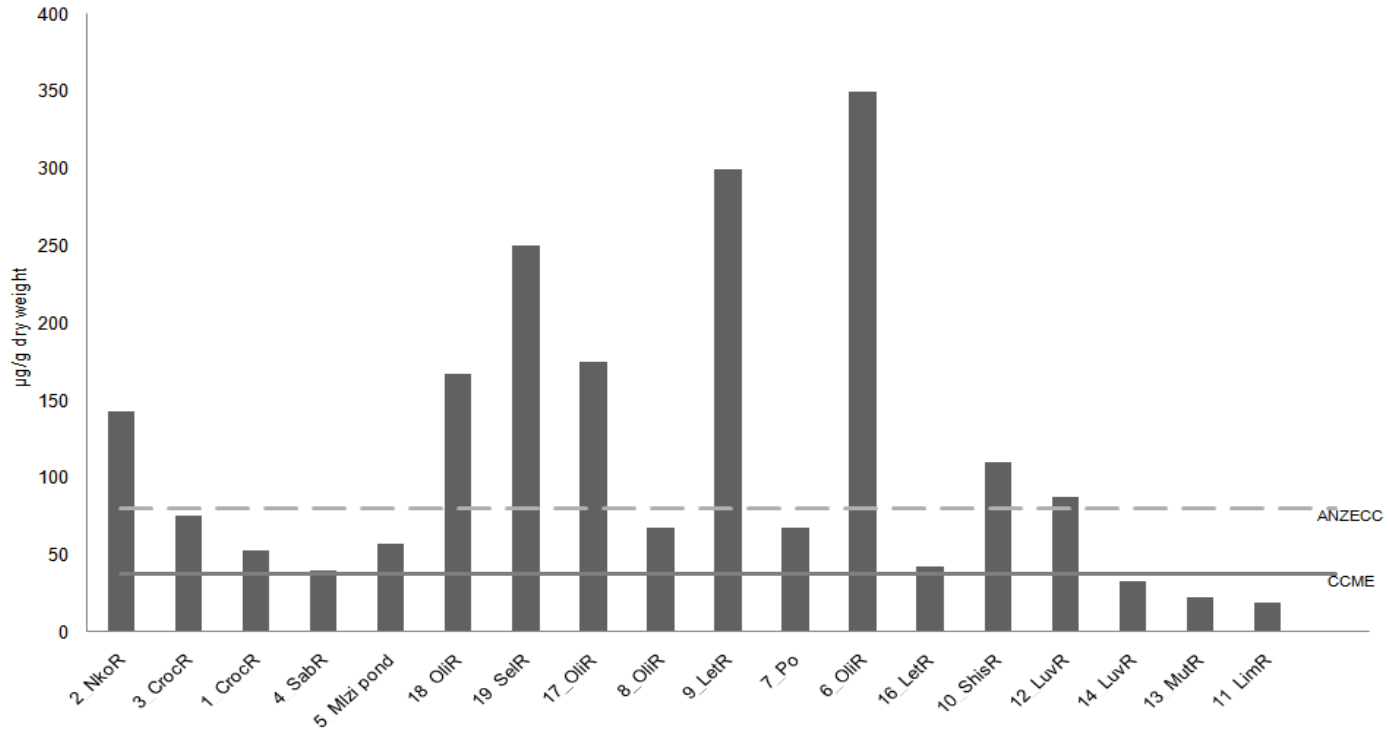


Figure 52: Chromium concentrations compared with the Australian/New Zealand and Canadian SQGs.

The normalised concentrations indicated that sites 17_OliR, 18_OliR, 19_SelR, 6_OliR and 10_ShisR had the highest concentrations and could have been from the coal, copper, platinum, phosphate, magnetite, gold and vermiculite mines as well as other industries found within the Phalaborwa area (Figure 53; Mbendi, 2014; Van Vuuren, 2010; Van Vuuren, 2009; Asthon *et al*., 2001). The health effects of Cr exposure include a weakened immune system; prolonged exposure can cause death (Lenntech, 2011). These high concentrations quantified in the catchment where the mortalities occurred were also supported by the SQP, that indicated Cr was in the top two hazard classes (Tables 12; 14 & 15).

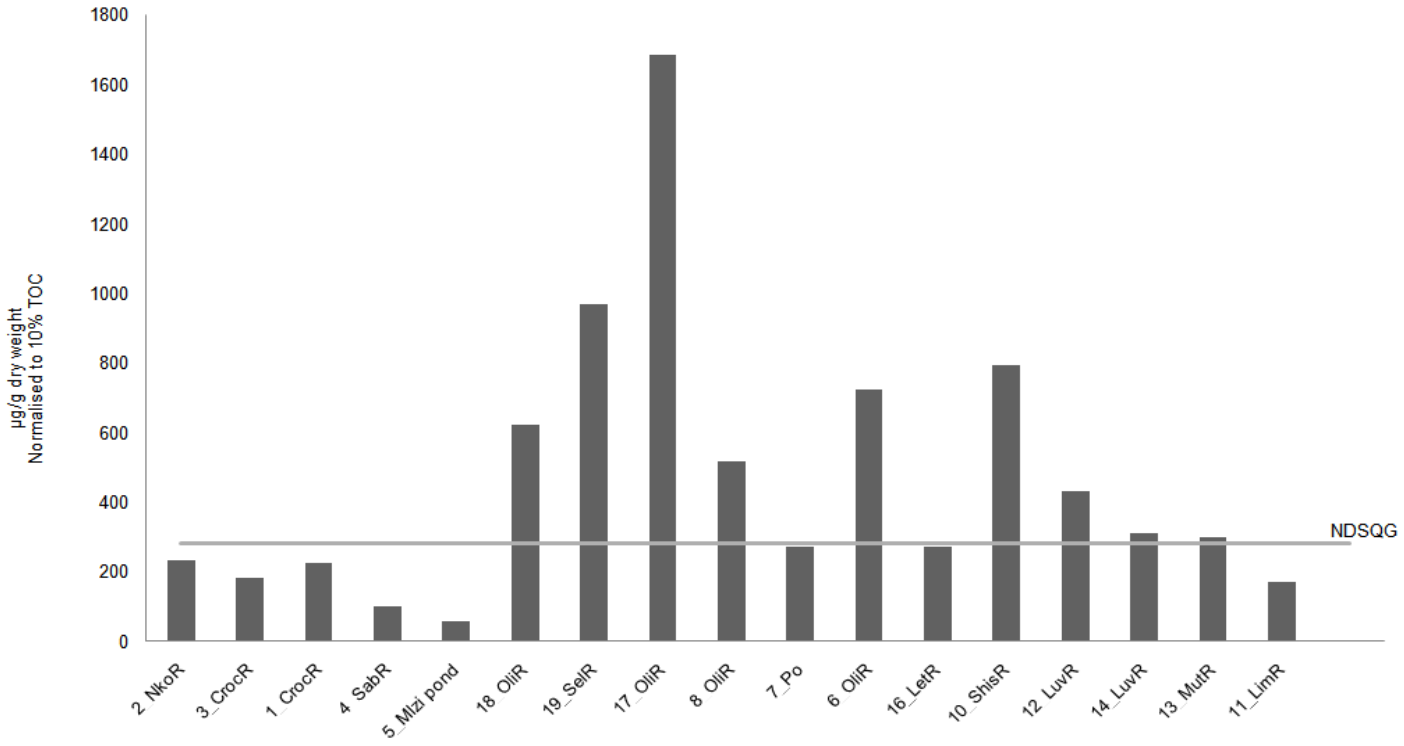


Figure 53: Chromium concentrations, normalised to 10% TOC, compared with the Netherlands' SQG value. No TOC value was available for 9_LetR.

5.2.7 Copper (Cu)

The highest concentration of Cu was detected at site 5_Mlzi pond, which was the pond in the dried up river bed. Concentrations quantified at sites 2_NkoR, 5_Mlzi pond, 18_OliR, 19_SelR, 9_LebR, 6_OliR, 10_ShisR and 12_LuvR (Figure 54) exceeded the Canadian guideline concentration of 37.7 µg/g dw (CCME, 2012). The Australian guideline, 65 µg/g dw was exceeded at 5_Mlzi pond, 9_LetR and 6_OliR (Figure 55; ANZECC, 2000). Two of the sites were in the area where the crocodile mortalities occurred and both exceeded the Canadian and Australian guideline concentrations. After normalisation to 10% TOC, the Dutch guideline, 180 µg/g dw, was exceeded at 18_OliR, 17_OliR, 10_ShisR and 12_LuvR (Figure 55; NDSQG, 2011).

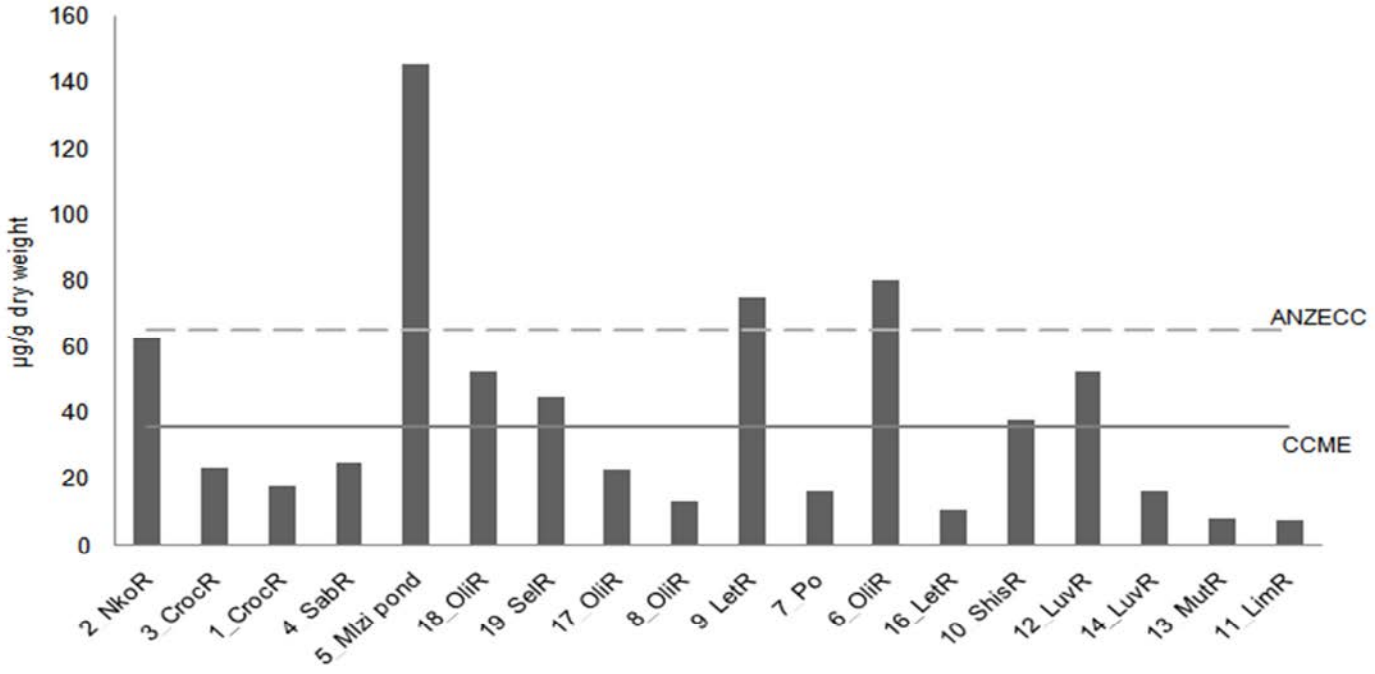


Figure 54: Copper concentrations compared with the Australian/New Zealand and Canadian SQGs.

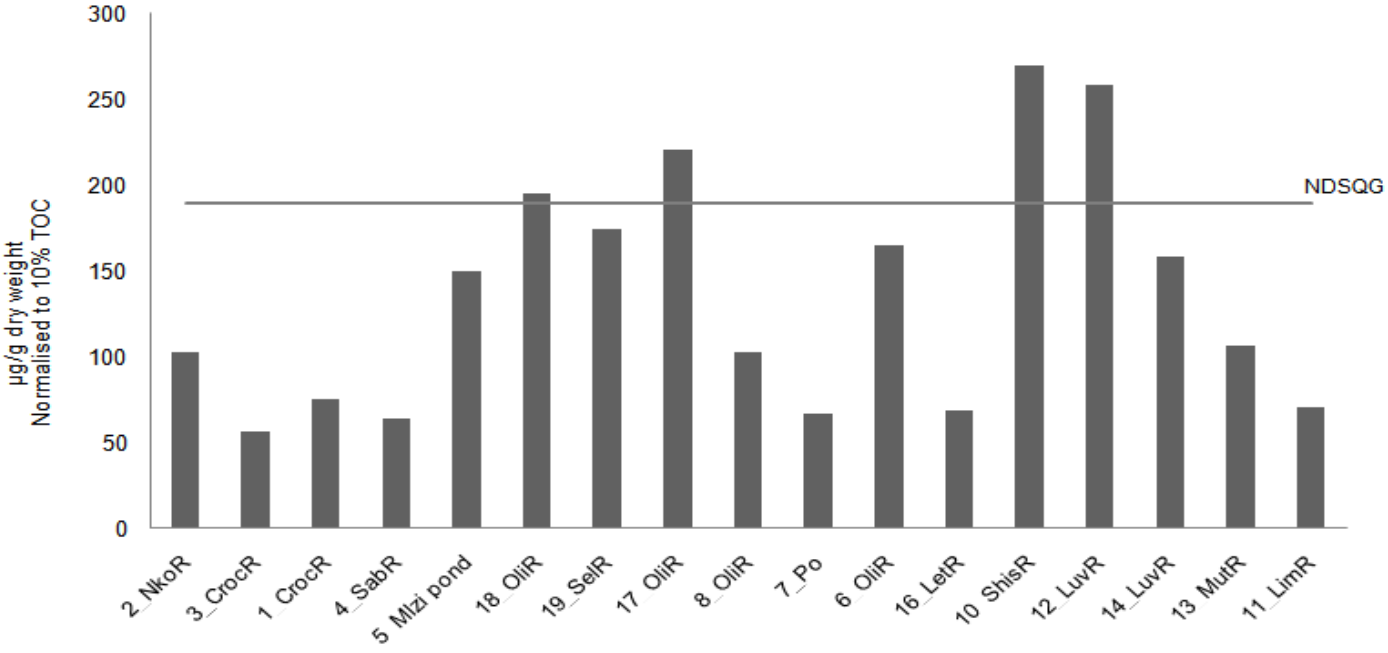


Figure 55: Copper concentrations, normalised to 10% TOC, compared with the Netherlands' SQG value. No TOC value was available for 9_LetR.

A dilution effect could be seen downstream of site 2_NkoR (62.5 µg/g dw)>3_CrocR (23.5 µg/g dw)>1_CrocR (17.8 µg/g dw; Figure 29 & Table 18).

The highest Cu concentration was quantified at 9_LetR and could be from other rivers or streams that flow into the greater Letaba River downstream of the 16_LetR locality (Figure 29 & Figure 54). The concentrations of Cu were also high at 6_OilR, the area where the crocodile mortalities occurred, and the upstream rivers seemed to contribute to the higher concentrations at 6_OliR. This accumulating effect can be seen from 8_OliR (13.50 µg/g dw)<9_LetR (75 µg/g dw)<6_OliR (80 µg/g dw; Figure 29 & Table 18). The normalised data indicated that the highest concentrations were at 10_ShisR and 12_LuvR (Figure 55). This is in contrast to what the SQP indicated, as 12_LuvR was classified as having low contamination and no pollution. The enrichment factor indicated that there was moderate enrichment of Cu in this area. The CF for Cu classified site 5_Mlzi pond as at “very high contamination” that correlates with the high quantifiable concentration at this site. Various sites throughout the KNP was classified as having “considerable contamination” (2_NkoR, 6_OliR, 9_LetR, 12_LuvR, 18_OliR and 19_SelR; Table 12). The Igeo corresponds with the CF as 5_Mlzi pond was classified as part of the “moderately to heavily polluted” and 2_NkoR, 6_OliR, 9_LetR, 12_LevR, 18_OliR and 19_SelR were in the “moderately polluted” range (Table 14). The EF classed sites 5_Mlzi pond and 11_LimR in the “very severe enrichment” range (Table 15). The Cu quantified in these areas may have been because upstream of the sampling sites, on the outside borders of the KNP, there are sugar farms, paper mills, wood processing industries and some smaller copper and coal mining areas (Figure 29; Lenntech, 2011; Van Vuuren, 2010; Van Vuuren, 2009; DWA, 2009b; Asthon *et al*., 2001).

According to the selected guidelines, there are situations with exceedances that may require investigations into possible Cu sources. If the sources are anthropogenic, then mitigation should be considered.

5.2.8 Iron (Fe)

There were no guideline concentrations for Fe in the selected international guidelines (Table 18). The highest concentrations were quantified at sites 6_OliR, 9_LetR, 5_Mlzi pond and 2_NkoR with moderate concentrations also quantified at 17_OliR, 18_OliR and 19_SelR (Figure 18). These concentrations could possibly originate from various upstream mines outside the border of the KNP.

The high concentrations at 9_LetR could have been from smaller rivers or streams that flow into the greater Letaba River, downstream of 16_LetR (Figure 26). Two of these sites were in rivers that flow towards the area where the mortalities occurred and the highest concentration was in the area where the crocodile deaths occurred.

The CF classified for Fe indicated moderate contamination at sites 2_NkoR, 5_Mlzi pond, 6_OliR, 9_LetR, 12_LevR, 17_OliR, 18_OliR and 19_SelR (Table 12). According to the Igeo, Fe ranged between the "practically unpolluted class" to "moderately polluted class" at all the sites (Table 14). The EF again classified Fe had "moderate to moderately severe" enrichment at all of the sites (Table 15). This may be due to the fact that Fe is commonly found in the earth's core (Railsback, 2008) and Fe had the highest concentration of all the elements quantified (Figure 18). The geological background indicated that Fe together with Al would be expected to be of the most abundant elements in sediments from the KNP. However the reference site 5_Mlzi pond had very high concentrations in relation to the 7_Po reference site. Thus the high Fe at 2_NkomR may be explained by the natural geology of the specific area (KNP, 2014). The 7_Po site was lower compared to the 6_OliR and 9_LetR, which supports the possibility of upstream pollution outside the KNP borders. The presence of Fe at localities, 17_OliR, 18_OliR and 19_SelR may be an indication of mining pollution, as Fe is associated with acid mine drainage, which is one of the most common type of pollution associated with the mining industries (Figure 26; Lenntech, 2011; Van Vuuren, 2010; Van Vuuren, 2009; Asthon *et al*., 2001).

5.2.9 Iodine (I)

Iodine was quantified at trace amounts in the sediment at all the localities (Figure 19). There were no guideline concentrations for iodine in the selected international guidelines (Table 18). In accordance with the rest of the sampled sites in the KNP, 2_NkoR had very high iodine concentrations (Figure 25).

From the CF the concentrations of iodine classed site 2_NkoR in the "very high contamination", 1_CrocR as "considerable contamination" ranges. Igeo recorded the iodine concentrations of 2_NkoR in the extremely polluted class, with 1_CrocR in the moderately polluted class (Table 14). The EF also indicated iodine at 2_NkoR in the "extremely severe enrichment", with 1_CrocR as "very severe enrichment" and 10_ShisR as well as 13_MutR as "severe enrichment" (Table 15). Only the

CF indicated “moderate contamination” of iodine in the area where the crocodile mortalities occurred (6_OliR and 9_LetR; Table 12). The reference pool (5_Mlzi pond) had quantifiable iodine concentrations that may indicate the presence of iodine in the natural geology; however it was a lot less than the quantified concentrations at 2_NkomR (Table 18). Iodine is not commonly found in fresh water sediment as it is more related to marine environments (Railsback, 2008). The concentrations quantified at 2_NkoR may be an indication of iodine pollution as it was commonly used as a disinfectant in drinking water (WHO, 2011), animal feed and industrial disinfectants (EPA, 2013). The problem with using the iodine as disinfectant was that normal activated carbon filters could not remove the iodine effectively (WHO, 2011).

5.2.10 Lead (Pb)

Quantified Pb concentrations were below both the Canadian (35 µg/g dw) and Australian (220 µg/g dw) guideline concentrations (Figure 56). The normalised data was also below the Dutch guideline; 530 µg/g dw (Figure 57; NDSQG, 2011). The highest normalised concentrations were at 17_OilR, 10_ShisR and 12_LuvR (Figure 57), which is different to the non-normalised data that indicated the highest concentrations were at sites 2_NkoR, 5_Mlzi_pond, 9_LetR and 6_OliR (Figure 19, 29 & 56). According to the aforementioned guidelines, no acute affects are expected and remediation is not required at the time of sampling (NDSQG, 2011; ANZECC, 2000).

The CF classified only one site, 2_NkoR in a “moderate contamination” class with Pb and the rest of the sites in the “low contamination” class (Table 12). The Igeo index indicated that all the sites were “practically unpolluted” by Pb (Table 14). This was in contrast to the EF, which classified 11_LimR as “moderately severe” enriched with the rest of the sites between “minor to moderate” enriched (Table 15).

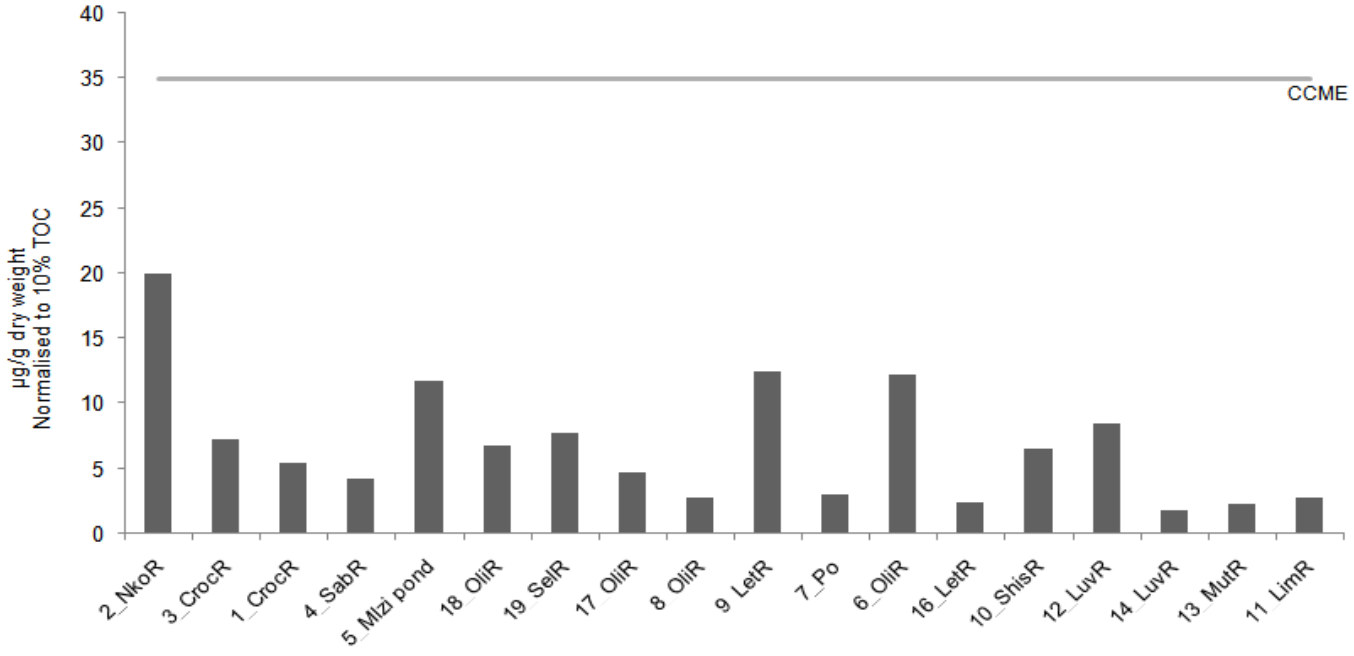


Figure 56: Lead concentrations compared with the Canadian SQG.

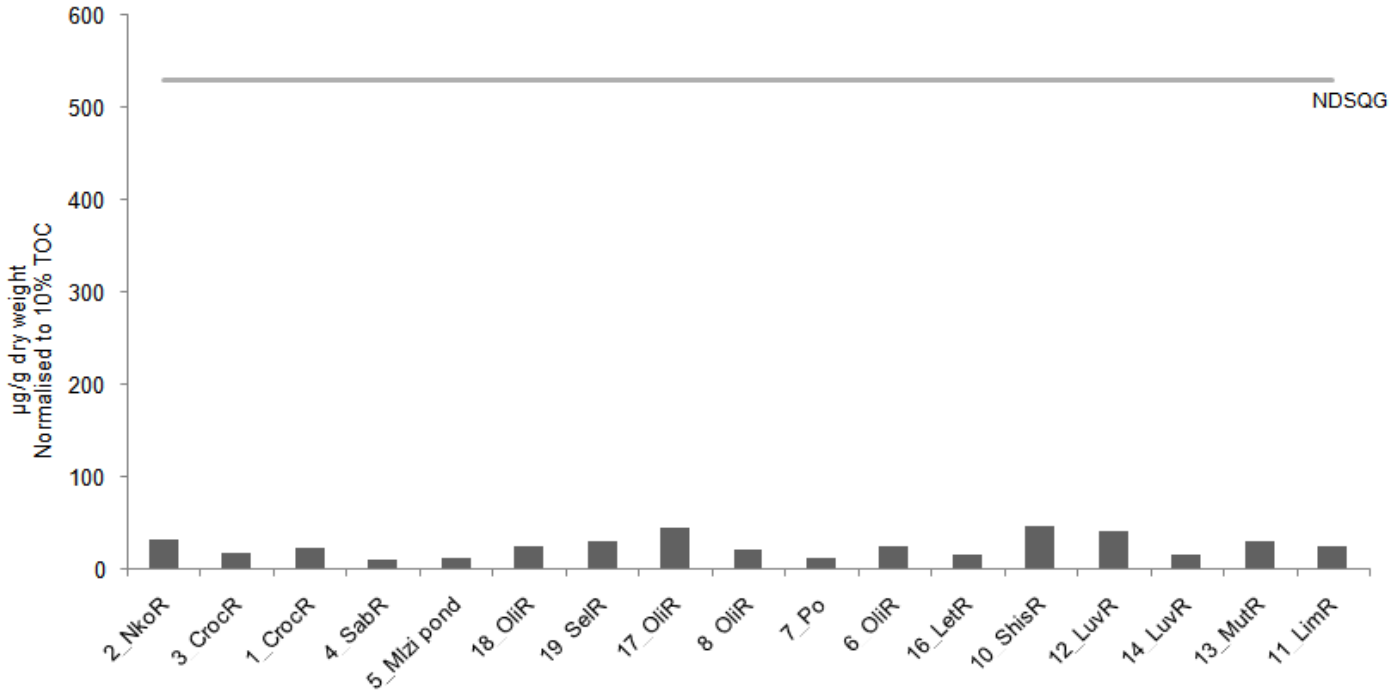


Figure 57: Lead concentrations, normalised to 10% TOC, compared with the Netherlands' SQG value. No TOC value was available for 9_LetR.

Chemicals such as Pb are released into the atmosphere mainly by industrial processes. When released into the atmosphere it can travel to non-emission areas where it can settle out and bioaccumulate in biota (WHO, 2007). Lead can also be released from waste water. This element rarely occurs in its elemental state. It tends to accumulate in the sediment and soil environments (DWAF, 1996b). The most common type of Pb mineral ore is galena (lead sulphide), cerussite (lead carbonate) and anglesite (lead sulphate). Lead will most likely be deposited in the bony skeleton of vertebrate organisms when absorbed at high concentrations (DWAF, 1996b). The concentrations at these sites can be from historic release as the use of Pb in petroleum, paints and plumbing pipes decreased over the years causing a decrease in environmental contamination (WHO, 2011).

5.2.11 Manganese (Mn)

There were no guideline concentrations for Mn in the selected international guidelines (Table 18). The highest concentrations were quantified at sites 9_Po, 6_OliR and 2_NkoR (Figure 19), which included the area where the crocodile mortalities occurred (Figure 30). The CF classified Mn contamination at site2_NkoR as “very high contamination”, whereas the sites where the crocodile mortalities occurred (6_OliR and 9_LetR and 19_SelR) were within the “considerable contamination” to “moderate contamination” categories (Table 12). Igeo classified Mn as “unpolluted to heavily polluted” in the KNP. “Moderate to heavily polluted” levels were classified at 2_NkoR and within the area where the crocodile mortalities occurred (6_OliR and 9_LetR). “Unpolluted to moderately polluted” levels were classified at 11_LimR (Table 14). The EF indicated that the area where the mortalities occurred (9_LetR and 16_LebR) were in the “moderately severe enrichment” class (Table 15). However the other rivers upstream from the sites where the crocodile mortalities occurred had fairly low concentrations. At 5_Mlzi pond the Mn concentrations were in comparison lower than what was measured at 2_NkomR, because of the area’s natural geology (KNP, 2014) this may be indicative of Mn pollution from upstream sources outside the KNP borders (Table 18). The reference site, 7_Po had fairly low concentrations compared to the 6_OliR and 9_LetR sites that may also indicate a contrast between natural geology and possible pollution (Table 18). Manganese is one of South Africa’s most abundant metals in the soil and is actively mined because it is used in the production of iron and steel products (Ashton *et al*., 2001). It is also part one of the essential minerals needed by all vertebrates (Railsback, 2008).

5.2.12 Mercury (Hg)

There were guideline levels for Hg in all the SQG consulted in this study: Canadian, 0.2 µg/g dw (CCME, 2012), Australian, 1 µg/g dw (ANZECC, 2000) and Dutch, 10 µg/g dw (NDSQG, 2011; Table 18). All of the non-normalised and normalised data were below the guideline concentrations (Figure 58 & Table 18). The CF classed all of the sampled sites in the "low contamination" range except at 2_NkoR that was classed in the "moderate contamination" range (Table 10). The Igeo classified all the sites as "practically unpolluted" levels, with only 2_NkoR as "unpolluted to moderately polluted" levels (Table 12). The EF classed 11_LimR as "severe enrichment" with the rest of the sites in the KNP at "minor" to "moderate enrichment" (Table 15).

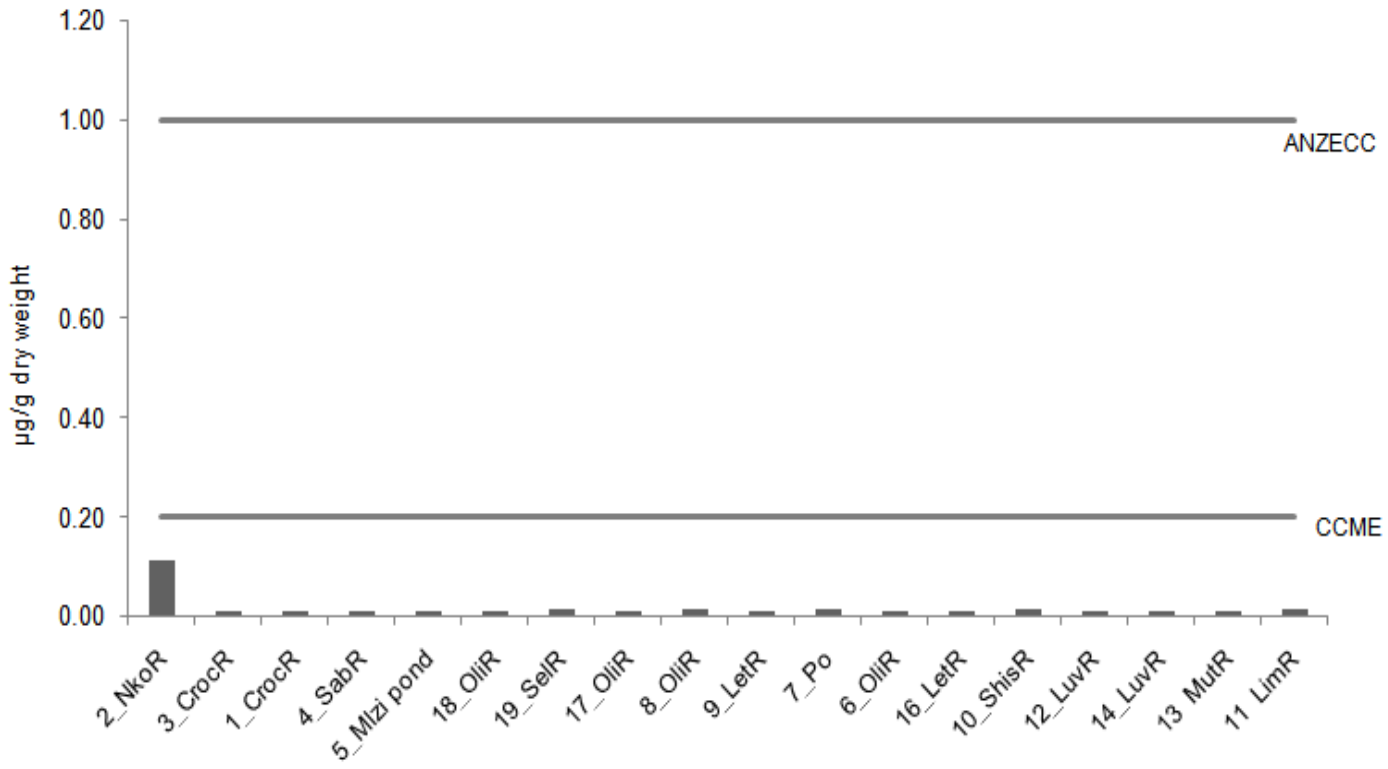


Figure 58: Mercury concentrations compared with the Australian/New Zealand and Canadian SQGs.

Most of the sites had trace amounts of Hg in the sediment and this could be an indication from historic release as Hg was used as a bactericide in paper mills in paints and food containers (Figures 25 & 58; Lenntech 2011; Hilscherova *et al*., 2000; Ayres, 1992). Some of the old mines

outside of the KNP could have used mercury amalgamation as a method for copper, iron, and gold (Au) mining recovery processes (Williams, 2001)

5.2.13 Nickel (Ni)

Numerous sites had concentrations that exceeded the Australian guideline, of 21 µg/g dw with only some sites (16_LetR, 14_LevR, 13_MutR and 11_LimR) below the guideline concentration (ANZECC, 2000, Figure 59). Again, there was a large difference in the concentrations measured at 9_LetR and 16_LetR. In terms of the normalised data, the Dutch guideline (210 µg/g dw) was exceeded at the following sites 18_OliR, 19_SelR, 17_OliR, 8_OliR, 6_OliR, 10_ShisR, 12_LuvR and 13_MutR (NDSQG, 2011; Figure 60). According to these exceedances of the international guidelines action needs to be taken in terms of Ni concentrations in the sediment as it may cause acute affects in the environment (NDSQG, 2011; ANZECC, 2000).

A dilution effect can be seen from sites 2_NkoR (90 µg/g dw) >3_CrocR (37.5 µg/g dw) >1_CrocR (32.5 µg/g dw; Figure 26). "Very high contamination" factors were classified for Ni at 6_OliR, 9_LetR, 10_ShisR and 19_SelR, with 2_NkoR, 12_LevR, 17_OliR and 18_OliR as "considerable contamination" (Table 13). "Moderate contamination" of Ni was classified for 1_CrocR, 3_CrocR, 4_SabR, 5_Mlzi pond, 7_Po, 8_OliR and 13_MutR (Table 13). The Igeo classed 6_OliR, 9_LetR, 10_ShisR and 19_SelR in the "moderate to heavy pollution" categories with sites 2_NkoR, 12_LevR, 17_OliR and 18_OliR in the "moderately polluted" category (Table 14). According to the calculated EF, it classed 10_ShisR in the "extremely severe enrichment" category (Table 15). The following sites; 7_Po, 11_LimR, 12_LevR and 13_MutR were classed in the "very severe enrichment" and the rest of the sites in the "severely enriched" range (Table 15).

The background concentrations quantified at 7_Po and 5_Mlzi pond could possibly be because Ni is one of the ten most abundant HM in the earth's crust and makes up most of the earth's core (Railsback, 2008). With this in mind, the concentrations quantified in the mortality area (6_OliR & 9_LetR) may be indicative of upstream pollution as the natural geology indicates lower concentrations in comparison. Some of the higher concentrations were detected upstream from the area where the mortalities occurred (17_OliR, 18_OliR and 19_SelR). This could possibly have originated from the large coal, gold and platinum mines upstream of the KNP and in the Phalaborwa area (Van Vuuren, 2010; Van Vuuren, 2009; Asthon *et al*., 2001).

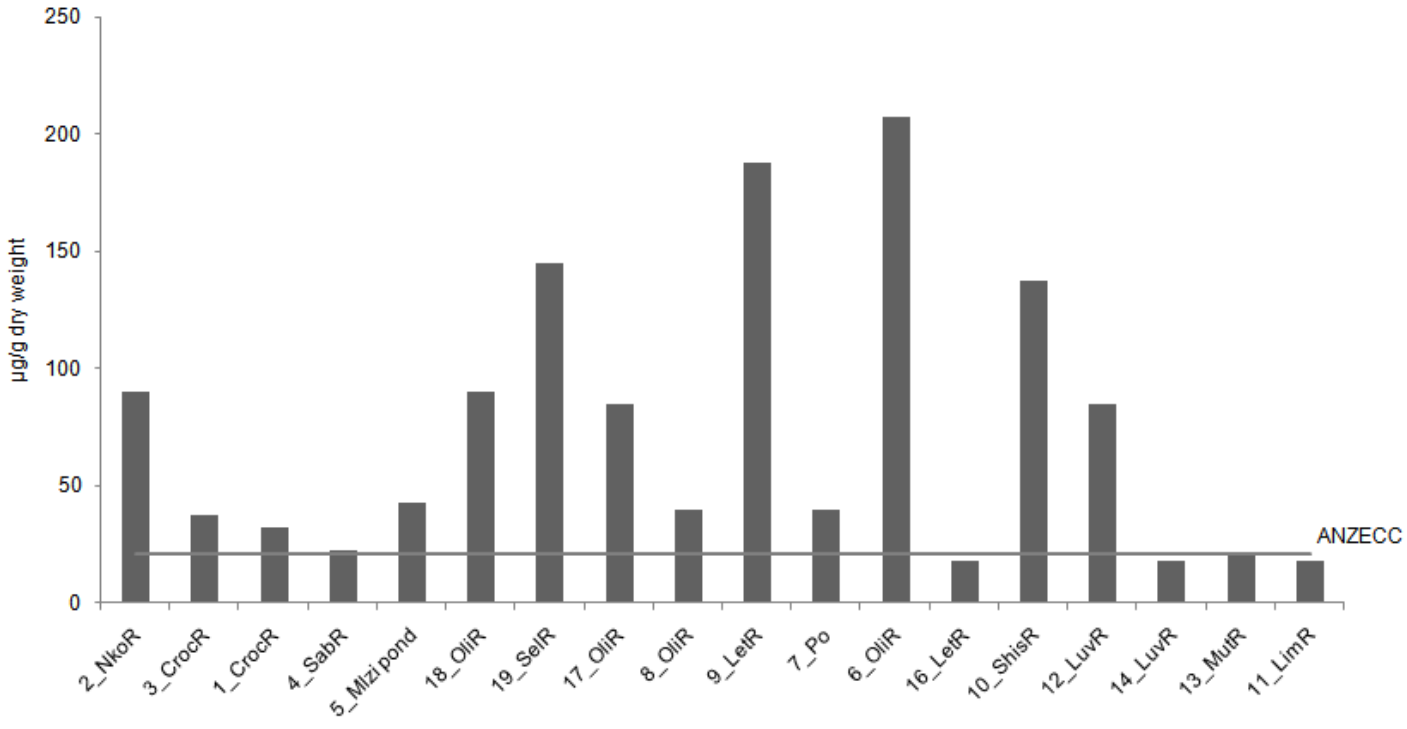


Figure 59: Nickel concentrations compared with the Australian/New Zealand SQG.

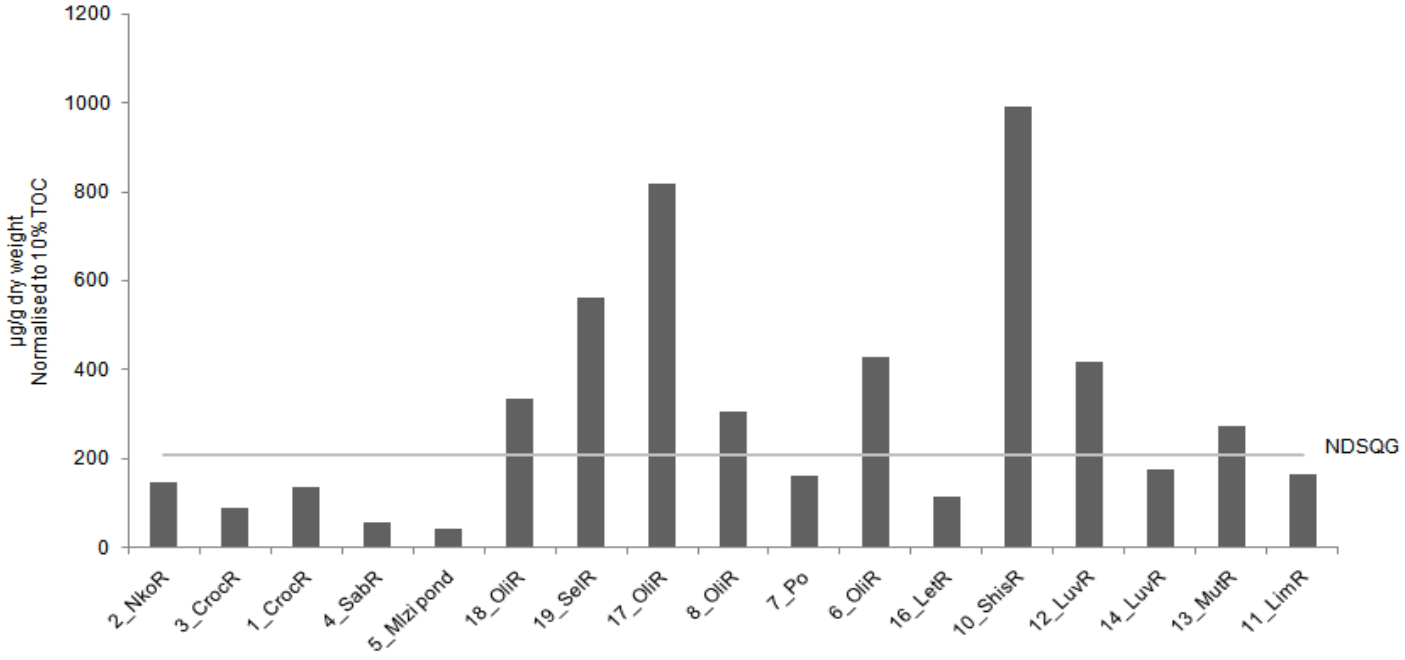


Figure 60: Nickel concentrations, normalised to 10% TOC, compared with the Netherlands' SQG value. No TOC value was available for 9_LetR.

Nickel is commercially used in the preparation of alloys and can be found in a variety of steel-associated products. The presence of old and operational mines may have contributed to the quantified concentrations (Lenntech, 2011; Asthon *et al*., 2001). This was also within the area where the mortalities occurred.

5.2.14 Selenium (Se)

The only guideline concentration for Se was the Dutch guideline, 100 µg/g dw (NDSQG, 2011, Figure 61). For all of the normalised data it was below this concentration. The site where the mortalities occurred also had quantifiable concentrations of Se (Figures 19 & 27). Selenium had very high classifications for all the SQP at all of the sites. The SQP concurs with Figure 61, as having the highest normalised concentration at 10_ShisR (Table 12; 14 & 15). The CF classified all of the sites in the "very high contamination" range (Table 12). Igeo classifications ranged between "moderate to heavily polluted" at 4_SabR, 14_LuvR and 17_OliR.

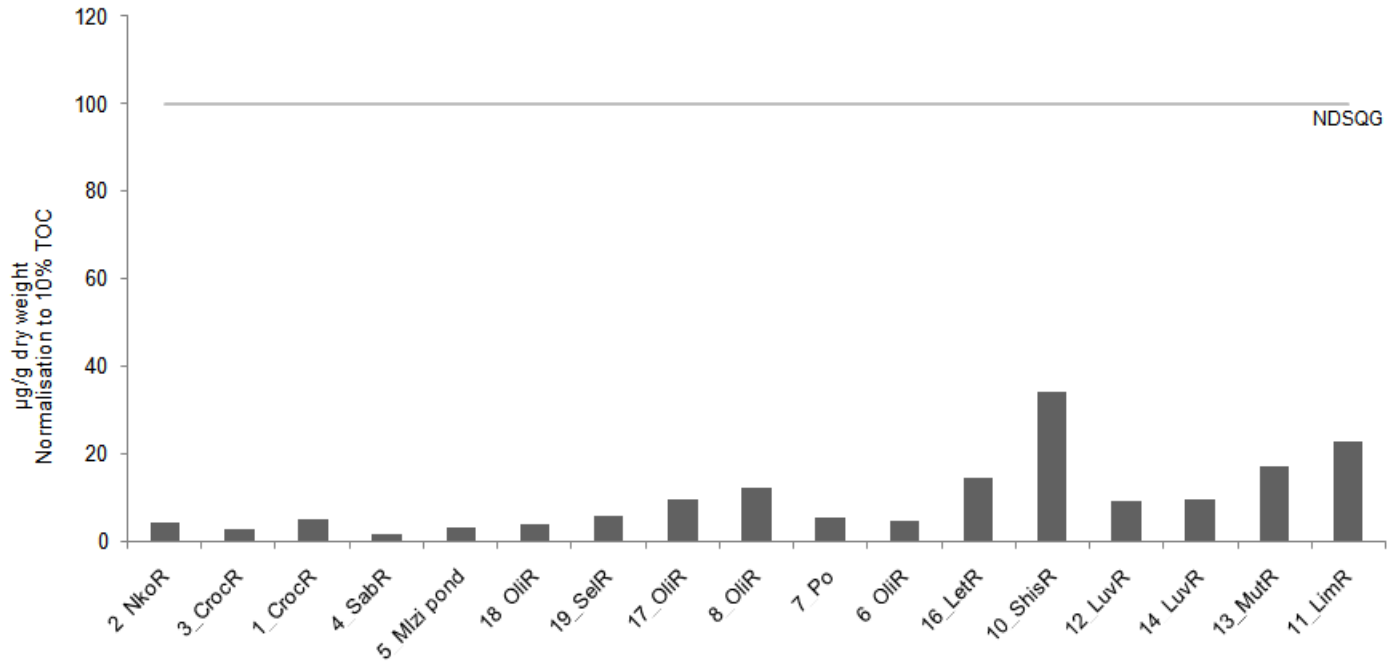


Figure 61: The Se concentrations, normalised to 10% TOC, compared with the Netherlands' SQG value. No TOC value was available for 9_LetR.

The highest Igeo classification was at 10_ShisR and agrees with Figure 61, as having the highest normalised concentration (Table 14). The EF indicated that most of the sites classified as “very severe to extremely severe enriched” (Table 15).

Quantifiable concentrations were found at both the reference sites indicated the natural geology does contain Se. The geology of the northern part of the KNP is slightly different to the rest of the KNP (KNP, 2014) that may indicate the overall higher Se concentrations in relation to the rest of the sampled rivers. Selenium is a natural occurring element in the earth’s crust (Railsback, 2008). This element is mobilised by (1) coal mining and (2) combustion, (3) agricultural irrigation, (4) gold, silver, nickel and phosphate mining, (5) metal smelting, (6), municipal landfills, to name some of the anthropogenic activities (Lemly, 2002 and 1999). The detected concentrations of Se were the highest in sites 2_NkoR, 5_Mlzi pond, 10_ShisR and 11_LimR. It has been found that elevated concentrations of Se can cause defects in the reproductive systems in fish (WHO, 2011; Lemly 2002).

5.2.15 Uranium (U)

There were no guideline concentrations for U in the selected international guidelines (Table 18). Low concentrations of U were detected at all the sampled sites (Figure 19) of which the highest of these was at 12_LuvR. Two trends that can be seen for the quantified U (Figure 28); the first was in the area where the mortalities occurred and the second in the southern part of the KNP. In the area where the mortalities occurred, the concentrations of U seems to concentrate towards the Olifants Gorge from the upstream sampled sites; 8_OliR (0.09 µg/g dw)<9_LetR (0.28 µg/g dw)=6_OliR (0.28 µg/g dw; Table 18 & Figure 29). This indicates that the largest contribution of U in the area where the crocodile mortalities occurred predominantly came from the Letaba River catchment (Figure 29). In the southern area there also seems to be a dilution effect from 2_NkoR (0.30 µg/g dw)>3_CrocR (0.14 µg/g dw)=1_CrocR (0.14 µg/g dw; Table 18). Both the CF and Igeo had low pollution scores for U; all of the sites were classified in the low contamination and indicated that the sites were practically unpolluted with U (Table 12 & 14). The EF also indicated minor enrichment at all the sites (Table 15). This maybe indicated of natural U found at all the sampled localities, but U could also be from the uranium and gold mining activities (leaching, subsequent re-precipitation and ore mining) in upper reaches of the Limpopo and Olifants catchments (WNA, 2011; Winde & Van der Walt, 2004).

5.2.16 Vanadium (V)

The only guideline concentration for V was the Dutch guideline; 250 µg/g (NDSQG, 2011, Table 18). The 10% normalised data exceeded the Dutch guideline at the following sites 18_OliR, 19_SelR, 8_OliR, 6_OliR, 10_ShisR, 12_LuvR, 14_LuvR and 13_MutR (Figure 62). Non-normalised data indicated that the highest concentration was at 17_OliR and the second highest at 18_OliR (Figure 19). Again with the normalised data, 17_OliR had the highest concentration but the second highest normalised concentration was 8_OliR (Figure 62). According to the Dutch guideline pollution abatement and/or clean-up is needed of the aforementioned localities (NDSQG, 2011). It seems that the concentration of V at 6_OliR could have accumulated from the upstream rivers 8_OliR=112.5 µg/g<9_LetR= 117.5 µg/g<6_OliR=125 µg/g (Figure 28 & Table 18). In contrast to the western side of the KNP, a dilution effect can be seen from 2_NkoR (80 µg/g dw)>3_CrocR (37.5 µg/g)>1_CrocR (25 µg/g dw) (Figure 28 & Table 18).

The CF for V classified 17_OliR as having “very high contamination”, whereas the second highest category was “moderate contamination” at the following sites; 2_NkoR, 5_Mlzi pond, 6_OliR, 8_OliR, 9_LetR, 10_ShisR, 12_LevR, 18_OliR and 19_SelR (Table 12). Igeo calculations indicated that 17_OliR was “moderately polluted” and the same sites that had “moderate contamination” for the CF were in the “unpolluted to moderately polluted” Igeo range (Table 14). With the EF sites; 7_Po, 8_OliR, 10_ShisR, 11_LimR, 13_MutR, 14_LuvR, 16_LetR and 17_OliR were classified in the “severe enrichment” category. The rest of the sites classified between “moderate to moderately severe” enrichment (Table 15). Vanadium is used in the production of steel alloys especially reactors, high speed airframes and jet engines as the metal does not deform under high temperatures (Lenntech, 2011). Concentrations of V in these rivers maybe from vanadium mines upstream of the above mentioned areas or mining activities that release V during the mining extraction process (Panichev *et al*., 2006; Moskalyk & Alfantazi, 2003). It has been found that vanadium mining causes large scale V pollution as the compound is released into air and water near the mining area (Panichev *et al*., 2006; Moskalyk & Alfantazi, 2003) which might explain the elevated concentrations in the two ponds (7_Po & 5_Mlzi pond).

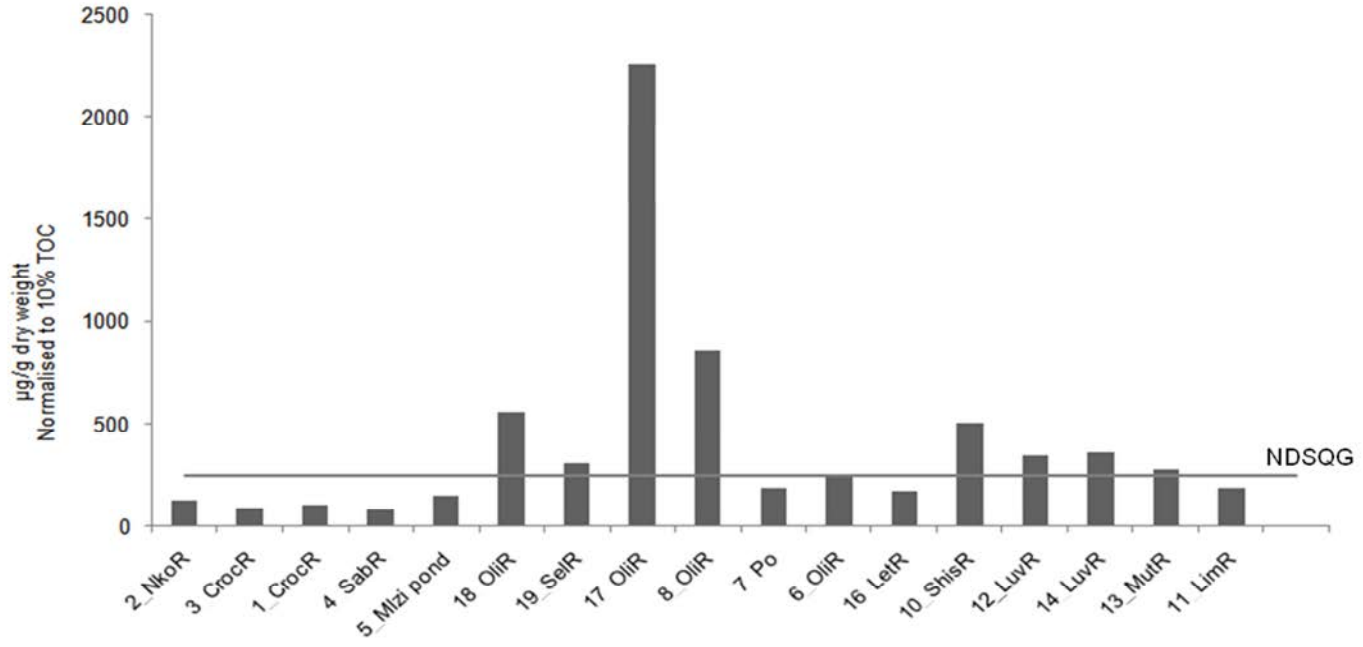


Figure 62: Vanadium concentrations, normalised to 10% TOC, compared with the Netherlands' SQC value. No TOC value was available for 9_LetR

5.2.17 Zinc (Zn)

The highest guideline for Zn is the Australian guideline at 200 µg/g dw (ANZECC, 2000), with the Canadian guideline at 123 µg/g dw (CCME, 2012), and lastly the Dutch guideline at 720 µg/g dw (NDSQG, 2011; Table 18). All of the sampled sites were below the guideline concentrations except for 5_Mlzi pond that exceeded the Canadian guideline concentration (Figure 63). The normalised data was below the Dutch guideline concentration for all of the sites (Figure 64). The highest concentrations quantified were at 2_NkoR and 5_Mlzi pond to the south of the KNP (Figure 19 & Figure 29). The concentration of Zn decreases from 2_NkoR (75 µg/g dw)>3_CrocR (32 µg/g dw)> 1_CrocR (24 µg/g dw; Figure 28 & Table 18). The area where the mortalities occurred (6_OliR) also had higher concentrations of Zn compared to some of the other sites. There was an accumulating effect that could be seen in the Olifants Gorge as the upstream sites had lower concentrations compared to the downstream sites 8_OliR (21.5 µg/g)<9_LetR (65 µg/g)<6_OliR (70 µg/g; Table 18).

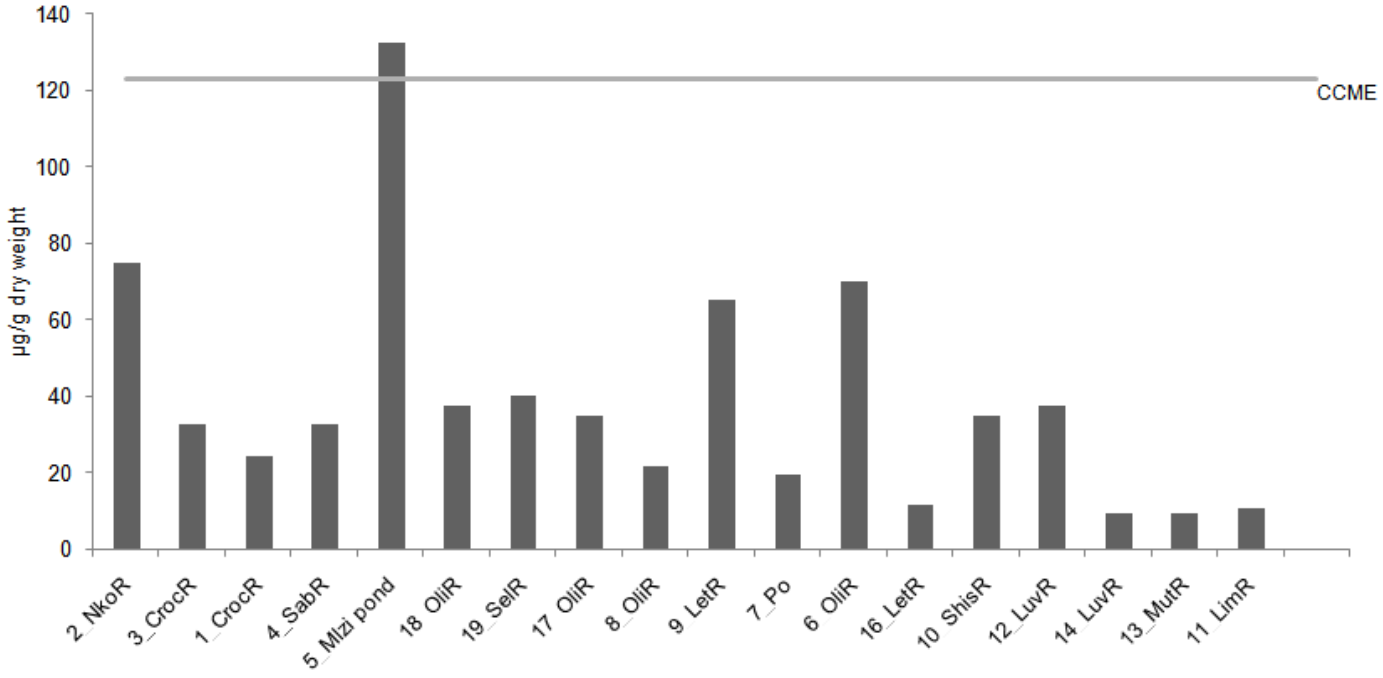


Figure 63: The Zn concentrations compared with the Canadian SQG.

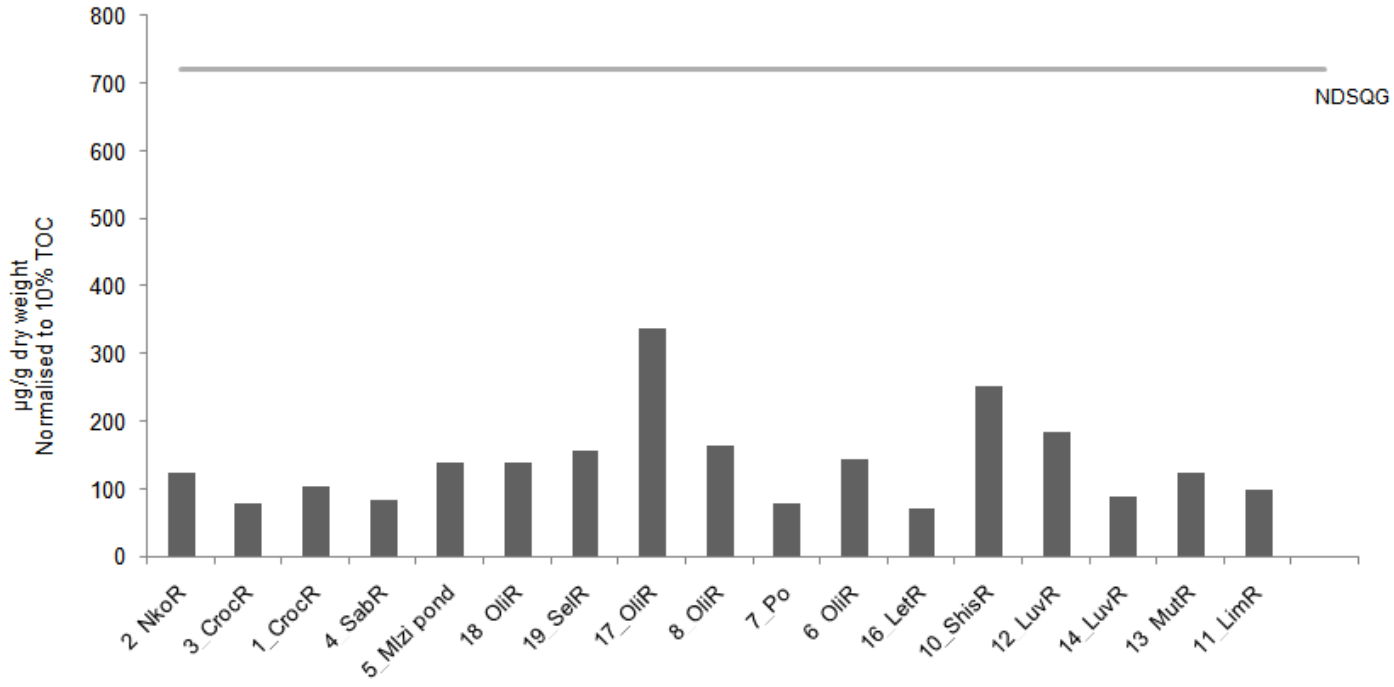


Figure 64: The Zn concentrations, normalised to 10% TOC, compared with the Netherlands' SQG value. No TOC value was available for 9_LetR.

The CF classed the sites (6_OliR and 9_LetR) where the crocodile mortalities occurred, in the "moderate contamination" range as well as at 2_NkoR and 5_Mlzi pond (Table 12). Site 17_OliR was the only locality that was classified by the Igeo as "unpolluted to moderately polluted", whereas the rest of the sites in the KNP were "practically unpolluted" (Table 14). The EF classified sites 4_SabR, 5_Mlzi pond, 7_Po, 10_ShisR, 11_LimR and 13_MutR in the "moderately severe enriched" range and the rest of the sites as "minor to moderate enrichment" (Table 15). The very high Zn quantified at the 5_Mlzi pond may indicate that this element most likely forms part of the natural geology. Zinc is commonly used in the production of galvanised steel and iron, it is also released as a by-product of coal mining and combustion processes (Lenntech, 2011; Pone *et al*., 2001).

5.3 Discussion of the PCA analysis of all the analysed compounds

Factors that can influence the chemical profile at the different localities are river flow, flow velocity, sediment mobility as well as the historic use of the rivers (Dallas & Day 2004). The PCA that combined all the analysed data (Figure 31; 32 & 33) was mainly influenced by the organic chemical pollutants. Various organic chemical compound classes contributed to factors 1 – 3 (Figure 31 & 32) explaining 64% of the variance in the data. The elements only contributed to factor 4 which explained 9% (Figure 33). Regarding the organic compound classes, the chlorinated pesticides did not contribute highly to any of the factors, except for single DDT metabolites and chlordane isomers that featured in factors 2 and 3 (Figure 31 & 32).

Factor 1, on the positive side, mainly consisted of PCBs, PBDEs and V, whereas the negative side consisted of PCDD/F, HMW PAHs and iodine (Figure 31). This factor seems to distinguish between more industrially associated pollutants (e.g. PCBs and PBDEs) and the compounds usually released by any combustion process where carbon is a source (e.g. PCDD/Fs and the PAHs). The sites that were mainly influenced by factor 1 on the positive side were 17_OliR, 16_LetR and 7_Po, indicative of a more industrially polluted area, with the negative side having high scores for sites 1_CrocR, 2_NkoR and 3_CrocR, indicative of an area exposed to combustion sources. The PCA containing only the unintentionally produced compounds (Figure 34) supported the findings of the sites that associated with the PCDD/F and PCBs. The sites on the southern border (2_NkoR, 1_CrocR, 3_CrocR) were loaded strongly with the larger PCDD/Fs whereas the sites on the western side (19_SelR, 18_OliR and 17_OliR) and 7_Po were associated strongly with the larger PCBs (Figure 34). The PAHs recorded at 17_OliR and 16_LetR were part of the LMW PAHs and can be

confirmed with the PCA consisting of only PAHs (Figure 35; positive side of factor 1). These localities are all subjected to large scale industries and agricultural activities, which are well-known sources of organic chemical pollution (ATSDR, 2012; Mallory & Beater, 2009; USEPA, 2008). Since site 7_Po was an isolated pond when sampled, these pollutants were most likely deposited there via air. Iodine recorded at 1_CrocR and 2_NkoR in this PCA (Figure 31) can be supported by the PCA that consisted only of the elements (Figure 38). This may indicate a possible source of iodine pollution in this area, as iodine is not commonly found in fresh water sources (Railsback, 2008). The loading of V in this PCA is supported by the PCA that consisted only of the elements as it also associated with site 19_OliR (Figure 38).

Factor 2 had high positive loadings for LMW PAHs, *o,p'*-DDE, EC, TDS and HCB with negative loadings of the PCBs, *p,p'*-DDD and one hexa-PCDF (Figure 31). This factor also distinguished between PCBs on the one side contrasting with PAHs on its opposite side, similar to factor 1 (Figure 31). However, in factor 2, it was the LMW PAHs that had high loadings. The PCB congeners with high loadings were different from the PCBs in factor 1. Two of the environmental characteristics, EC and TDS, made an appearance as high loaders on this factor, associated with the LMW PAHs, and some organochlorine pesticides; *o,p'*-DDE and HCB (Figure 31). This association was confirmed in the PCA of the chlorinated pesticides only (Figure 37). The sites that scored highly on factor 2 on the positive side were 14_LuvR and 19_SelR, indicating a higher exposure here to LMW PAHs than at any of the other sites in the study (Figure 31). This was confirmed with the PCA that contained only the PAHs, as these two sites were influenced by the LMW PAHs on the positive side of factor 1 (Figure 35). The site with high negative scores included 17_OliR, 1_CrocR, and 4_SabR, indicating that they too were exposed to industrial pollution (PCBs; Figure 31). The PCA that contained the PCBs, DL–PCBs and PBDEs (Figure 36) confirmed that the upstream river locality (17_OliR) of the crocodile mortality area associated strongly with industrial pollution (PCBs). Both factors 1 and 2 indicated that site 17_OliR had high scores, definitely signifying that this site was mostly exposed to PCB pollution, more than any other of the compounds investigated in this study (Figure 31). However, the PCA for the pesticides only, had high scores for sites 17_OliR and 19_SelR, indicating a pesticide exposure, albeit historic one.

High loadings were recorded for the medium sized dioxins (TCDD/F, and hexa PCDD/Fs) on the positive side of factor 3 (Figure 32). This was in contrast to the HMW PAHs, TDS, EC, PCBs, cChlor, tChlor, tNChlor, and cNChlor on the negative side of this factor (Figure 32). This factor seems to

distinguish between the dioxins and the LMW PAH which clustered together on the same side of factor 1. The various chlordane isomers made a noteworthy contribution and appeared together with the TDS and EC characteristics. These environmental characteristics seemed to be associated with the organochlorine pesticides, like it did in factor 2 (Figure 31 & 32). The localities that scored highly on the positive side of factor 3 were 14_LuvR and 1_CrocR in contrast to the high negative scores of sites 3_CrocR, 2_NkoR and 6_OliR. The 2_NkoR site corresponded with the findings in factor 1 and 3 because the PCA that consisted of only the PAHs indicated a strong relationship with the HMW PAHs on the negative side of factor 1. Site 1_CrocR associated with the dioxin-PAH side of factor 1 and in this factor it became clear that its inherent association is really with the dioxins, because it is now associated with the dioxin side of factor 3, and not its opposite PAH side (Figure 32). Both the factors in the PCA that consisted of the PCBs, DL–PCBs and PBDEs (Figure 36) showed that locality 6_OliR had very strong loadings with different PCBs congeners. This same locality associated with HMW PAHs on the positive side of factor 2 in the PCA that consisted only of PAHs (Figure 35).

Factor 4, the first factor to which the elements contributed, distinguished between mostly transitional metals on its negative side and a selection of organic compounds on its positive side, among them a hexa-TCDD, a variety of PCBs, chlordane isomers and LMW PAHs (Figure 33). It therefore seems, based on the PCA data, that these sites, where the crocodiles died, were more influenced by metal contamination, rather than the organic compounds. The high loading of LMW PAHs recorded in this factor was supported by the positive side of factor 1 in the PCA containing only the PAHs (Figure 35). The sites that associated with the negative side of this factor were 6_OliR and 9_LetR, both sites in the centre of the crocodile mortalities. This is supported by the negative side of factor 2 in the PCA containing only the elements (Figure 38). Site 6_OliR however, also seemed to be characterised by an organic component of HMW PAHs (see above at factor 3's discussion). A possible source for both types of contamination is the mining activity on the western border of the KNP, on the banks of the Olifants River, just before it entering the KNP.

When 7_Po and 5_Mlzi pond are studied—which had their own small catchment areas, not connected to any large riverine systems—it is possible that the elements that strongly associate with these site can be a part of the natural geology of the area (Railsback, 2008). With this in mind, the PCA that contains all the elements that were analysed provided a single two-dimensional model with only factors 1 and 2 (Figure 38). This factor combination contributed 79% of the variance in the data. On the positive side of factor 1 the Pb, iodine, Mn, As, and Zn grouped together with sites 1_CrocR

and 2_NkoR, indicating that these compounds may be active at high concentrations in these sites. In the PCA that only contained the elements, As grouped together with Pb, Mn and iodine at 1_CrocR, 2_NkoR, 4_SabR and 12_LuvR on the positive side of factor 1 (Figure 38). The presence of As was also confirmed with the enrichment factor as it indicated that site 4_SabR was "severely enriched" with this element. With factor 4 of the PCA containing all the elements, As clustered with the environmental factors (pH, temperature, EC and TDS; data not shown). This could be because arsenic is largely influenced by pH in the environment (Williams, 2001). The CF indicated that iodine and Mn caused "very high contamination" whereas Pb and Zn were in the "moderate contamination range" at site 2_NkoR (Table 10).

The negative side of factor 1 consists mainly of the association between Ag, Hg, Cd, and Se with the environmental factors (Figure 38). Cadmium could have grouped together with the environmental factors because Cd is assimilated by anoxic sediments with high organic matter content, which generates the potential for bioaccumulation through dietary uptake (Okafar & Opuene, 2007). The sites that were influenced by this factor were 11_LimR, 10_ShisR, 16_LetR and 7_Po. This can indicate that there are high concentrations of these compounds in the natural environment, because this was the situation for site 7_Po too which is the isolated pool that doesn't form part of any one of the rivers and could therefore not have been contaminated by up-stream anthropogenic activities. The elements at 7_Po must have come from its natural environment. The CF recorded "very high contamination" of Se at all of the sites (Table 12), whereas the Igeo ranges for Se was "extremely polluted" at 10_ShisR and "moderate to heavily polluted" at 7_Po, 11_LimR and 16_LetR (Table 14). The EF also indicated that Se was present at "extremely severe enrichment" at all of the above mentioned localities (Table 15). The CF indicated that Cd concentrations were in a "moderate contaminant" value range at sites 10_ShisR, 11_LimR and 12_LevR (Table 12). Only sites 10_ShisR and 12_LevR indicated an "unpolluted to moderately polluted" range for Cd with the Igeo index (Table 14). The EF classifications were the second highest for Cd at 10_ShisR and 12_LevR in the "severe enrichment" category and the highest was at 11_LimR in the "extremely severe enrichment" category (Table 15).

The second factor of the PCA containing only the elements indicated on the positive side, that Ag, Se and Hg clustered together with the environmental factors (temperature and pH) with site 11_LimR (Figure 38). None of the indices indicated that Ag may be considered as pollutants at the sampled

localities. According to the indices, the only locality that had any significant pollution of Hg was at 2_NkoR (Tables 12; 14 & 15).

The PCA containing only the elements indicated on the negative side of factor 2, that the following compounds; V, Ni, Co, Cr, Fe, Al, Zn and Cu strongly associated with the river system where the crocodile mortalities occurred (6_OliR, 9_LetR, 17_OliR, 18_OliR en 19_SelR; Figure 38). This corresponds with the composition concentrations graph of the elements, with the highest composition of elements at 6_OliR and 9_LetR (Figure 19). The highest concentration quantified for Cr was at 9_LetR and 6_OliR (Figure 52). The higher concentrations measured at 9_LetR may have been introduced by other rivers or streams within the greater Letaba River catchment downstream of the 16_LetR locality. The concentrations at 6_OliR, the area where the crocodile mortalities occurred, were also relatively high compared to the guideline values. It seems that the upstream localities may have had a large influence on the Cr load quantified at 6_OliR (Figure 54). The high loadings of Al in this PCA factor could most likely be because this compound is very abundant in the geological composition of the area. The SQP also indicted that these elements V, Ni, Co, Cr, Fe, Al, Zn and Cu had concentrations that could have been involved in the mortalities of the crocodiles (Table 12; 14 & 15). Because the site 7_Po (the isolated pool) sample was collected within the same geological area as where the mortalities occurred, it was possible to compare its elemental composition to that of the nearby river sediments. It may be assumed that the elements recorded in the rivers area were enriched by anthropogenic activities and less so from natural occurrence in the rock. The area where the crocodile mortalities occurred, 9_LetR and 6_OliR, grouped together with V, Ni, Co, Cr, Fe, Al, Zn and Cu in the PCA that contained all the elements (Figure 38). These two localities also exceeded the international guidelines for the aforementioned elements.

5.4 Distribution of pollutants

I am aware that there are several factors in play about how much of the underlying geology and sedimentology may influence eventual toxicology, however, existing geological knowledge and the predictive ability of sedimentology there is not such that acceptable predictions can be made about the elemental compositions of any site including history of sedimentation. This study aims to compare at a gross level the elemental compositions of different sites from different catchments in relation to crocodile toxicology.

The high Fe and Al concentrations at all of the sites may be more related to the natural geological composition as these elements are commonly found in the earth's crust (Railsback, 2008).

The SQPs indicated that Se concentrations all of the sampled localities could possibly have caused harm to biota. Of the four international sediment quality guidelines consulted in this study, it was only the Netherlands that had a value for Se of 100 ug/g dw. Reviewed literature suggests that the toxic threshold is in the region of 2.5 µg/g dw, but adverse effects will only be observed at 4 µg/g dw in sediments (NIWQP, 1998). Taking this into consideration, only sites 2_NkoR, 5_Mlzi pond, 10_ShisR and 11_LimR exceeded the 2.5 µg/g threshold (Table 18), while only 10_ShisR exceeded the 4 µg/g. Despite what the SQPs indicated, the Se concentrations in the area where the crocodile mortalities occurred were less than the 2.5 µg/g dw threshold concentration. Although studies have associated high intake of Se with a number of diseases and that there are potential adverse health effects (WHO, 2011; Lemly 2002), it does not seem that the Se concentrations, in the area where the mortalities occurred, were the main cause of the crocodile deaths.

This summary distinguishes between *four regions in the KNP*, briefly describing the compounds (POPs, PAHs and elements) that exceeded the guideline concentrations and also indicating possible contamination using the SQP. These areas include the northern section (10_ShisR, 11_LimR, 12_LuvR, 13_MutR and 14_LuvR), the southern portion (1_CrocR, 2_NkoR, 3_CrocR, 4_SabR and 5_Mlzi pond), the area where the mortalities occurred in the eastern border (8_OliR, 9_LetR, 7_Po and 6_OliR) and lastly the industrialised area in the western section (16_LetR, 17_OliR, 18_OliR and 19_SelR) of the KNP.

5.4.1 Northern Section

Barium had the highest normalised value at the 10_ShisR together with 12_LuvR, which exceeded the Australian/New Zealand guideline concentration (Figure 39). The EF for these elements: As, Cd, Co, Cr, Cu, Hg, I, Mn, Ni, Se and V (Table 13) placed sites 10_ShisR and 11_LimR in the "moderately severe" range. On the other hand, the CF and Igeo indicated "low contamination" or "pollution" only in terms of Ni, Cr and Se (Table 10 & 12). These apparent contradictions in the SQP could have been caused by the normalisation factor in the EF equation, which was absent in the CF and Igeo calculations (Chapter 3; Section 3.7).

The highest concentration of ΣDDT was quantified in the northern section of the KNP at 14_LuvR and exceeded both the Australian and Canadian guidelines. As mentioned, the DDT/metabolite concentration ratios (Table 9) indicated that the application of this pesticide was recent, which is expected as DDT is still used in SA to control the vectors for malaria (Basel Convention, 2011; Stockholm Convention, 2011; Bouwman *et al*., 2006).

Of the number of sites in the northern section, it was only the sediment from 14_LuvR that was analysed for organic components and was therefore the only site from this area participating in the PCA. In the PCA containing all the quantified variables (Figure 20), 14_LuvR scored with the LMW PAHs, mirex as well as the DDT, HCH and chlordane isomers. This site also scored in factor 3 on the positive side with medium sized dioxins (TCDD/F, and hexa PCDD/Fs; Figure 33). This indicated that this river is mostly influenced by disease vector insecticides, and to some extent, industrial pollution (Lenntech 2011; Ayres, 1992).

5.4.2 Southern Section

SQPs also indicated that at these localities had the highest contamination, pollution and enrichment factors for As. Locality, 5_Mlzi pond, showed the highest Zn concentrations of all the sites and exceeded the Canadian guideline (Figure 63 & 64). The same locality (5_Mlzi pond) had the maximum concentration of Cu which exceeded both the Australian and Canadian guidelines, although it was far below the Dutch guideline (Figure 54 & 55). In terms of the Cu concentrations at 5_Mlzi pond the SQP classified it “very severe” enrichment (Table 14 & 15) with localities 5_Mlzi pond and 2_NkoR classified as “moderate” contamination for Fe and Zn (Table 12).

DDT was the only POP that exceeded both the Australian/New Zealand and Canadian guidelines in the southern part of the KNP (Figure 39). The ΣHMW PAHs had the highest concentrations in the Nkomati River system. Arsenic concentrations exceeded both the Australian and Canadian guideline concentrations at 1_CrocR, 2_NkoR and 3_CrocR with 4_SabR exceeding the previous mentioned guidelines as well as the Dutch guideline (Figure 46), thus indicating that the highest As pollution was within the southern part of the KNP.

The PCAs indicated that the localities in the southern area could be subjected to a variety of pollution could have ranged from industrial, combustion, agricultural as well as mining activities (ATSDR, 2012; Lenntech, 2011; MOEW, 2006; Ayres, 1992).

5.4.1 Eastern section

High concentrations of Al, Fe, Cr, Cu, Ni, V, Zn and Co were quantified at 9_LetR and 6_OliR (Table 18). Sediment from both localities (9_LetR and 6_OliR) exceeded all three the Australian, Canadian and Dutch guideline concentrations for Cr with Ni exceeding the Australian/New Zealand and Dutch guidelines. Copper also exceeded the Australian/New Zealand and Canadian guidelines, and V was greater than the Dutch guideline. Although Al and Fe were very high in this area, no guideline concentrations were available in the selected international guidelines. The SQP indicated that all the aforementioned elements were present in this area at “moderate” to “very severe” concentrations. Arsenic exceeded the Canadian guideline concentration at 9_LetR and 6_OliR with the SQP, indicating that As was within the “moderate” to “severe” concentrations. The As concentrations in the KNP could arise from gold and base metal mining practices in the upstream catchments (Williams, 2001).

Very low concentrations of POPs and PAHs were recorded for the eastern part of the KNP. The elements, however, did contribute more to the pollution load of this area. The localities in the east and south scored high on the negative side of factor 2 with the ΣHMW PAHs in the PCA containing only the PAHs (Figure 35). Factor 4 of the PCA that contained all the compounds had a very strong association with the ΣLMW PAHs at both sites 6_OliR and 9_LetR (Figure 38). The highest concentrations of Ant and BaP were quantified in 7_Po, the isolated pool. This indicates that Ant and BaP was transported most likely to this site via the atmosphere, although some of the other OMP and element concentrations were less in the east than in the southern area.

The fact that these sites were severely influenced by the damming of the water, and consequent sedimentation at these sites, might have amplified the effect that the pollutants had on the crocodiles. The increased sedimentation prevented these pollutants to be flushed away, allowing longer exposure time.

5.4.1 Western Section

The highest concentrations of elements in the western part were Al, Fe, Cr, Cu, Ni, V, Zn and Co (Table 18). The Australian/New Zealand, Canadian and Dutch guidelines were exceeded in terms of Cr (Figures 54 & 55), whereas Cu exceeded the Canadian and Dutch guidelines (Figures 56 & 57). According to the Igeo, Fe classified between "practically" unpolluted to "moderately" polluted for all the sites (Table 11), whereas the EF classified these sites at "moderate" to "moderately severe" enrichment. The Ni concentrations exceeded the Dutch and Australian guidelines (Figure 58 & 59) with V exceeding the Dutch guideline concentration (Figure 61). The SQP calculations indicated that the elements listed above at these sites in the western part of the KNP had "extremely high" to "considerable" concentrations that may influence the health of the ecosystem (Tables 12; 14 & 15). It has been found that higher concentrations of elements especially found in sediment near industrial and urban areas are a good indication of man-made contamination rather than the natural enrichment from geological weathering (Davies et *al.,* 1991).

The highest concentration of PCBs (Figure 40) and ΣLMW PAHs were recorded in the western part of the KNP. From these OMPs, only the ΣPAHs (Figure 43) and ΣLMW PAHs (Figure 44) exceeded the Australian/New Zealand and Dutch guidelines at 19_SelR. PAHs can be released from petrogenic and pyrogenic sources. The ratios indicated that the concentrations at 19_SelR are most likely from petrogenic and grass, wood and coal combustion (Figure 14). These high concentrations were most likely from the large industrial town Phalaborwa outside the KNP border where there are large phosphate, copper, gold and antimony mines (Mbendi, 2014). This was also seen with the PCA that contained all the analysed compounds (Figure 31). Site 19_SelR scored very strongly with ΣLMW PAHs. This indicated that 19_SelR was most likely influenced by industrial combustion and mining activities rather than agricultural pollution.

Generally, there was a distinct pattern in the type of contamination associated with various sources. Possible agricultural and industrial contamination were more localised in the southern part of the KNP, whereas suspected industrial and mining related contamination were largely located in the western section of the KNP border. Lastly, the rivers in the northern part of the KNP indicated contamination mostly associated with malaria control and agricultural activities. The intensity of suspected pollution tended to be more on the southern and western side of the KNP. The rivers that showed the highest concentrations of agricultural pollution were at the Nkomati and Crocodile River

or near Phalaborwa area in the Selati and Olifants Rivers. The highest amount of OMP contamination were at sites 1_Croc, 2_NkoR, 3_CrocR, 14_LuvR, 17_OliR and 19_SelR with the elements being more dominant in the eastern area where the mortalities occurred.

The selected elements were analysed in sediments from all of the sites; however some elements were below the detection limit (Figure 18 & 19). Locality 7_Po had low concentrations of Cu, Co, Cr, Fe and Al indicating that these elements probably occurred naturally in the geology of the area which is in the same geographical area as where the mortalities occurred. However, the rivers that flow towards the Olifants Gorge had higher concentrations compared to this isolated pool, indicating that the elevated concentrations quantified in the rivers are from anthropogenic origin and not from the natural geology.

Chapter 6: Conclusions

This study was conducted to determine if the OMPs and/or selected elements, quantified in the sediment of selected rivers, could indicate possible causes of the mass crocodile mortalities in the Olifants Gorge. In assessing the sediment quality, selected international guidelines were used to determine whether or not the concentrations at the different localities could possibly influence the health of the crocodiles. With the use of selected international SQGs together with the PCA and SQP, it was possible to determine that some of the selected elements could have affected the health of the crocodiles in the Olifants Gorge.

Rivers are a major pathway for the transport of elements (Mohiuddin *et al*., 2010). The use of sediments for element analyses is more effective than water sampling analyses as long term partitioning to the sediment could result into higher concentrations than what may have been measured in the water (Davies et *al.,* 1991). Sediment quality guidelines provide considered benchmarks or so called reference points. Comparing the quantified concentrations to the guideline concentrations assisted in identifying areas of concern (CCME, 2001, Reimann & De Caritat, 2000).

One of the largest contributing factors to the contaminant load in the KNP is most likely the presence of large industrial areas outside the Park's borders especially in the Olifants River (Botha *et al*., 2011; Aston *et al*., 2001; Avenant-Oldewage & Marx, 2000). These industrial areas play a large role in the economy of South Africa and at the same time create large impacts on the environment (Adler *et al*., 2007). This study indicated that the rivers in the KNP are contaminated with different OMPs and elements. The extent of the pollution varied between the different catchment areas. The northern part of the KNP, which includes the Mutale, Limpopo and Luvuvhu Rivers are more subjected to vector control compounds and to a small extent mining pollution. The Crocodile and Nkomati Rivers had less agricultural pollution with more industrial and mining pollution. Lastly, the rivers in the Olifants Catchment had higher mining and industrial pollution compared to all the other areas, with a lesser degree of agricultural pollution.

Because so little is known about crocodile toxicology, the main premise for this study was that the sites where the Crocodiles died should have markedly higher concentrations and exceedances of guideline limits of the OMPs and selected elements compared with other sites in the KNP where crocodiles have not died *en masse*. The crocodiles died in the Olifants Gorge but not in the Nkomati

River which, according to the data, also had a high contaminant load. This can be explained by the difference in the physical characteristics of the two river systems. The Nkomati River is a free-flowing system without any major dams that may restrict the flow of the river. The pollutants are constantly transported to areas downstream of the KNP and towards the Indian Ocean. The Olifants River in the KNP is a free-flowing system until it enters the Olifants Gorge where the water slows down and the sediment that was transported in the water, is deposited, making the pollutants more available in that specific area due to the large Massingir Dam. This for example can be seen with the Cr, Ni, Fe and Co concentrations (Figure 26) at the Nkomati River system, as a dilution effect may have occurred although at the Olifants River there was a possible enrichment effect, possibly due to the differences in the systems.

The *in situ* water quality data indicated fairly stable conditions and as long as the pH remains high (alkaline) then the metals will remain in the sediment and is not expected to be harmful to the environment. As soon as the pH drops, the metals will become more bioavailable and can then cause harm to the environment (DWAF, 1996a; Dallas & Day 2004). Also most elements, especially the heavy metals are more adsorbed and precipitated in sediments at an alkaline pH (DWAF, 1996a)

According to Bouwman (2014), the POPs that were quantified in crocodile eggs were unlikely to have caused the mortalities observed in the Olifants Gorge area. On the other hand, studies on elements conducted on different fish species (*Oreochromis mossambicus* and *Clarias gariepinus*) in the Olifants River indicated that elevated concentrations of elements in the aquatic environment can accumulate in the liver, gills, skin and muscle (Oberholster *et al*., 2012; Avenant-Oldewage & Marx, 2000; Kotze *et al*., 1999).

The hypothesis of this study was: “The concentrations of OMPs and selected elements in the sediment of the Olifants Gorge contributed to the localised mass mortality in its Nile crocodile population. The null-hypothesis (H_0) could not be conclusively discarded as it stated: “There are no marked differences in concentrations of the OMPs and selected elements between sites with affected and non-affected crocodiles. Very low quantities of OMPs were measured even in accordance to the international guidelines and studies conducted in SA (Nieuwoudt *et al*., 2011; Batterman *et al*., 2008; Bornman *et al*., 2007, Bouwman *et* al., 2006; Bouwman *et al*., 1990; Kimbrough, 1985) The OMP concentrations quantified in the Olifants Gorge were the lowest compared to all the other sampled sites. These concentrations were also below the listed international guidelines, thus indicating that

OMPs could not have contributed in any major way to the deaths of the crocodiles. The alternate hypotheses (H_1) stated that the compounds will be more abundant in the areas where the affected crocodiles occurred. This study did indicate that the following elements; Fe, Co, Cu, Cr, Pb, V, As, Se and Ni, may have contributed to the mass fatalities, but in all likelihood did not cause the mortalities, as there were other areas with somewhat lower concentrations but within the same order of magnitude where the crocodiles seemed unaffected. Although high concentrations of Al were quantified in the area where the mortalities occurred, this element is unlikely to have contributed to the deteriorating health of the crocodiles as un-published data indicated that the concentrations of Al in the crocodile tissue of affected crocodiles were very low and comparable with unaffected crocodiles. Because this study was conducted in the major rivers of the KNP, the current contamination load in the sediments of these rivers is now known. This study contributed in identifying areas in the KNP that is specifically contaminated with OMPs and elements. Areas such as the southern (2_NkoR, 1_CrocR and 3_CrocR) as well as the western (19_SelR, 18_OliR and 17_OliR) part of the KNP is of great concern as these localities had the highest contamination load in terms of OMP and elements.

The dead crocodiles were diagnosed with pansteatitis of the lipid tissues. As previously mentioned, pansteatitis is the result of lipid peroxidation due to oxidative damage in an organism. Oxidative damage can be caused by different compounds, such as chlorinated pesticides, PAHs, PCBs, and elements that have been connected to the production of reactive oxygen species (ROS) that causes oxidative damage (Stohs & Bagchi, 1995).

The data indicated that the aforementioned elements had the highest concentrations in the Olifants River as well as the area where the crocodiles died. The only OMPs that were detected at relative high concentrations were some of the PCBs and PAH congeners. Literature indicates that oxidative stress is a result of complex mixtures of pollutants within the aquatic environment (Vasseur & Cossu-Leguille, 2003; Klaunig *et al*., 2010; Klaunig *et al*., 1998; Livingstone, 1993; Kimbrough, 1985). High concentrations of Selenium were also measured at all sampled sites. Although this element was indicated by the SQP as a highly contaminating element, no close association with the sites in the Olifants Gorge were found in the PCA's. Selenium is an essential element needed by plants, animals and humans to maintain a healthy body and ecosystem. High concentrations of Se intake can be associated with a number of diseases and can possibly have adverse effects (WHO, 2011; Lemly 2002). In laboratory studies, research has found that animals with a vitamin E deficiency can be

supplemented with Se as it is an antioxidant and an O_2 scavenger (Hafeman & Hoekstra, 1977). These O_2 scavenger function is to remove ROS, thus protecting the organisms from oxidative stress (Doytte *et al*., 1997; Klaunig *et al*., 1998). Theoretically one would believe that high concentrations of Se in a vitamin E-deficient aquatic animal will be beneficial. However other laboratory studies have shown that excess dietary selenium may not necessarily protect an animal with vitamin E-deficiency as the concentrations needed, can also be lethal to the receiving animal (Levander *et al*., 1977; Menzel, 1979). Despite the presence of cell antioxidant defence systems to counteract the oxidative damage from ROS, oxidative damage can also accumulate during the life cycle (Valko *et al*., 2006). Because crocodiles are long-lived and change their eating habits during their development (Huchzermeyer, 2003; Guggisberg, 1972), the high concentrations of the elements quantified in this study could have affected the health of the crocodiles over an extended time period. The combination of high concentrations of elements (Fe, Co, Cu, Cr, Pb, V, As and Ni) that exceeded the selected guidelines in the Olifants River can also be associated with lipid peroxidation, DNA damage, oxidative stress, and production of free radicals and reactive oxygen species (ROS) (Valko *et al*., 2006; Livingstone, 1993; Stohs & Bgchi, 1995). These elements (Fe, Co, Cu, Cr, Pb, V, As and Ni) may therefore have had a negative influence on the crocodiles in the Olifants Gorge over an extended time period. This, in combination with the dramatic change in the physical environment due to the changes in the dam, could have caused an additional stressor that may have contributed to the decline in the crocodile population in the Olifants Gorge.

A word of caution should be expressed about the use of SQGs from countries where crocodiles do not occur or have not been taken into account when setting these limits. Crocodiles represent a trophic level higher than the normal fish predators (e.g. pike, trout, eel, and bass) common to European, Canadian, and New Zealand's freshwaters. It is not clear whether crocodiles were taken into account with the determination of the Australian guidelines. This additional trophic level could account for additional orders of magnitude in bio-accumulation, meaning that protective guidelines elsewhere might not be protective of ecosystems with "super", long-lived, predators such as the Nile crocodile. Further research is therefore warranted to determine if these guidelines are indeed applicable to freshwater systems that host crocodiles.

6.1 Recommendations

This study was a cross-sectional assessment to determine the concentrations of organic and inorganic compounds within the different rivers in the KNP, a large-scale and long-term project would be more informative. A more extensive monitoring programme is necessary and the different sites should be monitored over a period of two to three years to determine the effect of seasonality on the distribution and fluctuations of the pollutants in the rivers (Van Vuren *et al*., 1994). Seasonal variations could help to determine how the different rivers influence each other at confluences, or how the concentrations of compounds differ before and after heavy rainfall. The sites that are recommended for such a study will need to be the previously sampled sites in the area were the crocodile mortalities occurred (6_OliR, 7_Po, 9_LetR, 16_LetR, 17_OliR, 18_OliR and 19_SelR) as well as an additional site between 16_LetR and 9_LetR. The data between 16_LetR and 9_LetR was contradictory and should be further investigated. Site 16_LetR, in the Lesser Letaba River had low concentrations of elements, whereas site 9-LetR, in the Great Letaba River, had higher concentrations than expected, thus it is possible that there may have been an additional pollution source between 16_LetR and 9_LetR. This will help rule out whether or not the Letaba River did in fact contribute to the quantified concentrations at 6_OliR or was 9_LetR compromised by a back flush after heavy rainfall. Furthermore, the use of modelling programs to determine the high transport rates of both suspension and bed load sediments during flood events (Roca *et al*., 2009) will be a useful exercise.

The following compounds need to be considered: For the POPs, DDT and its metabolites, PeCBs, PCBs, and the 16 priority PAHs. For the elements: Al, As, Ba, Cu, Cr, Co, Fe, Mn, Zn, Ni, Se, and V. The additional analysis such as the different states of Fe, Cr, As and Se should be considered and analysed as these have different toxicities in the environment (ANZECC, 2000) For example hexavalent Cr is the oxidative state of Cr which is very toxic and does not behave toxicologically in the same manner as other heavy metals (Avenant-Oldewage & Marx, 2000). More in depth study of the geology of these areas will be a great advantage in order to determine if these compounds listed are in fact part of the natural geology or caused by anthropogenic activities.

The bioavailability of elements plays an important role in the metal loads of sediments. It would be advantageous if metal speciation analyses can be included in future studies. This will give more clarity on the toxic effects of the metals in the aforementioned sites. Given that most of these compounds are associated as contaminants with industries as well as agricultural activities, it would

be advantageous for future studies to include up- and downstream studies of some of the industrial, mining, and agricultural activities mentioned in this project.

It will also be worthwhile to include the new POPs such as PFOS and the others mentioned, in order to determine their presence in the system (Stockholm Convention, 2011). They were not part of this study as these analyses are very expensive and some of the compounds were only added to the Stockholm's list after the initial study was prepared. Further studies could include the analysis of PAHs in the water, sediment, biota and fauna within the KNP. This will be advantageous because of the widespread presence of PAHs and the high concentrations reported in this study.

The use of a suitable screening tool such as a cell-based bio-assay should also be included to determine if sediments and surface water are capable of eliciting a dioxin-like response or even estrogenic and androgenic responses. If these screening tools are incorporated, it can act as a first step in assessing the presence of xenobiotics. Thereafter, if a site did elicit a response with the bio-assays, further analyses can be performed.

Continued census of the crocodiles and tracking of specific individuals, together with regular monitoring of water chemistry and other dependable environmental factors, will help prevent, or limit, unnatural decline of the crocodile population.

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