Elemental compositions of Nile crocodile tissues *(Crocodylus niloticus)* from the Kruger National Park

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ABSTRACT

A SANParks programme assessed the health of wild Nile crocodiles in the Kruger National Park (KNP) during 2010, with appropriate permits and ethical clearance. Samples were collected from sixteen shot crocodiles (eight males and eight females) from the Sabie, Olifants, Crocodile, Levuvhu, Shingwedzi, Nwaswitsontso, and Letaba rivers, collected by SANParks staff. The samples were collected to obtain more information on the biology, pathology, and ecotoxicology of wild crocodiles, based on the 2008/09 mass crocodile mortality events in the Olifants and Letaba rivers. The aim of the current study was to provide an assessment of the elemental composition of legacy samples already collected and analysed. To achieve this aim, the elemental composition of five different tissues of 16 Nile crocodiles (*Crocodylus niloticus*) collected in the Kruger National Park was measured and assessed. Additionally, it was determined which tissue(s) would be representative for possible future biopsies from catch and release crocodiles to assess any changes in environmental concentrations. Muscle (from the tail) and tail fat tissue is relatively easily assessable after live capture, while liver, kidney and abdominal fat are more difficult to sample.

Most of the elements that were not statistically different between muscle and the other tissues were with kidney (31 elements) and liver (34 elements) tissues. Muscle tissue had no differences with 21 elements for both tail fat and abdominal fat. Kidney and liver shared 38 elements with no significant different concentrations, but only 10 and 16 with abdominal fat and tail fat, respectively. Only three elements were comparable between liver and tail fat, but had no significant different concentrations for 16 elements with abdominal fat. Tail fat and abdominal fat, on the other hand, had no differences whatsoever for any of the 47 elements thus compared. There were surprisingly little direct associations of concentrations between mass and length. This may be due to differences in individual life histories of long-lived animals and feeding preferences.

It is clear that there is little pattern of prediction or consistency of elemental concentrations between tissues, except between the two fatty tissues, and kidney and liver to some extent. To a lesser extent, muscle and liver, and muscle and kidney had corresponding concentrations. These tissues may therefore be useful when taking biopsies from live animals to determine pollutant loads in the crocodiles.

There were few patterns to discern, but mass, length, and sex did not discriminate between elemental concentrations with any confidence in any tissue. Due to the large variations in concentrations, proper scientific studies using live-captured animals would need a balanced sampling design. The numbers of individuals needed for such a scientific study for the targeted elements and organs can be calculated from my data. However, it seems that for regular and general surveys, the capture and biopsy of fewer animals with less consideration for mass, length and sex, may be appropriate.

This investigation provides the largest elemental concentration dataset and baseline for any African crocodile. The data and interpretations will assist in monitoring changes and comparisons with other regions and contribute to a better understanding of the biology, ecology, and threats faced by these apex predators, the largest in Africa.

Keywords: Nile crocodile, *Crocodylus niloticus*, Kruger National Park, elemental compositions, biopsies, elemental concentrations.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Elemental compositions of Nile crocodile tissues (*Crocodylus niloticus*) from the Kruger National Park

CHAPTER 1: INTRODUCTION & LITERATURE REVIEW

During the winters of 2008 to 2009, the Kruger National Park (KNP), an internationally renowned conservation area, experienced a phenomenon where Nile crocodiles (*Crocodylus niloticus*) numbers declined rapidly due to a mass die-off at the confluence of the Olifants and Letaba rivers (Department of Water Affairs and Forestry, 2006; Botha *et al*., 2011; Ferreira and Pienaar, 2011; Woodborne *et al*., 2012; Downs *et al*., 2015). Following the mass crocodile die-offs during the winters of 2008 and 2009, more information on this species was required urgently. The North West University (NWU) became involved at the request of SANParks Scientific Services to look into possible causes. Generally, some causes of the decline of Nile crocodile populations include the following:

- Loss of breeding habitat (Leslie and Spotila, 2001);
- Exploitation (Bourquin and Leslie, 2011);
- Environmental pollution (Botha *et al*., 2011); and
- Disease (Ferreira and Pienaar, 2011).

During the course of the operational investigations into the mass die-offs, it became clear that there was not enough information on what healthy wild crocodiles looked like compared with diseased crocodiles. There were plenty of dead crocodiles without obvious cause of death, but comparisons with healthy crocodiles were difficult. Although we took biopsy samples from captured live crocodiles, the sample numbers and masses that became available were too small for proper investigations.

There followed a SANParks programme that assessed the health of wild crocodiles in the KNP during 2010, with appropriate permits and ethical clearance (Threatened or Protected Species Registration South African National [SAN] Parks, S 21201). Samples were collected from sixteen shot crocodiles (eight males and eight females) from the Sabie, Olifants, Crocodile, Levuvhu, Shingwedzi, Nwaswitsontso, and Letaba rivers, collected by SANParks staff (discussed in more detail in Fig. 3.1). The samples were collected to obtain more information on, *inter alia*, the biology, pathology, and ecotoxicology of wild crocodiles, based on the 2008 and 2009 mass crocodile mortality in the Olifants and Letaba rivers. The 2010 Crocodile Survey project provided a unique sample and dataset of wild crocodiles since most comparable data and information comes from captive bred crocodiles. The elemental composition of five tissues (abdominal fat, muscle, kidney, liver and tail fat) sampled during the 2010 crocodile survey were analysed for elemental composition in 2011. This study is focussed on analysing the data and comparing this data with other studies.

1.1 THIS STUDY

The present study is an assessment of the elemental compositions of five tissues of Nile crocodiles (abdominal fat, muscle, kidney, liver, and tail fat) sampled during 2010. These samples were collected to obtain more information on the biology, pathology, and ecotoxicology of healthy wild crocodiles, since no baseline exists on what healthy wild crocodiles from the KNP look like. To some extent, this study will mirror that of Nilsen *et al*. (2017), who also determined elemental concentrations in blood, scutes, muscle, and liver, while we used muscle, liver, kidney, tail fat, and abdominal fat.

1.2 AIMS & OBJECTIVES

I aim to provide an assessment of the elemental composition of legacy samples already collected and analysed. No such study has ever been conducted on this scale for any of the African crocodiles. To achieve this aim, I will:

- Measure and assess the elemental composition of five different tissues of 16 Nile crocodiles (*Crocodylus niloticus*) collected in the Kruger National Park.
- Determine which tissue(s) would be representative for possible future biopsies from catch and release crocodiles to assess any changes in environmental concentrations. Muscle (from the tail) and tail fat tissue is relatively easily assessable after live capture, while liver, kidney and abdominal fat are more difficult to sample.

Hypotheses:

Selected elemental concentrations in crocodile muscle and/or tail fat biopsied tissues will allow inference of concentrations in other tissues.

1.3 REPTILES AND CROCODILIANS

Due to habitat loss and degradation, invasive species, pollution, disease, unsustainable use of recourses, and global climatic change, reptile species are declining on a global scale (Whitefield Gibbons *et al*., 2000). Together with lizards, snakes, tuataras, and chelonians, crocodilians form part of the class Reptilia also known as reptiles. These classifications are based on the exothermic characteristics and skin style these animals share (Huchzermeyer, 2003). Nevertheless, a few aspects separate crocodilians (Order Crocodilia) from the rest of the Reptilia including, heart morphology, the presence or absence of a fat body, and behaviour during parental care. The largest extant freshwater reptilians are collectively known as crocodilians (Huchzermeyer, 2003).

1.3.1 Reptiles as bio-indicators

Reptiles can be used as bio-indicators for numerous contaminants in the natural environment, especially for mercury (Hg) (Schneider *et al*., 2013). One of the reasons is that many reptiles are long-lived animals that could lead to accumulation of contaminants in their tissues over long periods. This accumulation occurs from exposure to and uptake of contaminants in sediments, air, water, and food (Schneider *et al*., 2013). The large range of diets of reptiles also makes them good bio-indicators, but this aspect is highly dependent of their position in the trophic web. For instance, alligators and crocodiles are bio-indicators because they are top predators. However, to exhibit all the requirements for being useful bio-indicators, they must also exhibit a correlation between their tissue contaminant concentration and the concentration of specific contaminant in the surrounding environment. This correlation must also be comparable among individuals of the species, between sites, and under any condition (Burger *et al.,* 2006).

Reptiles possess traits that make them good bio-indicators (Schneider *et al*., 2013). A list of these follows, but not all of them are applicable to all reptiles.

- High energy conversion efficiencies i.e. the amount of ingested energy converted to biomass;
- Ingestion of large meals at infrequent intervals and therefore a dietary pattern that would result in pulse exposure to contaminants (Burger *et al*., 2002);
- The enzymatic detoxification system of reptiles is less developed than in their endothermic counterparts (Burger *et al*., 2002). Existing literature advises that reptiles have the major components of the vertebrate mixed function oxygenase system, but the concentrations

and activity of these components are often lower in reptiles than in other vertebrates (Burger *et al*., 2002);

- Can accumulate contaminants;
- Are relatively sessile, but crocodiles can be quite mobile;
- Are found on all continents except Antarctica;
- Most are relatively easy to collect; and
- Sufficient tissues and eggs are available for analysis for the larger species.

The accumulation of contaminants in reptiles such as crocodiles, turtles, and to some extent, snakes, is a serious health issue as humans have been consuming reptiles for generations (Schneider *et al*., 2013). This way, humans can accumulate contaminants through their diet that include reptilian tissues and eggs.

Within the Order Crocodilia, there are three extant families: Gavialidae, Alligatoridae, and Crocodylidae, with 23 extant species (Please note that there are several different classification systems). The subfamily Crocodylinae contains three genera (Huchzermeyer, 2003):

- *Crocodylus*, also referred to as the true crocodiles containing 12 species;
- *Osteolaemus*, two species, and
- *Mecistops*, one species.

As listed in Huchzermeyer (2003), the genus *Crocodylus* includes the following species:

- *C. rhombifer* - Cuban crocodile
- *C. moreletii* - Morelet's crocodile
- *C. acutus* - American crocodile
- *C. cataphractus* - African slendersnouted crocodile
- *C. niloticus* - Nile crocodile
- *C. intermedius* - Orinoco crocodile
- *C. porosus* - Indo-Pacific crocodile (Saltwater crocodiles)
- *C. johnsoni* - Johnston's crocodiie
- *C. palustris* - Mugger
- *C. siamensis* - Siamese crocodile
- *C. mindorensis* - Philippine crocodile
- *C. novaeguineae* - New Guinea crocodile
- *C. raninus* - Bornean crocodile

Crocodilians are robust animals and have proven a significant ability to recover from severely diminished population numbers (Webb *et al*., 2001). As long-lived and apex predators, crocodilians (gharials, caimans, alligators and crocodiles) in particular are first-rate subjects for researching environmental and ecosystem health (Campbell, 2003; Milnes and Guillette, 2008).

1.4 THE NILE CROCODILE (*Crocodylus niloticus*)

The Nile crocodile scientific classification is provided below (Huchzermeyer, 2003) but note that other classification systems may differ:

- Kingdom: Animalia
- Phylum: Chordata
- Class: Reptilia
- Order: Crocodilia
- Family: Crocodylidae
- Genus: Crocodylus
- Species: *C. niloticus*

The Nile crocodile (*Crocodylus niloticus*) is one of largest crocodilians after the Saltwater crocodile (*Crocodylus porosus*). These giants are the largest freshwater predators in Africa. The Nile crocodile is also Africa's largest non-marine predator. An adult crocodile can reach a maximum size of about six meters, a mass of up to 780 kg, and live up to approximately 45 years in the wild (NGS, 2018). The body of a Nile crocodile is dark olive to grey coloured with dark cross bands and markings (Huchzermeyer, 2003). They are covered with thick, protective scales consisting of keratin, except on their backs where the scales are strengthened by bony plates called osteoderms (Burnie, 2004). These attributes contribute to these animals' abilities to tolerate varying degrees of salinity and therefore has allowed them to spread across the African continent to different river systems and even as far as islands (Huchzermeyer, 2003).

Nile crocodiles are apex predators and therefore play an important role in the maintenance of freshwater ecosystem structure and functions (Ross, 1998; Leslie and Spotila, 2001; Glen *et al*., 2007). Being apex, large predators, crocodiles can be described as important 'umbrella' species for the conservation of freshwater ecosystems (Seddon and Leech, 2008). Diets of Nile crocodiles vary as they are considered opportunistic hunters. Juvenile crocodiles eat mostly aquatic invertebrates (Platt *et al*., 2002) and move on to larger vertebrates as they reach adulthood when their diet mostly consists of freshwater fish and larger terrestrial animals inclusive of humans and livestock (Cott, 1961; Ross and Garnett, 1992; Tucker *et al*., 1996; Wallace and Leslie, 2008; Radloff *et al*., 2012).

Nile crocodiles are exothermic animals and tend to maintain their internal body temperature by means of basking in the sun or cooling down in the deep water (Thorbjarnarson, 1999). Nile crocodiles are considered valuable assets economically and ecologically. Crocodile meat and skins command high prices on the international market and this maintains the farming of captive crocodiles today (Thorbjarnarson, 1999). Being a valuable resource, Nile crocodile population numbers have overall increased, despite die-offs in the KNP, and is still listed as "Least Concern" by the International Union for Conservation of Nature (IUCN) (IUCN, 2018).

Figure 1.1: A Nile crocodile (*Crocodylus niloticus*) basking in the sun. Prominent scales visible. (Photo taken at Malelane Gate, Kruger National Park, 2016. D. van der Westhuizen)

Crocodiles do not possess any sex chromosomes and instead, the sex of the crocodile is determined by the temperature of the nest (Huchzermeyer, 2003). Sexual dimorphism is prevalent as females are almost 30% smaller than males; however, it remains difficult to sex crocodiles externally.

There are a few differences between crocodiles and alligators concerning their anatomy and physiology. However, the three most important and obvious differences are listed below (Huchzermeyer, 2003):

- **Cold resistance**: alligators tend to be more cold resistant than crocodiles and caimans. This can be geographically explained simply by the distribution of these animals. Alligators are found much further north than caimans and crocodiles.
- **Teeth:** crocodiles' fourth mandibular tooth fits into a notch in the upper jaw and thus remains visible even when the mouth is closed. Alligators and caimans' lower jaw teeth fit into pits in the upper jaw, resulting in no visible teeth when the mouth is closed.
- **Sensory pits:** crocodiles and gharials possess sensory pits in their ventral scales which are absent in scales of alligators and caimans.

1.4.1 Crocodilian Anatomy:

The anatomy of crocodiles is important not only to identify them amongst other crocodilians but also to understand the processes of the body and contributes to identification of organs during post-mortem autopsies.

Muscles

Long dorsal muscles within the trunk are extend all the way into the tail. These muscles a used to provide the power to swim, together with the ventral tail muscles (Huchzermeyer, 2003). Tail muscle was sampled during this study.

Liver

The liver is located between the two transverse membranes. These are all located in the hepatic coelom. The liver consists of two lobes of almost similar size, although the right lobe often is slightly larger than the left lobe (Huchzermeyer, 2003).

Kidney

Located in the furthest posterior part of the abdomen, attached to the abdomen wall, the two kidneys can be found. The multiple folds which contain the kidney results in a triangular shape on transverse section (Huchzermeyer, 2003).

Fat tissues

Fat cells usually have large nuclei indicating that they have the ability to activate rapidly stored fat. Supplementary fat may be stored in somatic fat cells between the muscles in the tail. The composition of fatty acids usually depends on the sources of fat in the food that is consumed (Huchzermeyer, 2003). There are three main fatty tissues in crocodilians; subcutaneous fat, tail fat, and the fat body. Previous results showed that the three adipose tissue types did not differ in fatty acid composition (Osthoff *et al*., 2010).

Subcutaneous fat

Subcutaneous fat refers to the layer of fat tissues located directly under the skin of the crocodile. Nile crocodiles are exothermic animals, meaning that they do not need fat for insulation of their bodies, as subcutaneous fat layers would hinder thermoregulation (Huchzermeyer, 2003).

Fat body (abdominal fat)

Steatotheca is the name for the abdominal fat body in crocodiles and other reptilians (Huchzermeyer, 2003; Osthoff *et al.*, 2010). The fat body is a white visceral fat body in Nile crocodiles which size relates to the nutritional state of the crocodile. It is located close the heart, and about the same size. The size may depend on the condition of the crocodile (Huchzermeyer, 2003; Osthoff *et al*., 2010).

Tail fat

The amount of tail fat in crocodiles is related to the nutritional state of the crocodile (Osthoff *et al*., 2010). Few studies have been done on this fat tissue and therefore more research is needed on this topic.

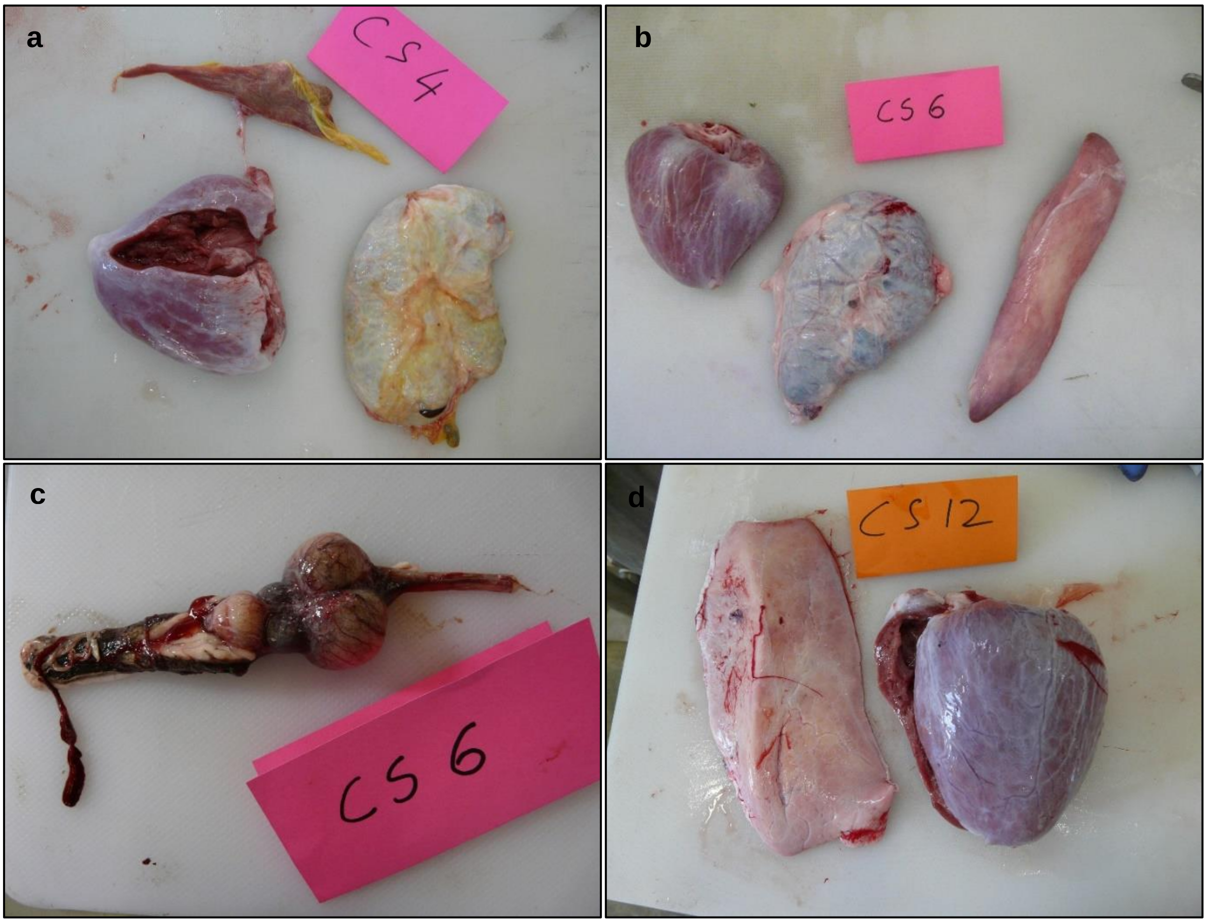

Figure 1.2: Different organs dissected from sampled crocodiles for analysis of elemental composition. (a) Heart, fat body, and liver of CS 04. (b) Heart, liver, and fat body of CS 06. (c) brain of CS 06. (d) Heart and spleen of CS 12.

1.5 PANSTEATITIS

1.5.1 Disease

Pansteatitis is an inflammatory reaction that affects fat depots which leads to necrosis (damage to fat cells caused by disease or infections) and hardening (also known as saponification) of the fat cells. The condition is easily recognisable on gross morphology by yellowish-brown colouration of the normally white fat (Huchzermeyer, 2003). Inflammation and discolouration of the fat can be the effect from a lack in vitamin E (Osthoff *et al*., 2010), which can result in a deteriorating the cycle due to malnutrition of the animal. This condition makes the animal stiff and can cause severe cell-damage and finally death in many species, including Nile crocodiles (Roberts *et al*., 1979; Herman and Kircheis, 1985; Ladds *et al.,* 1995; Wong *et al*., 1999; Niza *et al*., 2003; Goodwin, 2006; Roberts and Agius, 2008; Neagari *et al*., 2011). This disease is relatively rare (Woodborne *et al*., 2012). However, pansteatitis is presumed to have caused the death of most of the Nile crocodile population at the Lake Loskop in the

Upper Olifants Valley (Ashton, 2010; Botha *et al*., 2011). It leaves the crocodiles rigid and indolent, unable to swim, walk, or hunt. Death is believed to be ultimately caused by starvation or drowning (Woodborne *et al*., 2012). The sharptooth catfish (*Clarius gariepinus*) has also been diagnosed with pansteatitis and these incidents are discussed below.

Even though the origin of the condition may be diet related, this co-occurrence is not related to a trophic association, as the disease in one organism does not necessarily cause similar effects in those that consume them (Woodborne *et al*., 2012). Pansteatitis in crocodiles, however might be caused by the consumption of rotten, deceased fish (Ladds *et al*., 1995; Huchzermeyer, 2003). The cause of pansteatitis may be due to the fundamentally changed fatty composition of rotten, rancid fish (Brooks *et al*., 1985; Goodwin, 2006) rather than pre-existing pansteatitis in the fish.

1.5.2 The 2008/2009 crocodile mortalities in the Kruger National Park (KNP)

During the winters of 2008 to 2009, the KNP experienced an occurrence where Nile crocodiles (*C. niloticus*) died (Osthoff *et al*., 2010; Ferreira and Pienaar, 2011; Woodborne *et al*., 2012). Indicated by post mortems, the mass die-off of crocodiles that occurred in rivers including Sabie River, as well as the confluence of the Letaba River and Olifants River were caused by pansteatitis (Woodborne *et al*., 2012). This disease killed 170 crocodiles in the Olifants River Gorge alone (Bouwman *et al*., 2014). Due to the remote character of the area, the rough estimated total number of crocodile die-offs is probably closer to 500 that are half of the entire estimated population of about 1000 crocodiles. The incidents reported in the KNP initiated a series of research to elucidate the cause of pansteatitis (Ashton, 2010; Osthoff *et al*., 2010; Ferreira and Pienaar, 2011; Woodborne *et al*., 2012; Bouwman *et al*., 2014; Osthoff *et al*., 2014; Du Preez *et al*., 2016; Gerber *et al*., 2017).

Figure 1.3: Mass Nile crocodile die-offs in the Kruger National Park during 2008 and 2009. (a) Crocodile carcass floating in river after death by pansteatitis. (b) Dissection of crocodile carcasses by SANParks' staff.

As the cause or causes of this condition in the KNP crocodiles have yet been defined, some suspected causes and possible contributing factors are described below (Du Preez *et al*., 2016).

- Microcystins from cyanobacteria (Myburgh and Botha, 2009).
- Pollutants settling out of the water as the river slows down entering the Massingir Dam in Mozambique (Osthoff *et al*., 2010).
- Crocodiles consuming rancid fish (Ashton 2010; Huchzermeyer *et al*., 2011).
- Environmental decline and pollution (Ferreira and Pienaar, 2011).
- Crocodiles feeding on steatitic African Sharp-toothed Catfish (*Clarias garipienus)* (Huchzermeyer *et al*., 2011).
- Ecosystem changes combined with extra-limital fish species as vector of the cause (Woodborne *et al*., 2012).
- High concentrations of aluminium in the fat of the Nile tilapia (*Oreochromus mossambicus*) that may interfere with cellular metabolism such as lipid-peroxidation (Oberholster *et al*., 2012).
- Seasonal change in diet due to potamodromic migrations of the invasive Silver carp (*Hypophthalmichthys molitrix*) that has a fatty acid composition different from indigenous fish (Huchzermeyer, 2012).

- Lipid peroxidation due to oxidative damage in an organism (Kotin and Falk, 1963).

1.6 METALLIC ELEMENTS (METALS AND METALLOIDS)

Almost all elements are found in the natural environment. They are part of the building blocks of our world (Newman, 2010). Metallic elements refer to the collection of chemical elements that share properties like shiny in solid form, poses a high melting point, conductors of heat and electricity, usually solid forms at room temperature, low ionization energies, low electronegativity, malleable, ductile, usually have high density, corrodes when introduced to air or seawater, and loses electrons in reactions (Newman, 2010). These elements, as displayed in the periodic table (Table 1.1), are located in groups 1-2 and 4-16 in the periodic table. Please note that I do consider selenium (Se) as well, but is not considered a metal or metalloid.

Most metallic elements form part of the Earth's crust. These elements include silver (Ag), nickel (Ni), copper (Cu), and gold (Au). Mining and many other anthropogenic activities are sources of most metallic elements in the natural environment, above their natural background. Physiologically, metallic elements can be divided into two groups, namely those that are essential and non-essential for life. Essential elements include iron (Fe) and magnesium (Mg). Non-essential elements include mercury (Hg), lead (Pb), and cadmium (Cd). In the following paragraphs, the metallic elements that were analyzed in this study will be discussed. More information on each element is given in Table 1.2 including symbol, atomic number, group, elemental category, and atomic weight (Emsley, 2003; Newman, 2010; EniG. Periodic Table of the Elements, 2018).

Many metallic elements are toxic in the environmental when occurring at excess concentrations. Humans and wildlife that are exposed to toxic elements face two types of poisoning, namely acute poisoning and chronic exposure (Emsley, 2003; Newman, 2010). When organisms are exposed to high concentrations over a short period, acute poisoning may occur, often resulting in death. Chronic poisoning refers to the exposure to a toxicant with low concentrations but over a longer period, often resulting in extended disease. Elements such as Cd, Pb, Zn, Cr, and Hg can have toxicity in humans even at trace amounts found in pipes, drains, old paint supplies, batteries, and pesticides. The result hereof is mostly sub-lethal (Emsley, 2003; Newman, 2010). Toxic elements are discussed in more detail in the sections below.

Rare earth metals are an important category to consider in ecotoxicology. They are used, inter alia, in modern day computers, television screens, cell phones, and optical networks. The mining of these rare earth metals as well as the processing and disposal thereof has increased significantly, as the use of technology expands (Newman, 2015).

Table 1-1: Periodic table of elements (EniG. Periodic Table of the Elements. 2018)

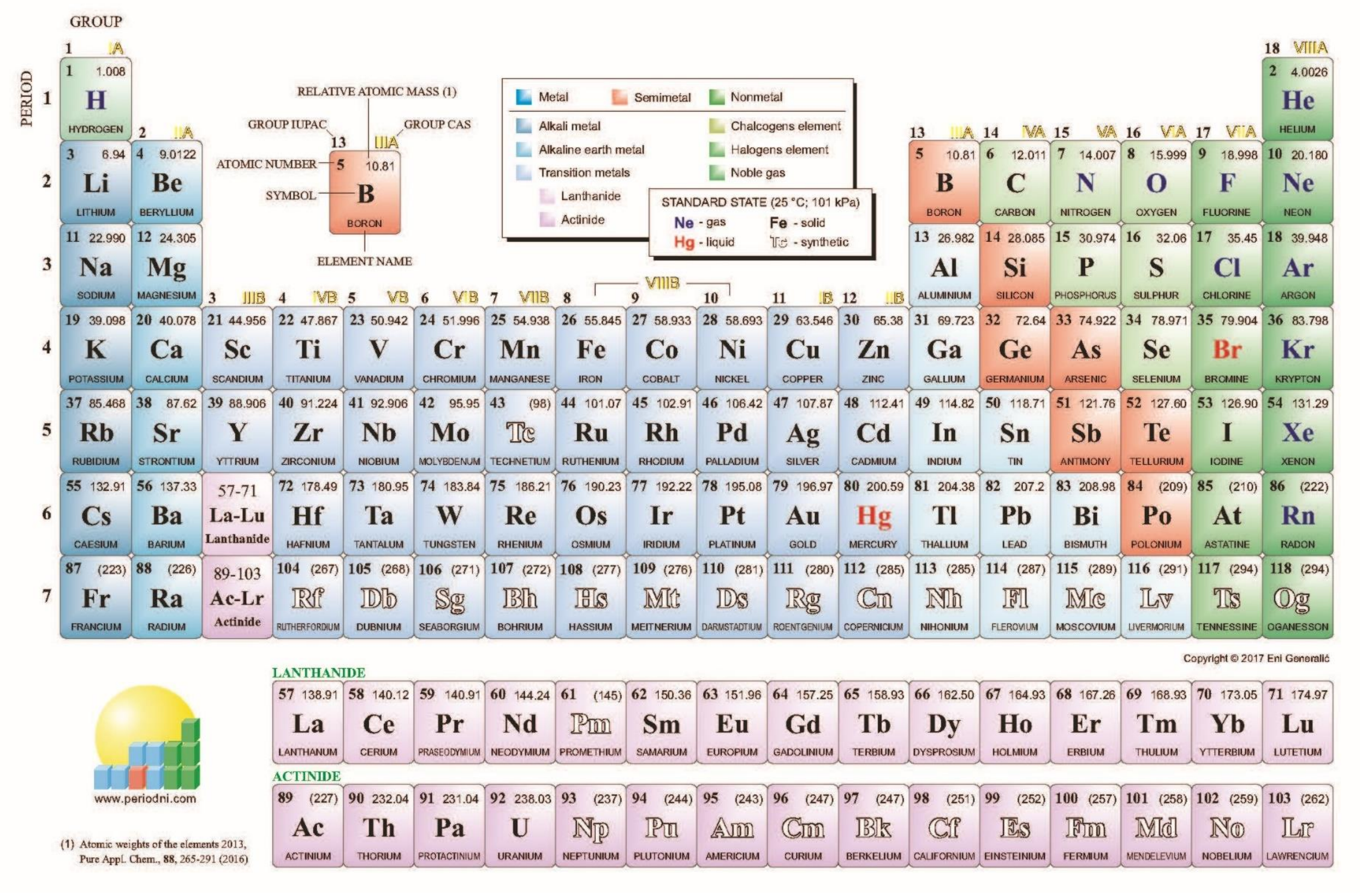

Table 1-2: All elements that were measured, with details.

Element	Symbol	Atomic number	Group	Elemental category	Atomic Weight
Lithium	Li	3	Group 1	Alkali metal	6.939
Beryllium	Be	4	Group 2	Alkaline Earth Metals	9.0122
Boron	B	5	Group 13	Metalloids	10.811
Magnesium	Mg	12	Group 2	Alkaline Earth Metals	24.305
Aluminium	Al	13	Group 13	Post-Transitional Metals	26.982
Silicon	Si	14	Group 14	Metalloids	28.086
Scandium	Sc	21	Group 3	Transitional Elements	44.956
Titanium	Ti	22	Group 4	Transitional Elements	47.867
Vanadium	V	23	Group 5	Transitional Elements	50.942
Chromium	Cr	24	Group 6	Transitional Elements	51.996
Manganese	Mn	25	Group 7	Transitional Elements	54.938
Iron	Fe	26	Group 8	Transitional Elements	55.845
Cobalt	Co	27	Group 9	Transitional Elements	58.933
Nickel	Ni	28	Group 10	Transitional Elements	58.693
Copper	Cu	29	Group 11	Transitional Elements	63.546
Zinc	Zn	30	Group 12	Transitional Elements	65.39
Gallium	Ga	31	Group13	Post-Transitional Metals	69.723
Germanium	Ge	32	Group 14	Metalloids	72.59
Arsenic	As	33	Group 15	Metalloids	74.922
Selenium	Se	34	Group 16	Other Non-Metals	78.96
Bromine	Br	35	Group17	Halogens	79.904
Rubidium	Rb	37	Group 1	Alkali metal	85.468
Strontium	Sr	38	Group 2	Alkaline Earth Metals	87.62
Yttrium	Y	39	Group 3	Transitional Elements	88.906
Zirconium	Zr	40	Group 4	Transitional Elements	91.224
Molybdenum	Mo	42	Group 6	Transitional Elements	95.94
Palladium	Pd	46	Group 10	Transitional Elements	106.42
Cadmium	Cd	48	Group 12	Transitional Elements	112.41
Indium	In	49	Group 13	Post-Transitional Metals	114.82
Tin	Sn	50	Group 14	Post-Transitional Metals	118.71
Antimony	Sb	51	Group 15	Metalloids	121.76
Iodine	I	53	Group 17	Halogens	126.9
Caesium	Cs	55	Group 1	Alkali metal	132.91
Barium	Ba	56	Group 2	Alkaline Earth Metals	137.33
Hafnium	Hf	72	Group 4	Transitional Elements	178.49
Tantalum	Ta	73	Group 5	Transitional Elements	180.95
Tungsten	W	74	Group 6	Transitional Elements	183.84
Platinum	Pt	78	Group 10	Transitional Elements	195.08
Gold	Au	79	Group 11	Transitional Elements	196.97
Mercury	Hg	80	Group 12	Transitional Elements	200.59
Thallium	Tl	81	Group 13	Post-Transitional Metals	204.38
Lead	Pb	82	Group 14	Post-Transitional Metals	207.2
Bismuth	Bi	83	Group 15	Post-Transitional Metals	208.98
Uranium	U	92	Group n/a	Actinoides	238.03

1.6.1 Lithium (Li)

Lithium is a soft, silvery-white alkali metal that is one of the lightest of all metals. It reacts robustly with water, found in minor amounts in almost all rocks, and is common in spring waters. Whether on land or in the sea, Li poses little threat to plants or animals as it only occurs in trace amounts, though its physiological functions are uncertain. The physiological action of Li is unknown, but it might help improve mental disorders by increasing the activity of chemical messengers in the brain (Emsley, 2003).

1.6.2 Beryllium (Be)

Beryllium is of a shiny silvery colour and can be a serious health issue for exposed workers that can lead to the development of the chronic beryllium disease (CBD) (Emsley, 2003). Be is chemically similar to magnesium (Mg) as they are both in group 2 of the periodic table. The human toxicity of finely divided Be (dust or powder, mainly encountered in industrial settings where beryllium is produced or machined) is well documented.

1.6.3 Boron (B)

Boron is characterised as a dark powder that is unreactive to oxygen, water, acids, and alkalis. B compounds may be used in treatment of brain tumours (Emsley, 2003). Borates have low toxicity in mammals but are toxic to arthropods. B is therefore commonly used as an insecticide (Emsley, 2003). B is an essential plant nutrient, increasing crop production while protecting plants from pests. B compounds such as borax and boric acid are used as fertilizers in agriculture. B compounds also have a strengthening effect in the cell walls of plants. B is necessary for plant growth, but an excess of boron is toxic to plants (Emsley, 2003).

1.6.4 Magnesium (Mg)

Magnesium is a silvery-white, soft-solid metal and carries a close resemblance to the other elements in the second column (group 2) of the periodic table (Emsley, 2003). It is the third most abundant element in the Earth's crust. Mg plays an important role in the chlorophyll molecule in plants. Mg is highly flammable and is almost impossible to extinguish once it catches fire (Emsley, 2003). An overdose from only nutritive sources is improbable because excess magnesium in the blood is filtered by the kidneys almost straight away. Overdose is more probable in the presence of weakened renal function. Mg is used and sourced in fertilizers, plastic, cattle feed, mining, alloys, electronics, batteries, and beverage cans. Mg is categorised as an essential element for life but at high intakes it can cause muscle weakness, lethargy, and confusion (Emsley, 2003).

1.6.5 Aluminium (Al)

Aluminium in its purest form is a silvery-white, malleable, nonmagnetic, and ductile metal. It is the most abundant metal (Newman, 2015) in the earth's crust, and the third most abundant element overall (Emsley, 2003). Although Al is not essential for life, some plants absorb it and can make up to 1% of the dry weight (Emsley, 2003). Once entered into the blood stream, Al is difficult to remove from the body. Unusually high concentrations can cause the death of aquatic species (Newman, 2015). Although Al is abundant in nature, excess can be caused by sources like mining, and use of aluminium foam.

1.6.6 Silicon (Si)

Silicon has a hard and brittle crystalline solid appearance with a blue-grey metallic shine and is relatively unreactive (Emsley, 2003). It is the second most prevalent element in soil. Si occurs in all tissues but does not accumulate in any particular organ (Lenntech, 2018). Si is an essential element in biology, although only minor amounts are required by animals. However, it is considered a non-essential but beneficial nutrient for plants as it improves pest and drought resistance (Richmond and Sussman, 2003).

1.6.7 Scandium (Sc)

Scandium has a silvery-white metallic appearance. When exposed to air, it becomes a yellowish-pink colour. It was discovered in 1879 in Scandinavia, hence the name. It is rarely found in the natural environment. It has no known biological role and only trace amount can be located in the food chain. Sc however, might, pose a threat to the liver (Lenntech, 2018). Environmental sources of Sc include petrol-producing industries. Sc causes damage to cell membranes in water animals (Lenntech, 2018).

1.6.8 Titanium (Ti)

Titanium is a soft, silvery-white metal that is ten times more abundant than silver (Emsley, 2003). A dose of Prussian blue ink is the antidote for Ti poisoning (Emsley, 2003). Ti is resistant to corrosion in seawater and chlorine. The two most useful properties of the metal are corrosion resistance and strength-to-density ratio, the highest of any metallic element. Sources of release to the body and environment include medical implants and devices, aircraft industry, chemical plants, power plants, alloys, oil rings, ships, paint, plastics and papers, and ceramics and enamels (Lenntech, 2018). Ti is non-poisonous even in large doses and does not play any biological role (Emsley, 2003).

1.6.9 Vanadium (V)

Vanadium is a hard, silvery-grey, ductile, and malleable transition metal (Emsley, 2003). The elemental metal is rarely found in nature. South Africa is one of the largest sources of V in the world (Emsley, 2003; Lenntech, 2018). V is usually obtained as a by-product of other ores and only as little as 2% V can increase the strength of steel (Emsley, 2003). V is an essential element to humans and certain other species but it is important to know that V in certain forms can cause health problems (Lenntech, 2018). Environmental sources of V include steel production, a by-product of other ores, alloys, and polymer production (Emsley, 2003).

1.6.10 Chromium (Cr)

Chromium has a silvery colour and is hard. It is found in the precious gem, alexandrite, and gives the gem its blue-green colour. Anthropogenic sources of Cr include mining, pigments, wood preserves, tanning, anticorrosives, and the production of refractory bricks (Newman, 2015). Cr is considered a toxic and carcinogenic (Newman, 2015) element although essential in the adequate concentrations (Emsley, 2003; Lenntech, 2018). Cr toxicity include health effects like respiratory problems, weakened immune, lung cancer, alteration of genetic material and even death (Lenntech, 2018).

1.6.11 Manganese (Mn)

Manganese is a hard, silvery, and brittle metal (Emsley, 2003). Mn ores are mined and South Africa and is one of the major countries in this production. The ocean floor is where the most Mn is located (Emsley, 2003). Environmental Mn sources include fertilisers, animal feed, mining, rubber production, and glass production. It is considered an essential element but in certain forms and in high concentrations it can be a health hazard (Emsley, 2003; Lenntech, 2018).

1.6.12 Iron (Fe)

Iron is a lustrous, silvery, and soft metal. It is therefore easily workable (Emsley, 2003). There is an entire part of human history named after Fe, namely The Iron Age. Fe is the most mined ore in the whole world (Emsley, 2003). Fe is essential to all life but, as so many other elements, are poisonous and toxic in excess and high concentrations. Sources of Fe include pharmaceuticals, industries, construction, and manufacturing of weapons, jewellery, and cutlery (Emsley, 2003).

1.6.13 Cobalt (Co)

Cobalt is a hard lustrous, silvery blue metal (Emsley, 2003). The most Co is located in the earth's core and was once commonly used for making "invisible ink". Interestingly, Co remains invisible, until it is heated (Emsley, 2003). Co is an essential element, but in high concentrations is subject to health problems. Environmental sources of Co include mining, colour agents, and food preservatives (Emsley, 2003; Lenntech, 2018).

1.6.14 Nickel (Ni)

Nickel is silvery coloured, lustrous, malleable, and ductile metal. The ‘nickel itch” is a condition of inflammation of the skin, also known as dermatitis, that is caused by unproductive contact with the skin (Emsley, 2003). Most Ni is found in the iron-nickel molten core of the Earth and is therefore mostly inaccessible (Emsley, 2003). Ni is toxic and carcinogenic (Newman, 2015). Environmental sources thereof include alloys (stainless steel, and Ni plating), battery production, mining, food and chemical processing industries, paints, and coinage (Emsley, 2003; Newman, 2015).

1.6.15 Copper (Cu)

Copper is a malleable and ductile metal of orange-gold colour. Cu is the second best electrical conductor and a component of our diets (Emsley, 2003). Cu is absorbed by plants through their roots were it is accumulated. Unlike most metals, Cu occurs in nature in a directly usable metallic form known as native metals and is not extracted from an ore (Emsley, 2003; Lenntech, 2018). It is described as an essential life element, but toxic at high concentrations (Newman, 2015). Environmental sources of Cu include mining activities, construction, alloys, water purification, agrochemical pesticides that are used to control the growth of algae, bacteria and fungi (Emsley, 2003; Newman, 2015; Lenntech, 2018).

1.6.16 Zinc (Zn)

Zinc has a bluish-white colour. Zn oxide is used as an active ingredient in sunblock. Environmental sources of Zn include mining activities, alloys, industry, batteries, pigments, rubber industries, and paints (Newman, 2015). Zn is an essential to life in small concentrations but can be toxic, although less so than most metals (Emsley, 2003; Newman, 2015).

1.6.17 Gallium (Ga)

Gallium is a soft, blue-silvery coloured metal with orthorhombic crystalline structure (Lenntech, 2018). Elemental Ga is a brittle solid at low temperatures and does not occur as a free element in the natural environment (Lenntech, 2018). Some forms of Ga are known as by-products of zinc ores and therefore zinc mining activities can be environmental sources of Ga (Emsley, 2003).

1.6.18 Germanium (Ge)

Germanium is lustrous, hard, grey-white, with metalloid characteristics (Lenntech, 2018). It is chemically similar to its group neighbours tin (Sn) and silicon (Si). Ge is not considered an essential element for any living organism although it accumulates biologically in tissues (Emsley, 2003). Ge ores are rare (Lenntech, 2018) but it is widely distributed in ores of other metals, especially zinc. It is considered to have a negative impact on aquatic ecosystems (Lenntech, 2018)

1.6.19 Arsenic (As)

Arsenic is grey coloured and brittle in its metallic form and also tarnishes and burns in oxygen (Emsley, 2003). As has been used as poison and weed killer but soil contaminated by As can be cleaned up by growing *Pteris vittata* that is a plant that absorbs As (Emsley, 2003). As can make up 5% of the plants dry weight (Emsley, 2003). Environmental sources of this element include pesticides, herbicides, wood preservatives, coal ash, mining (gold and lead), and plant desiccants. As is toxic and carcinogenic (Emsley, 2003; Newman, 2015). It causes health effects like anaemia, lung irritation, skin changes, heart disruptions, and can even alter DNA. It can cause liver cancers (Pershagen, 1981; Ayres, 1992; Lenntech, 2018).

1.6.20 Selenium (Se)

Selenium has two forms, namely as a silvery metal and as a red powder. It is one of the rarer elements from the surface of the Earth and can be used as an antagonist as it counters effects other toxic metals such as mercury and arsenic (Emsley, 2003; Newman, 2015). Se deficiency is linked to low sperm counts (Emsley, 2003). Environmental sources of Se include pigments, alloys, electronics, and by-product of mining (gold, copper, and nickel), food supplements, animal feed, and coal power stations. Se is an essential to life element for humans but in high doses it can be poisonous (Emsley, 2003; Newman, 2015).

1.6.21 Bromine (Br)

Bromine is a burning red-brown liquid at room temperature that evaporates quickly to form a likewise coloured gas. Elemental Br is very reactive and does therefore, not occur freely in the natural environment (Emsley, 2003).

1.6.22 Rubidium (Rb)

Rubidium is a soft, white coloured metal that is silvery when first cut. The rubidium-strontium dating method is used to date rocks and earth layers. Rb is more valuable than gold and platinum per kilogram (Emsley, 2003). Sources of Rb include the manufacturing of a special kind of glass and due to its use in research. Rb has no biological role (Emsley, 2003).

1.6.23 Strontium (Sr)

Strontium is silvery-white coloured and relatively soft. Crystals of Sr titanite shine brighter than diamonds due to their high refractive index (Emsley, 2003). Environmental sources of Sr include mining, warning flares, fireworks, and glass manufacturing. Sr is a non-toxic element (Emsley, 2003).

1.6.24 Yttrium (Y)

Yttrium is a silvery-metallic transition metal, chemically similar to the lanthanides. Y is not found as a free element in natural environments. Y has no known biological role (Emsley, 2003).

1.6.25 Zirconium (Zr)

Zirconium is a lustrous, grey-white, strong transition metal. It closely resembles hafnium (Hf), and Ti to a lesser extent. Zr compounds have no known biological role (Emsley, 2003).

1.6.26 Molybdenum (Mo)

Molybdenum is a lustrous, soft metal. Environmental sources of Mo include mining, glass manufacturing, lubricants, anti-corrosion additives, pigments, and alloys. Mo is an essential element in low concentrations but can be toxic at high doses. It can cause foetal deformities (Emsley, 2003).

1.6.27 Palladium (Pd)

Palladium is a lustrous, silvery-white coloured, malleable, and ductile metal. Out of all four platinum group metals, Pd is the least dense and has the lowest melting point. Pd has a significant resistance to corrosion (Emsley, 2003). Environmental sources of Pd include alloys, fuel, electrical appliances, chemical industry, fertilisers, and the production of polyester. Pd has a low toxicity but as most others can be poisonous and carcinogenic at high doses (Emsley, 2003).

1.6.28 Cadmium (Cd)

Cadmium is a very soft silvery metal. Cd is poisonous and can cause death if not properly treated when exposed to it (Emsley, 2003). Environmental sources of Cd include various industrial processes like alloy production, electroplating, galvanizing, pigments, batteries, plastic, zinc ore processing, and mining (zinc and lead mines as it is a by-product thereof) (Newman, 2015; Lenntech, 2018). Tobacco smoking releases Cd in the general population (Erie *et al*., 2007;

Newman, 2015). Cd is categorised as a toxic and carcinogenic element and non-essential to life (Goering *et al*., 1995; Emsley, 2003; Newman, 2015; Lenntech, 2018). It is also known as an endocrine disrupting compound. Higher Cd concentrations are absorbed through the intake of Cd in crops grown on land that was fertilized by human waste or which was previously was mined, or from metal processing plants (Godt *et al*., 2006).

1.6.29 Indium (In)

Indium is a soft, silvery metal that is stable in air and in water but dissolves in acids. In is scarce in its distribution throughout the environment and it poses no threat to land or marine life (Emsley, 2003). Cultivated soils are richer in indium than non-cultivated sites. This may have a somewhat inhibiting influence on particular soil micro-organisms such as nitrate-forming bacteria (Emsley, 2003). In sources to the environment include mining activities, glass manufacturing, and research activities (Emsley, 2003).

1.6.30 Tin (Sn)

Tin is the 49th most abundant element. It appears a silvery-white or grey metal. Due to the low toxicity of inorganic Sn, it is used widely for food packaging like Sn containers. However, some organotin compounds can be very toxic (Emsley, 2003). Organic Sn compounds can stay in the natural environment for extended periods and are therefore considered persistent (Lenntech, 2018). They accumulate in water soils for many years and these concentrations still rise to this day. Organic Sn can spread through water systems and are known danger of causing serious harm to aquatic ecosystems. Exposure of organotin is known to disrupt growth, reproduction, enzymatic systems and feeding patterns of aquatic organisms (Lenntech, 2018).

1.6.31 Antimony (Sb)

Antimony is a bright, hard, brittle, and silvery coloured when in metal form. Sb was used in the 1990's to treat mattresses for flame resistance. The presence of Sb in the mattresses may have caused cot deaths during this time. Environmental sources of Sb include alloys and flame retardants. Sb is considered a toxic element (Emsley, 2003).

1.6.32 Iodine (I)

Iodine appears lustrous metallic grey as a solid, and violet as a gas. I is essential to life and the lack of it may result in cretinism (low IQ) of children whose mothers lacked this vital element. It is an essential element for humans and animals and the body can recycle I to some extent but still a little is lost through urine and therefore it is crucial to have in diet (Emsley, 2003). I is not an essential element for plants but plants have the ability to absorb this element through their roots from the soil water or from the atmosphere through their leaves (Emsley, 2003).

1.6.33 Caesium (Cs)

Caesium is a soft, silvery-gold alkali metal. Cs clocks are essential around the world and enable us to know the exact time, as it is part of the internet and mobile phone networks. Cs has no biological role (Emsley, 2003). However, it may partly replace the essential element potassium (K), which it resembles chemically. Cs has been measured in certain plants and occurs in vegetables and fruits. In an experiment, rats that was fed Cs instead of K died within two weeks (Emsley, 2003). Therefore, it can be regarded as a toxic element for that species (Emsley, 2003). However, caesium chloride is probably no more toxic than sodium chloride. If taken in excess, there will, however be serious effects. The tests on rats showed that they experienced extreme irritability and seizures. Cs were specifically a perturbing environmental pollutant during the years that above-ground nuclear weapons tests were carried out (Emsley, 2003).

1.6.34 Barium (Ba)

Barium is soft and silvery of colour. It is abundant in the Earth's crust and is heavier in comparison with other elements when ores are mined. Ba forms insoluble salts and it has been documented that some algae such as *Closterium* thrive in Ba rich waters. These algae also stores Ba as sulphate crystals (Emsley, 2003). Environmental sources of Ba include mining activities, alloys, manufacturing of oil and grease additives, de-hairing agent, rubber production, fertilizer, and is used in fireworks. It is suspected that Ba is non-toxic in low concentrations (Emsley, 2003; Lenntech, 2018). High concentrations can cause reproductive defects (Lenntech, 2018).

1.6.35 Hafnium (Hf)

Hafnium has no known biological role and salts usually have low toxicity. Poisoning of Hf compounds are unheard of and the absorption of Hf by the body is very poor. Plants absorb minor amounts of Hf from the soil they grow in (Emsley, 2003).

1.6.36 Tantalum (Ta)

Tantalum is a rare, hard, blue-grey, lustrous transition metal and is highly corrosion-resistant. Today it is mainly used in electronic equipment as Ta capacitors (Emsley, 2003).

1.6.37 Tungsten (W)

Tungsten or wolfram is a rare metal found naturally on Earth. It has a greyish-white, lustrous appearance and is remarkable for its robustness as a free element (Emsley, 2003). W is known to be somewhat toxic to organisms.

1.6.38 Platinum (Pt)

Platinum is a lustrous, silvery-white coloured, malleable, and ductile metal that is commonly used in the jewellery manufacturing industry (more or less 50% of all Pt). Three-quarters of the planets Pt is derived from South Africa (Emsley, 2003). Environmental sources of Pt include mining activities, alloys, jewellery, catalytic convertors, chemical industry, electrical industry, glass industry, and aircraft industry. It is considered a non-toxic metal but it is important to know that some of its associated compounds are poisonous (Emsley, 2003).

1.6.39 Gold (Au)

Gold metal is a soft, shiny, yellow solid. Au can occur as grains, sheets, flakes, crystals, and as a solid metal. South Africa is one of the largest producers of Au worldwide and due to the value of Au, many cities and towns around the country has been established due to a gold rush (Emsley, 2003). Environmental sources of Au include mining, alloys, dentistry, jewellery, bullion, and electronics. It is categorised as a non-essential element, and can be toxic in high concentrations (Emsley, 2003).

1.6.40 Mercury (Hg)

Mercury is a heavy, silvery-white liquid at room temperature unlike other metals that are solid. Hg is poisonous in all forms and can cause diseases like “Hatter’s shake” and “mercury madness” and Minamata disease and is therefore one of the most poisonous metals (Emsley, 2003).

Methyl mercury is an enduring contaminant of the environment that threatens the health of organisms in ecosystems (Schneider *et al*., 2013). Since methylmercury bio-accumulates over time and biomagnifies at each trophic level, long-lived, carnivorous species such as reptiles are at greatest risk (Schneider *et al*., 2013). Reptiles are considered valuable bio-indicators of local Hg in ecosystems as they tend to accumulate large concentrations of Hg contamination in affected habitats (Schneider *et al*., 2013).

Due to the release of Hg into terrestrial and aquatic ecosystems increasing over the past 50 years, this element has become a major contaminant (Mason *et al*., 1994; Haines, *et al.,* 1995). Hg can occur in the environment in a variety of chemical species, as listed below (Schneider *et al*., 2013):

- Elemental Hg (Hg^0), in the form of gas which can be efficiently transported in the atmosphere around the globe;
- Inorganic Hg [Hg(II], one of the main forms of Hg in water;
- Methylmercury (CH_3Hg^+), one of the main forms of Hg present in water;
- Inorganic Hg in its ionic form (Hg^{2+}); or
    - can be combined forming mercury chloride ($HgCl^2$), mercury sulphide (HgS) and organic acids;
- Organic forms of Hg like methylmercury CH_3Hg^+; and
- Ethylmercury (CH_3CH_2 Hg^+).

Methylmercury is considered as the most toxic species found in aquatic environments that accumulate readily in biota (Bernhoft, 2011; Schneider *et al*., 2013; Newman, 2015). Due to the attraction of Hg has to adipose tissue, CH_3Hg^+ tends to bio-accumulate and bio-magnify in aquatic environments (Hund and Bischoff, 1960; Salonen *et al*., 1995; Padovani *et al*., 1996). Information on Hg concentrations in Nile crocodiles from the KNP area is important because of potential health risks to humans and animals (Tchounwou *et al*., 2003) as well as the potential to use these apex predators and bio-indicators. The concentration of mercury in food is increasing and this it is a worrying condition (Guallar *et al*., 2002; Clarkson *et al*., 2003). Hg poisoning affects all organs of the body as it disorders proteins and enzymes on cellular level (Emsley, 2003). The highest concentrations of Hg are usually found in organs like the kidneys, liver and spleen. Environmental sources of Hg include volcanic eruptions, chlorine-alkali production, gold mining, paints, industrial catalyst, biocide, burning of fossil fuels (coal and oil), and cinnabar ore mines. (Hansen and Danscher, 1997; Boylan *et al*., 2003; Emsley, 2003; Tchounwou *et al*., 2003; Zahir *et al*., 2005; Newman, 2015). Studies have shown that small meat-eating organisms are more sensitive to methyl mercury poisoning than larger species (Wren *et al*., 1988).

1.6.41 Thallium (Tl)

Thallium is a soft, silvery-white metal. It can be absorbed through the skin and contact with Tl with bare hands can lead to the complete loss of fingernails. Tl was once prescribed by doctors as treatment for the removal of ringworms from the scalp. This caused the patient to lose all his/her hair in order to make it easier for the doctor to remove the ringworms (Emsley, 2003). Environmental sources of Tl include pesticides, coal-fired power stations, metal processing industries, and rat poison. Tl is considered toxic and is a non-essential element (Emsley, 2003).

1.6.42 Lead (Pb)

Lead is a soft, weak, ductile, dull silvery-grey coloured metal that has a high malleability, low melting point, and is resistant to corrosion (Emsley, 2003). In the eighteenth and nineteenth centuries, Pb was used to sweeten wine and this most likely caused gout in consumers due to large concentrations of Pb in the wine (Phillips, 1984; Emsley, 2003). Pb accumulates in the body although it is considered a non-essential element. Organisms are primarily exposed to Pb through inhalation, ingestion, or absorption through the skin (Nadjafzadeh *et al*., 2013). Only an estimated 10% of Pb that passes through organisms via the digestive tract are eventually absorbed by the body (Emsley, 2003). The form of lead-phosphate usually accumulates in the bones and soft tissues (Ethier *et al*., 2007). Environmental sources of Pb include storage batteries, ammunition, leaded fuel, present and past mining activities (Mielke, 2002; Fisher *et al*., 2006; Newman, 2015), insecticides, cosmetics (hair gels), sheeting, cables, solders, lead crystal glassware, lead smelting, coal combustion (Flora *et al*., 2012), and from bearings (Pain, 1990, Mielke, 2002; Emsley, 2003; Fewtrell *et al*., 2004; Fisher *et al*., 2006;). Pb is considered extremely poisonous and is exceedingly persistent in tissues (Newman, 2015). The mining of Pb ores is commonly found worldwide.

Bio-accumulation of Pb in plants are usually in minor concentrations which are absorbed through the roots (Fewtrell *et al*., 2004).The increased bio-accumulation of Pb in Nile crocodiles are attributed to the ingestion of Pb sinkers (Newman, 2015; Warner *et al*., 2016).

1.6.43 Bismuth (Bi)

Bismuth is a heavy solid element with a silvery colour and faint pink tinge. Bi is too brittle to be used as a pure metal, but does occur as a metal itself. Bi is an active ingredient in the medication used to treat gastric disorders. Cu and Pb smelting produces Bi as a by-product (Emsley, 2003). Environmental sources of Bi include medicine, cosmetics, mining (Cu and Pb), alloys, the synthetic fibre industry, and rubber industries. It has no known biological role and has no real environmental threat (Emsley, 2003).

1.6.44 Uranium (U)

Uranium is a silvery, malleable, ductile, radioactive metal and is the only element in this study that falls in the Actinides group of the periodic table (Table 1.1). The famous atomic bomb, “Little boy” which was dropped onto Hiroshima in 1945 was comprised of U. Today, U is mostly only used in the generation of electricity in nuclear reactors. U is ten times more abundant than both Hg and silver (Ag) together (Emsley, 2003). Environmental sources of U include bomb manufacturing, mining, nuclear reactors, nuclear fuel, and nuclear powered submarines, and other naval vessels (Emsley, 2003).

CHAPTER 2: MATERIALS AND METHODS

2.1 BACKGROUND

As indicated in Chapter 1, during August 2010, a wider SANParks programme assessed the health of wild crocodiles in the KNP, with appropriate permits and ethical clearance (Threatened or Protected Species Registration South African National [SAN] Parks, S 21201). Samples were collected from 16 crocodiles (eight males and eight females; average weight of 186 kg; length 1.9 - 4.6 m) from rivers representing seven catchments including the six major rivers that run through the KNP (Sabie, Olifants, Crocodile, Levuvhu, Shingwedzi, Nwaswitsontso and Letaba rivers). This collection represents a unique set of samples, since previous studies concentrated on opportunistic samples from already diseased pansteatictic crocodiles. Despite analyses conducted at the time, comparisons with healthy crocodiles were difficult as there was little baseline data for healthy wild crocodiles.

2.2 SAMPLE COLLECTION

This project is based on data already analysed in 2011 from samples ethically obtained in 2010. This study is therefore based on legacy samples and data. For this dissertation, no biological samples were handled, only existing data. It is therefore categorised as Category 0 ethical clearance that implies no further ethical implications for this study (Ethical approval number: NWU-00176-18-S5). Since this project concerns only data already collected, all risks have been mitigated prior to this study.

After the sixteen crocodiles where shot by rangers, each were dissected and sampled at the Skukuza abattoir (2010) on the same day of being shot, or the day after (Fig. 2.1a).

Figure 2.1: Samples taken from Nile crocodiles (*Crocodylus niloticus*) during study. (a) Crocodiles were shot and taken to Skukuza abattoir (2010). (b) Biopsies taken from crocodile tail fat and muscle.

The dissections were done under the supervision of Dr Danny Govender (wildlife veterinarian of SANParks), the research scientist in charge of this project at the time, together with Prof Bouwman and other NWU students, as part of their training.

The samples were collected using clean equipment. It was ensured that sharpened autopsy equipment was cleaned from any metal residue after sharpening as metal filings and oil may remain after sharpening. All samples were thereafter stored in plastic, labelled, and frozen immediately. Sub-samples (2 g) were taken from thawed samples for analyses. Standardised records were kept, and autopsy results, collected by veterinarians present, will be presented later.

2.3 SAMPLE PREPARATION

Tissues of abdominal fat, liver, kidney, muscle, and tail fat were sampled from each of the sixteen crocodiles (CS 01 – CS 16). All the sampled crocodiles were dissected and samples were stored at -20°C. The concentrations of the analysed elements in each of the five tissues were then analysed at the NWU using the standard EPA 3050B method with Inductively Coupled Plasma Mass Spectrometry (ICP-MS), using 2 g of dried sample in a 50 mL mixture of HNO_3, H_2O_2, HCL and deionised water. The samples were analysed soon after collection, and the resultant data is now the subject of this study. Concentrations are expressed as mg/kg dry mass (dm). The samples have been completely digested and analysed in 2011.

Figure 2.2: An example of samples at North-West University Laboratory to be analysed (Photo: NWU Laboratory).

As mentioned above, this study was conducted in respect of identifying a set of fifty different elements. These elements, namely lithium (Li), beryllium (Be), boron (B), sodium (Na), magnesium (Mg), aluminium (Al), silicon (Si), potassium (K), calcium (Ca), scandium (Sc), titanium (Ti), vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), gallium (Ga), germanium (Ge), arsenic (As), selenium (Se), bromine (Br), rubidium (Rb), strontium (Sr), yttrium (Y), zirconium (Zr), niobium (Nb), molybdenum (Mo), palladium (Pd), cadmium (Cd), indium (In), tin (Sn), antimony (Sb), iodine (I), caesium (Cs), barium (Ba), cerium (Ce), hafnium (Hf), tantalum (Ta), tungsten (W), platinum (Pt), gold (Au), mercury (Hg), thallium (Tl), lead (Pb), bismuth (Bi) and uranium (U) were analysed.

Samples and SRM 1944 (Standard Reference Material) were analysed using EPA 3050B for ICP-MS analyses, by Eco-Analytica at the NWU. The tissue samples were using acid and heat using 65% nitric acid, 50% hydrogen peroxide, and 33% hydrochloric acid (reagent grades from Associated Chemical Enterprises). An Agilent 7500 CE ICP-MS fitted with a micromist-type nebulizer and a standard quartz chamber was used. Optimisation to reduce interference (≤ 1.5%) was with a solution containing Li, Y, Ce, and Tl (1 µg/L).

External calibrations were conducted using multi-element standard solutions with elements at different, certified, concentrations (ULTRASPkEC-certified; De Bruyn Spectroscopic Solutions). The concentrations of the certified elements were within 25% of specification. Concentrations are expressed as mg/kg dry mass (dm).

2.4 STATISTICAL ANALYSIS

Statistical data analysis was done using Graphpad Prism 7.02 (www.graphpad.com) and PC-ORD 7.02 (MjM Software, www.pcord.com). All column statistics (Col. Stats: included in

Appendix) tested for normality for both untransformed and log-transformed data. Wherever untransformed data were not normally distributed, log-transformed data were used in further statistical comparisons. Normality was tested using the D'Agostino and Pearson normality test ($p < 0.05$ if not normally distributed). Descriptive, univariate, and multivariate statistical analyses were be used during this study. Multiple comparisons were done via Kruskal-Wallis test inter alia other statistical tests to determine whether data is statistically significant or not. Regressions of elemental compositions of each of the five tissues were done versus length and mass to give perspective on the comparison between large crocodiles and age. Comparisons between elemental compositions of male versus female were also conducted. Data below detection limits were not used.

Multivariate analyses were used to determine the usability of muscle tissue as representative tissue for future biopsy samples. This will enable future research that catch, sample, and release methods can be used to assess changes in environmental elemental composition, often due to pollution. Nonmetric multidimensional scaling (NMS) were performed on data relativized per sample as compositional 'fingerprint', with crocodile mass and length used as descriptive variables. Gower (ignore 0) was used as distance measure. Six axes were allowed, with a maximum of 500 iterations. Starting conditions were random. There were 250 runs with real data, followed by 250 runs with randomised data to determine the p-value for the Monte Carlo test. This was done for all ordinations as displayed in Chapter 3 below.

CHAPTER 3: RESULTS

3.1 LOCATION AND CROCODILE DETAILS

3.1.1 Sampling area

The crocodiles of this study were sampled from seven river catchments of the KNP, namely Crocodile River, Olifants River, Shingwedzi River, Levuvhu River, Shiloweni Dam, Letaba River, Sabie River, and at the confluence of the Olifants River and Ga-Selati River.

Crocodiles were sampled from across the KNP ranging in rivers form the Levuvhu River located close to the Limpopo River north of the KNP, to the Crocodile River forming the southern boundary of the KNP (Figure 3.1). Most of these rivers flowing through the park originate in catchments far outside the park. These rivers flow through settlements, mining areas, and a mosaic of agricultural land-uses before crossing the boundaries of the park. Chemical contamination of rivers in South Africa is primarily associated with industries including mining, smelting, transportation, and chemical and synthetic manufacturing plants, but also has residential and agricultural inputs (Schutte and Pretorius, 1997; Heath *et al.,* 2010; Combrink, *et al.,* 2011). There are six major rivers in the KNP, namely the Crocodile River, Limpopo River, Olifants River, Sabie River, Letaba River and the Luvuvhu River. These rivers are displayed in Figure 3.1 and further discussed.

As mentioned above, the Limpopo River is the northern border of the KNP (Figure 3.1). The Limpopo River catchment receives run-off from the two largest cities in the country, namely Johannesburg and Pretoria (not indicated on map). Much agricultural and mining activity also forms part of major run-off of the Limpopo River (Roychoudhury and Starke, 2006; Winde, 2009). The type of mining activities within the Limpopo River catchment include diamonds, emeralds, coal, Ni, Cr, Va, Mn, dolomite, Au, As, pyrite, Pb, Fe, W, Co, Ag, Pt, and Cu (Ashton *et al*., 2001). The Luvuvhu River flows into the KNP as an individual river, and confluences with the Limpopo River when within the KNP (Figure 3.1). The Luvuvhu River flows through urban, agricultural, and mining areas before flowing into the KNP.

The Ga-Selati River joins the Olifants River, just before the KNP border on the western boundary near the city of Phalaborwa (Figure 3.1). It originates from the Drakensberg Mountain region and flows eastwards towards the KNP. Domestic run-off and seepage from tailing dams of mines are discharged into the upper reaches of the Ga-Selati River. Phalaborwa consists of 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 (Phalaborwa Mining Company Limited, 2018).

The Olifants River catchment is approximately 54 500 km^2 and covers 4.3% of the total surface area of South Africa (DWAF, 2005). The Olifants River begins near the town of Bethal and flows in an easterly direction before crossing the KNP, joining the Letaba River, and entering Mozambique (Figure 3.1). Thereafter, it flows into the Indian Ocean after its confluences with the Limpopo River (Van Vuren *et al*., 1994). The Olifants River is considered one of the most polluted rivers in Southern Africa (Myburgh and Botha, 2009; Heath *et al*., 2010). The mining activities that take place in the Olifants catchment include Au, Cr, Pt, Zn, Ag, Ti, Sn, Mn, coal, V, Tl, and Cu mining (Asthon *et al*., 2001; Van Vuuren, 2009; Van Vuuren, 2010), electricity (power generation), government activities, and agricultural activities (DWAF, 2005). The lower Olifants River enters the KNP at the Phalaborwa area (Figure 3.1) (Van Vuuren, 2009; Van Vuuren, 2010).

The Letaba River consists of two large rivers, namely the Letaba River and the Greater Letaba River (Figure 3.1). The Letaba River catchment covers an area of 13 670 km^2 and comprises of many dams built for agricultural and domestic purposes, including approximately 20 large dams in the Greater Letaba River alone. The catchment area of the Letaba River comprises of much gold, phosphate, and vermiculite mining activities. These two rivers finally flow into the Olifants River, approximately 30 km before it flows through the Olifants gorge into Mozambique (Asthon *et al*., 2001).

The Sabie River basin is one of three sub-basins of the Inkomati River, which is one of the most important river basins in South Africa. The Sabie River or Sand River originates in the Drakensberg Mountains where it flows through the province of Mpumalanga into the KNP where it confluences with the Komati River on its way to the Indian Ocean (Figure 3.1) (KNP, 2018).

With a catchment area of 10 440 km^2 and an initial altitude of 2000 m above sea level, the Crocodile River is classified as one of the country's most biological diverse systems with at least 49 fish species (Roux *et al*., 1999). The Crocodile River forms the southern border of the KNP (Figure 3.1). From an ecological point of view, the Crocodile River is one of the most important rivers in South Africa (Roux *et al*., 2013). This is due to the rivers broad range of riverine habitats and stretches from fast-flowing streams in the Drakensberg Mountains to slow down into mild waters where the river twists through the Lowveld. The Lowveld area where the Crocodile River slows down and meanders towards the town of Komatipoort has developed rapidly with an increase in agricultural activities along the river. Due to these continuing developments, and therefore increased abstraction of water from the river, water flow has reduced (Roux *et al*., 2013).

Most of the rivers of the KNP flow through densely populated villages and settlements before entering the park. These influences of the surrounding environments of the catchment areas have led to increased pollution of the water. This also has had a suspected increase in diseased animals (KNP, 2018).

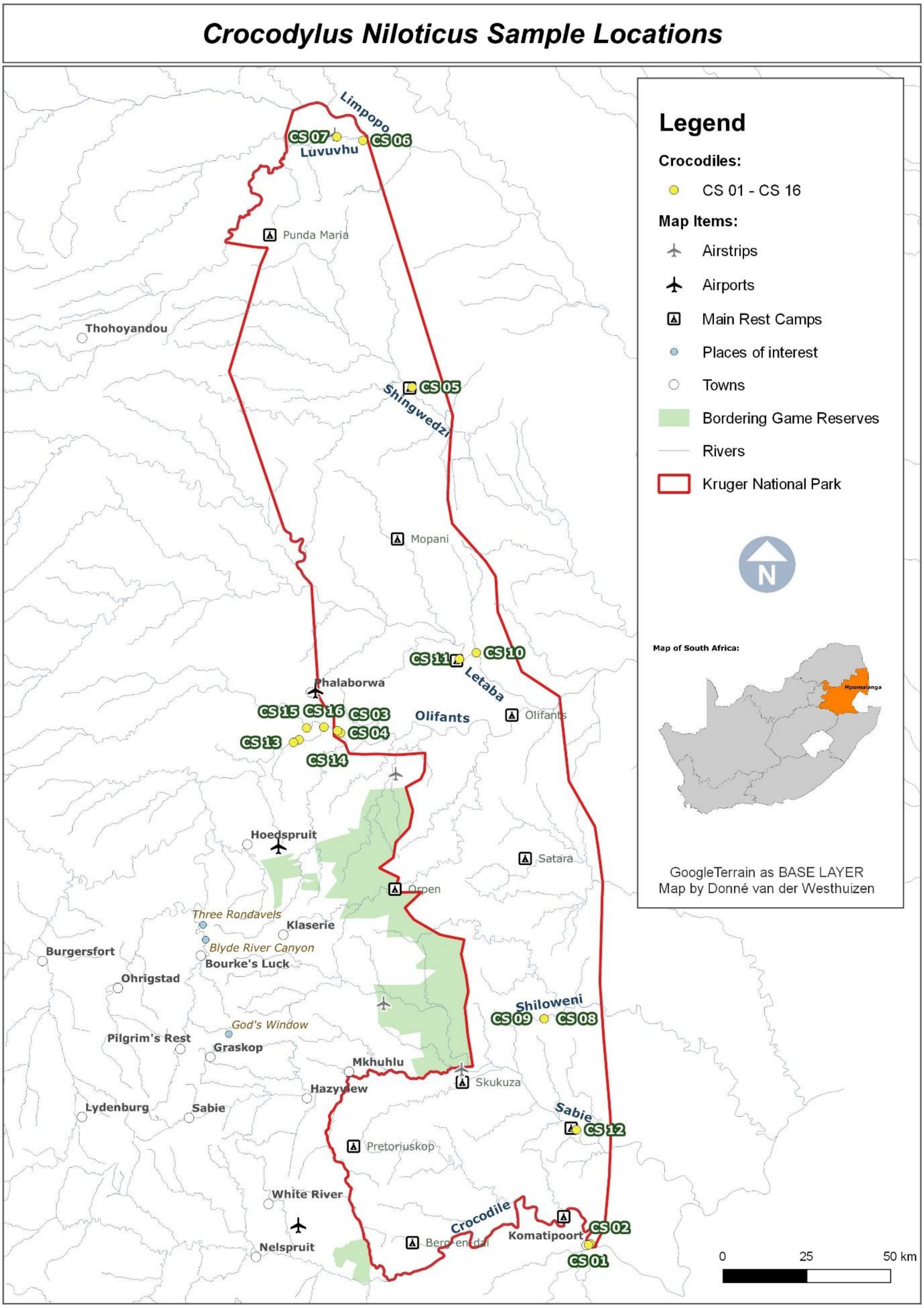

Figure 3.1: Sampling Locations of Nile crocodiles (*Crocodylus niloticus*) CS 01 – CS 16.

3.1.1.1 Crocodiles sampled

The samples were collected from Nile crocodiles, *C. niloticus,* that were shot at locations spread across the Kruger National Park (KNP) as part of a crocodile survey (CS 01 – CS 16). The exact coordinates of each crocodile individually is given in Table 3.1 and illustrated in Figure 3.1.

The crocodiles with references CS 01 – CS 12 were sampled within the same week, 13 July 2010 to 16 July 2010. The remainder of the sample (refer to crocodile referenced CS 13 – CS 16) were sampled during a second sampling week between 7 February 2011 to 10 February 2011.

The collection sites, dates of collection, and condition details of each sampled crocodile are provided in Tables 3.1, and 3.1-continued. The animal condition details were assessed by SANParks and veterinarians that attended the collection. Abdominal fat is abbreviated as Ab fat in the results.

Table 3.1: Details of sampled crocodiles in Kruger National Park

Reference	Date	Location	Sex	Length (m)	Mass (Kg)	Longitude	Latitude	Condition	Gonads	S : H : FB
CS 01	13/7/2010	Ressano Garcia Weir, Crocodile River	F	2.6	70	31.96674167	-25.43352778	Fair-poor	Maturing 20mm follicles	1.57 : 1 : 0.55
CS 02	13/7/2010	Ressano Garcia Weir, Crocodile River	F	2.92	102	31.97241667	-25.43085000	Fair	Maturing 20mm follicles	0.77 : 1 : 7.32
CS 03	14/7/2010	Mamba Weir, Olifants River	M	3.73	216.5	31.22048333	-24.04530000	Fair	Mature testes	0.93 : 1 : 0.33
CS 04	14/7/2010	Mamba Weir, Olifants River	M	3.23	110	31.22966667	-24.05128333	Emaciated	Immature	0.82 : 1 : 0.04
CS 05	14/7/2010	Kanidood Dam, Shingwedzi River	M	3.13	118	31.44250000	-23.10901000	Fair	Maturing testes	1.02 : 1 : 0.34
CS 06	14/7/2010	Old Pafuri Picnic Spot, Levuvhu River	M	3.23	127	31.29639000	-22.43260000	Fair	Mature testes	0.89 : 1 : 0.32
CS 07	14/7/2010	Bridge, Levuvhu River	M	3.85	267.5	31.21909000	-22.42282000	Good	Mature testes	0.89 : 1 : 0.63
CS 08	15/7/2010	Shiloweni Dam, Internal Catchment	F	3.4	210	31.83506667	-24.82503333	Fair	Mature: 40-mm follicles	0.97 : 1 : 1.07
CS 09	15/7/2010	Shiloweni Dam, Internal Catchment	M	2.82	85	31.83501667	-24.82498333	Fair-poor	Immature	0.96 : 1 : 0.15
CS 10	15/7/2010	Engelhardt Dam, Letaba River	F	2.8	80	31.63337833	-23.83346250	Fat	Mature 40-mm follicles	0.89 : 1 : 0.16
CS 11	15/7/2010	Hlanganini Mouth,Letaba River	F	2.8	83	31.58336833	-23.85026833	Fat	Mature 40-mm follicles	1.04 : 1 : 0.18
CS 12	16/7/2010	Lower Sabie Weir, Sabie River	M	4.6	337.5	31.93096667	-25.12436667	Fair	Mature	0.99 : 1 : 0.36
CS 13	7/2/2011	Selati Dam, Foskor, Phalaborwa	F	2.99	113	31.08893000	-24.03255000	Good	210-mm degenerating follicles present on ovary	1.57 : 1 : 0.78
CS 14	8/2/2011	Phalaborwa Barrage	F	3.3	160	31.10666000	-24.06977000	Good	Mature, inactive ovaries	1.52 : 1 : 0.6
CS 15	9/2/2011	Van Ryissen Dam, Foskor, Phalaborwa	F	1.9	22.5	31.09237000	-24.00168000	Fair	Immature	0.75 : 1 : 0.38
CS 16	10/2/2011	Tailings Dam, Phalaborwa Mining Company	M	3.46	170	31.17819000	-24.01161000	Good	Mature, inactive testes	0.88 : 1 : 0.17

Table 3.1: Continued: Comments on each sampled crocodile

CS 01	Crocodile in poor body condition, with minimal abdominal fat, fluid in abdomen and pericardial sack (hydro-pericardium). Tonsils enlarged. Catfish found in stomach.
CS 02	Increased density of the lungs, hydro-pericardium, ascites, and liver fibrosis, hypertrophy of the right ventricle and distention of the large veins as well as pulmonary artery. Backward failure of the heart most likely due to a primary lung disease. Snare and fish hook in stomach, no food items.
CS 03	Hydro-pericardium, otherwise negative post-mortem. *Paratrichosoma* trails. Kudu and impala pieces in stomach
CS 04	Crocodile in severely emaciated body condition, with distended proximal third oesophagus, fibrous liver and kidneys and adhesions throughout the abdominal and thoracic cavities. No subcutaneous fat, extremely little abdominal fat. Empty stomach and kidneys. Adhesions throughout the abdominal and thoracic cavities.
CS 05	Pleural adhesions with multifocal yellowish brown nodules in the parenchyma around the larger bronchioles in the cranial lung lobe. Little body fat in tail. Stomach empty.
CS 06	Hydro-pericardium, otherwise negative post-mortem. Little abdominal fat (white). Monkey found in stomach.
CS 07	Ascites, pancreas swollen and enflamed. Little body fat (white).
CS 08	Tonsillar hyperplasia. Little body fat (white). Stomach empty.
CS 09	Crocodile in poor body condition with hydro-pericardium, fibrossed atrophic liver, swollen and enflamed pancreas, enlarged budding spleen, left adrenal hyperplasia, and high internal parasite burden, with many nematodes free in the abdominal cavity. Abdominal fat slightly yellow with tiny ceroid nodules. No tail fat sampled. Stomach empty.
CS 10	Abdominal fat starting to take on yellow discoloration with multiple 1 mm dark brown and orange nodules present focally in different areas of the lower abdominal fat; tail and fat organ appeared normal. Splenomegaly with budding. Stomach empty.
CS 11	Multifocal to coalescing dark brown and orange 2 mm nodules in the abdominal fat over the left lower abdomen, peritoneal area as well as over the distal pancreas and in the fat body. Tail fat appeared normal. 2 mm white hard nodules in the mediastinal tissue (possibly parasitic). Serosanguinous hydro-pericardium. Kudu horn stuck in stomach wall, no food items.
CS 12	Multifocal 2 mm-5 mm dark brown and orange nodules in the fat of the right proximal third of the tail. Multiple 50 mm diameter fibroses around the lower left abdomen. Unilateral thickened ureter. Stomach empty.
CS 13	Crocodile with pronounced kyphosis of the spinal column as a result of possible blunt force trauma to the spine (external healed wound visible), resulting in a vertebral dislocation over the lumbar spine. The area was marked spinal cord compression at that point. Crocodile was found to be dragging his hind legs and tail. Healed scars on the ventral aspect of the tail and on the hind legs possibly due to the dragging or due to unrelated damage such as burns. Crocodile had multiple large, full thickness ulcers in stomach (>3 cm). Stomach contents: grasshoppers. Given the age of this crocodile, she was most likely struggling to hunt due to partial paralysis.

CS 14	Leeches noted in the oral cavity. Numerous healed and healing bite wounds on tail and around abdomen and thorax, with some contact into the internal cavity and associated with abscess formation or fibrous adhesions throughout thorax and abdomen. Partial involution of the thymus. Stomach content: two shoes and remains of one-month-old hippopotamus. Pancreas were slightly enflamed.
CS 15	*Paratrichosoma* tails found on belly skin. Algal growth on teeth. Prominent pharyngeal tonsils. Excessive serosanguinous fluid in peritoneal cavity (hydro-pericardium). Normal immature thymus extending from heart base to thoracic inlet. Crocodile had multiple medium sized, full thickness ulcers in stomach (2 cm). No stomach contents.
CS 16	Severe bruising in the musculature and along the pleural cavity of the ventral right thorax, possibly due to trauma. Healed and healing wounds along the tail and ventral aspect of the mandible. Crocodile had an excessive amount of subcutaneous fat. Stomach severe distended of small fish. Crocodile regurgitated large amount of gastric fluid with orange fish fat into lung. Hypertrophic reactive tonsils with multifocal petechial of the glottis and pharyngeal area. Gall bladder not distended- indicating that crocodile was actively feeding. Multiple focal areas of orange discoloration, with granular appearance in abdominal fat. Ruptured blood vessel close to lesion noted - possibly due to trauma and subsequent inflammatory reaction in the fat.

The sample of sixteen crocodiles consisted of eight males and eight females (Table 3.1). The crocodiles were in fair-good condition, except CS 04, which was rated as ‘emaciated’. CS 12 was the largest sampled crocodile at an estimated length of 4.6 m with a mass of 337.5 Kg. Crocodile CS 15 was clearly the smallest crocodile sampled, at a length of 1.9 meters and a mass of 22.5 Kg.

Of all the sampled crocodiles, four were in good condition (CS 07, CS 13, CS 14 and CS 16); seven were in fair condition (CS 02, CS 03, CS 05, CS 06, CS 08, CS 12, and CS 15); two were in fair-poor condition (CS 01, and CS 09); two were considered fat (CS 10, and CS 11); and only one crocodile was considered emaciated (CS 04) (Table 3.1). Most of the female crocodiles had maturing 20 mm to 40 mm follicles. Most of the male crocodiles had mature testes. All sampled crocodiles were large enough to be classified as adult, not juvenile, crocodiles. CS 13 had the largest spleen: heart: fat body ratio.

Hydro-pericardium refers to the condition where excessive fluid accumulates in the pericardial cavity. The pericardial cavity refers to the gap formed between the two layers of serous pericardium around the heart. In normal conditions, this sac contains only a small amount of serous fluid that acts to reduce surface tension and lubricate heart beating. Of all sixteen crocodiles sampled, seven crocodiles were diagnosed with this condition (CS 01, CS 02, CS 03, CS 06, CS 09, CS 11, and CS 15). In two crocodiles (CS 03 and CS 15), *Paratrichsoma* (Nematoda) trails were found in the skin, although these parasites are normal. Most crocodiles were in fairly good health.

I considered the sample size obtained adequate for the assessment of metals in healthy wild crocodiles for the purposes of this study. It represents a unique set of data on wild crocodiles.

3.1.2 Concentrations

The mean elemental concentrations in the five tissues are presented in Table 3.2. The results of the Kruskal-Wallis comparisons are presented in Table 3.3.1 – the medians that were statistically significantly different are highlighted in bold. Detailed statistics of the concentrations are given in Appendix A. The number of significant differences per organ, based on Table 3.3-1, is displayed in Table 3.3-2. It is clear that there were no statistically significant differences between abdominal fat and tail fat (Table 3.3-2). Kidney and liver tissues also indicated only seven statistically significant differences (Table 3.3-2).

Selected toxic elements were grouped in Table 3.3-3. This was done to provide a clear display of these toxic elements statistical significant differences, but the data are the same as in Table 3.3-1. As evident in Table 3.3-3, toxic elements in abdominal and tail fat had no statistically significant differences. In addition, kidney and liver tissues also indicated no statistically significant differences for the selected toxic elements.

Scatterplots, means, standard deviations, and comparisons of the data in the tables are illustrated in Section 2.3.

Table 3.2: Concentration means of all elements within each tissue

Concentration means (mg/kg dm)					
Element	**Ab Fat**	**Kidney**	**Liver**	**Muscle**	**Tail Fat**
Li	0.0082	0.014	0.016	0.016	0.0086
Be	0.011	0.012	0.052	0.052	0.020
B	4.4	5.9	9.0	34	3.9
Mg	240	960	810	1000	250
Al	17	65	330	13	14
Si	16	18	19.0	17	16
Sc	0.075	0.094	0.15	0.15	0.071
Ti	1.8	6.5	11	5.8	1.4
V	4.1	23	21	2.6	4.4
Cr	13	10.4	8.6	7.9	13
Mn	1.3	6.3	6.1	3.4	1.4
Fe	59	560	12000	210	48
Co	0.033	1.6	0.29	0.13	0.031
Ni	1.0	11	1.5	1.3	1.0
Cu	1.1	11	44	6.9	2.3
Zn	22	72	140	68	22
Ga	0.071	0.086	0.31	0.098	0.075
Ge	0.044	0.062	0.41	0.037	0.044
As	2.5	2.3	2.5	2.1	2.6
Se	1.7	14	19	5.85	1.7
Br	3.1	14	17	17.05	2.4
Rb	1.2	16	10	3.1	1.4
Sr	0.46	1.1	1.4	1.86	0.42
Y	0.0048	0.015	0.21	0.0055	0.0042
Zr	0.16	0.36	0.24	0.15	0.30
Mo	0.23	2.9	2.2	1.8	0.22
Pd	0.26	0.19	0.41	1.2	0.15
Cd	0.011	0.31	0.15	0.045	0.011
In	0.11	0.11	0.12	0.11	0.11
Sn	230	240	240	240	240
Sb	0.012	0.10	0.80	0.0077	0.012
I	0.68	0.55	0.45	0.65	1.0
Cs	0.012	0.059	0.016	0.0035	0.015
Ba	9.1	11	29	13	9.5
Hf	0.0030	0.0065	0.0044	0.0030	0.0048
Ta	0.0011	0.0012	0.0013	0.073	0.0010
Pt	0.13	0.15	0.17	0.44	0.082
Au	0.49	0.60	1.0	8.7	0.38
Hg	1.0	16	30	2.7	0.75
Tl	0.028	0.043	0.075	0.92	0.020
Pb	0.39	2.2	12	0.55	0.52
U	0.041	0.071	0.082	0.31	0.037

Table 3.3-1: Kruskal-Wallis comparisons of five tissues and selected elements, with multiple comparisons using the Dunn's method.[1,2]

Elements	Li	Log Be	Log B	Log Al	Log Si	Sc	Ti	V	Cr	Log Mn	Log Fe	Log Co	Log Ni	Log Cu	Zn
Kruskal-Wallis p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Muscle vs. Kidney	>0.9999	**0.0006**	**<0.0001**	**<0.0001**	>0.9999	**<0.0001**	>0.9999	**<0.0001**	**0.0436**	**<0.0001**	0.4305	**0.0034**	**<0.0001**	0.7521	>0.9999
Muscle vs. Liver	>0.9999	>0.9999	**<0.0001**	**<0.0001**	**0.0414**	0.9585	**0.0262**	**<0.0001**	>0.9999	**<0.0001**	**0.0009**	>0.9999	0.6893	**0.0017**	0.1882
Muscle vs. Tail fat	**0.0007**		**<0.0001**	>0.9999	**0.0056**	**<0.0001**	**0.0032**	**0.0009**	**<0.0001**	**<0.0001**	**0.0187**	**0.0151**	0.6893	0.2324	**0.0044**
Muscle vs. Ab fat	<0.0001		**<0.0001**	>0.9999	0.7504	**<0.0001**	**0.0298**	0.1164	**<0.0001**	**<0.0001**	0.09	**0.0216**	0.6893	**0.0056**	**0.0186**
Kidney vs. Liver	>0.9999	**<0.0001**	**0.0021**	0.6725	>0.9999	**<0.0001**	0.1305	>0.9999	0.139	0.6386	0.5384	0.3758	**<0.0001**	0.4704	0.3613
Kidney vs. Tail fat	**0.0154**		**0.002**	**0.004**	**<0.0001**	0.1439	**0.0004**	0.1159	**0.0064**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.0006**	**0.0017**
Kidney vs. Ab fat	**0.002**		**0.0213**	**0.0067**	**0.0127**	0.2231	**0.0049**	**0.0009**	0.2788	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.0078**
Liver vs. Tail fat	**0.0002**		**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.2687	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.1472	**<0.0001**	**<0.0001**
Liver vs. Ab fat	**<0.0001**		**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.0033**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.1472	**<0.0001**	**<0.0001**
Tail fat vs. Ab fat	>0.9999		0.3142	>0.9999	>0.9999	0.9315	>0.9999	>0.9999	>0.9999	0.6386	>0.9999	>0.9999	0.9783	>0.9999	>0.9999

Table 3.3-1: Continued

Elements	As	Log Se	Log Rb	Log Sr	Mo	Log Cd	Sn	Cs	Ba	Log Pt	Log Au	Log Hg	Log Tl	Log Pb	Log U
Kruskal-Wallis p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0002	0.0199	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Muscle vs. Kidney	0.4147	**<0.0001**	**0.0095**	0.2642	**<0.0001**	**0.0001**	>0.9999	**<0.0001**	0.6714	0.0572	**0.0002**	0.1491	**<0.0001**	0.0572	0.9358
Muscle vs. Liver	>0.9999	**<0.0001**	0.2753	>0.9999	0.114	**0.0254**	0.841	**0.0194**	**<0.0001**	0.1349	0.3345	**0.0166**	**<0.0001**	**<0.0001**	>0.9999
Muscle vs. Tail fat	**<0.0001**	**<0.0001**	0.1885	**<0.0001**	**<0.0001**		>0.9999	>0.9999	0.3536	**<0.0001**	**<0.0001**	**0.009**	**<0.0001**	>0.9999	**<0.0001**
Muscle vs. Ab fat	**0.0004**	**<0.0001**	0.1067	**<0.0001**	**<0.0001**		>0.9999	>0.9999	0.2688	**0.0009**	**<0.0001**	0.3453	**<0.0001**	0.6945	**0.0003**
Kidney vs. Liver	>0.9999	**0.0487**	>0.9999	>0.9999	**0.0018**	0.2616	>0.9999	0.8224	**<0.0001**	>0.9999	0.2945	>0.9999	**0.0046**	0.5473	>0.9999
Kidney vs. Tail fat	**0.0484**	**<0.0001**	**<0.0001**	**0.0045**	**<0.0001**		0.7999	**<0.0001**	0.8001	**0.0086**	**0.025**	**<0.0001**	**<0.0001**	0.2974	**0.0033**
Kidney vs. Ab fat	0.3535	**<0.0001**	**<0.0001**	**0.0114**	**<0.0001**		0.2244	**<0.0001**	0.7953	>0.9999	>0.9999	**<0.0001**	**0.0232**	**<0.0001**	0.0981
Liver vs. Tail fat	**0.0017**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**		0.1721	**0.0118**	**<0.0001**	**0.0029**	**<0.0001**	**<0.0001**	**<0.0001**	**0.0006**	**<0.0001**
Liver vs. Ab fat	**0.0232**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**		**0.0357**	**0.002**	**<0.0001**	>0.9999	**0.0039**	**<0.0001**	**<0.0001**	**<0.0001**	**0.0006**
Tail fat vs. Ab fat	>0.9999	0.9718	>0.9999	>0.9999	0.9853		>0.9999	>0.9999	0.8206	0.3501	>0.9999	>0.9999	0.0551	0.2016	>0.9999

[1]Items in bold indicates significant difference within that group and selected element.

[2]When "Log" appears before element, it indicates that untransformed data were not distributed normally. Instead log-transformed data were used.

Table 3.3-2: Total number of significant differences within each group based on Table 3.3-1 results

Group	Number significant differences
Liver vs. Ab fat	26
Liver vs. Tail fat	25
Kidney vs. Tail fat	23
Muscle vs. Tail fat	21
Kidney vs. Ab fat	20
Muscle vs. Kidney	16
Muscle vs. Ab fat	16
Muscle vs. Liver	15
Kidney vs. Liver	7
Tail fat vs. Ab fat	0[1]

[1]Tail fat and abdominal fat showed no statistical significant differences for any element

Table 3.4: Multiple ANOVA comparisons of selected, toxic, elements

Elements	V	Cr	Log Mn	Log Cu	As	Log Cd	Log Hg	Log Pb	Number significant differences
Kruskal-Wallis p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0002	<0.0001	<0.0001	n/a
Muscle vs. Kidney	**<0.0001**	**0.0436**	**<0.0001**	0.7521	0.4147	**0.0001**	0.1491	0.0572	4
Muscle vs. Liver	**<0.0001**	>0.9999	**<0.0001**	**0.0017**	>0.9999	**0.0254**	**0.0166**	**<0.0001**	6
Muscle vs. Tail fat	**0.0009**	**<0.0001**	**<0.0001**	0.2324	**<0.0001**		**0.009**	>0.9999	5
Muscle vs. Ab fat	0.1164	**<0.0001**	**<0.0001**	**0.0056**	**0.0004**		0.3453	0.6945	4
Kidney vs. Liver	>0.9999	0.139	0.6386	0.4704	>0.9999	0.2616	>0.9999	0.5473	0
Kidney vs. Tail fat	0.1159	**0.0064**	**<0.0001**	**0.0006**	**0.0484**		**<0.0001**	0.2974	5
Kidney vs. Ab fat	**0.0009**	0.2788	**<0.0001**	**<0.0001**	0.3535		**<0.0001**	**<0.0001**	5
Liver vs. Tail fat	0.2687	**<0.0001**	**<0.0001**	**<0.0001**	**0.0017**		**<0.0001**	**0.0006**	6
Liver vs. Ab fat	**0.0033**	**<0.0001**	**<0.0001**	**<0.0001**	**0.0232**		**<0.0001**	**<0.0001**	7
Tail fat vs. Ab fat	>0.9999	>0.9999	0.6386	>0.9999	>0.9999		>0.9999	0.2016	0

Tail fat and abdominal fat showed no statistical significant differences

Selected elements refer to elements of toxic interest

3.2 SCATTERPLOTS OF ELEMENTAL CONCENTRATIONS IN TISSUES

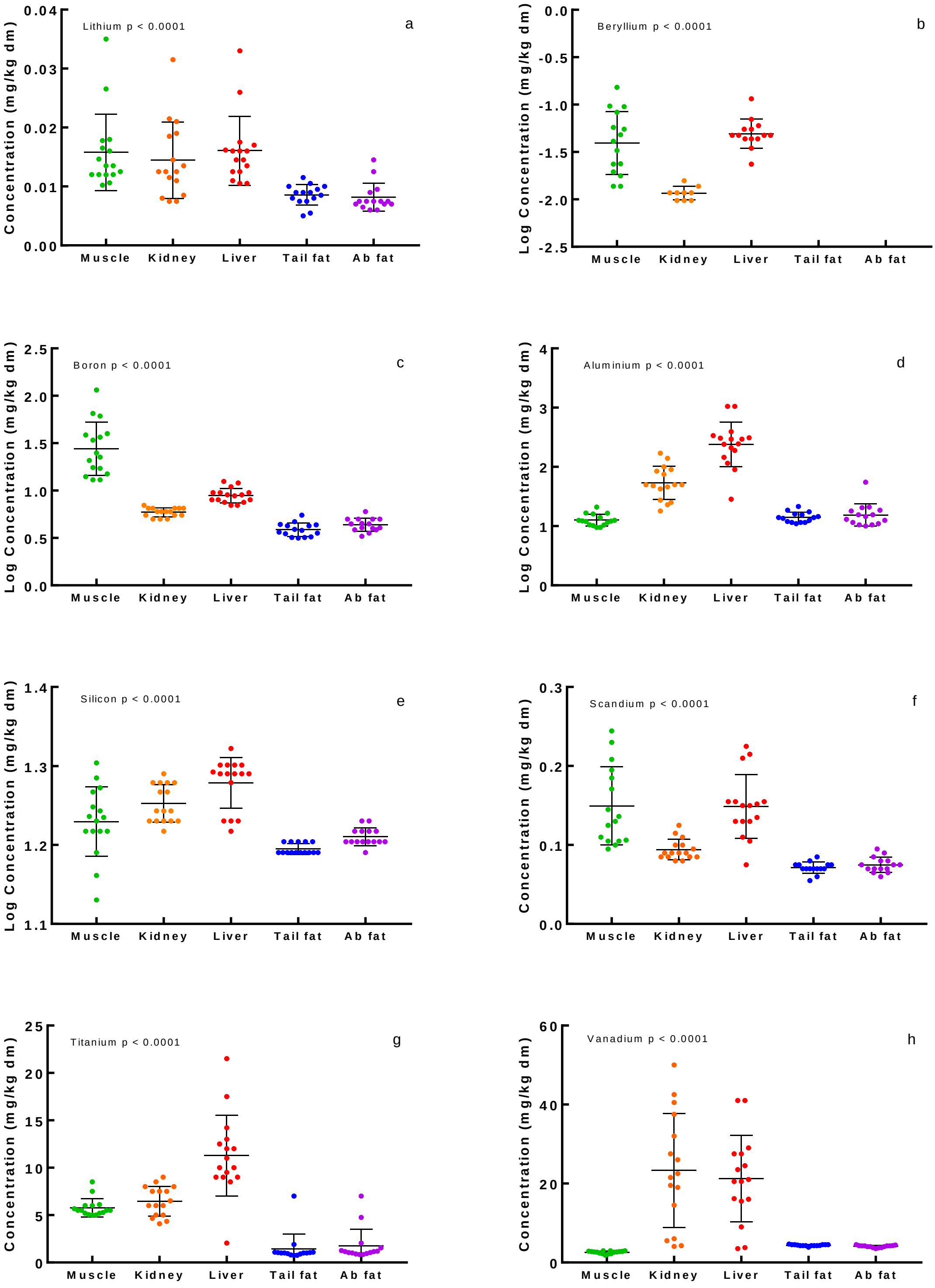

Figure 3.2-1: Scatterplots of elemental concentrations in five crocodile tissues. (a) Lithium, (b) beryllium, (c) boron, (d) aluminium, (e) silicon, (f) scandium, (g) titanium, (h) vanadium. The overall ANOVA p-values, means, and standard deviations are indicated in each plot. When data were not normally distributed, log-transformed data were used.

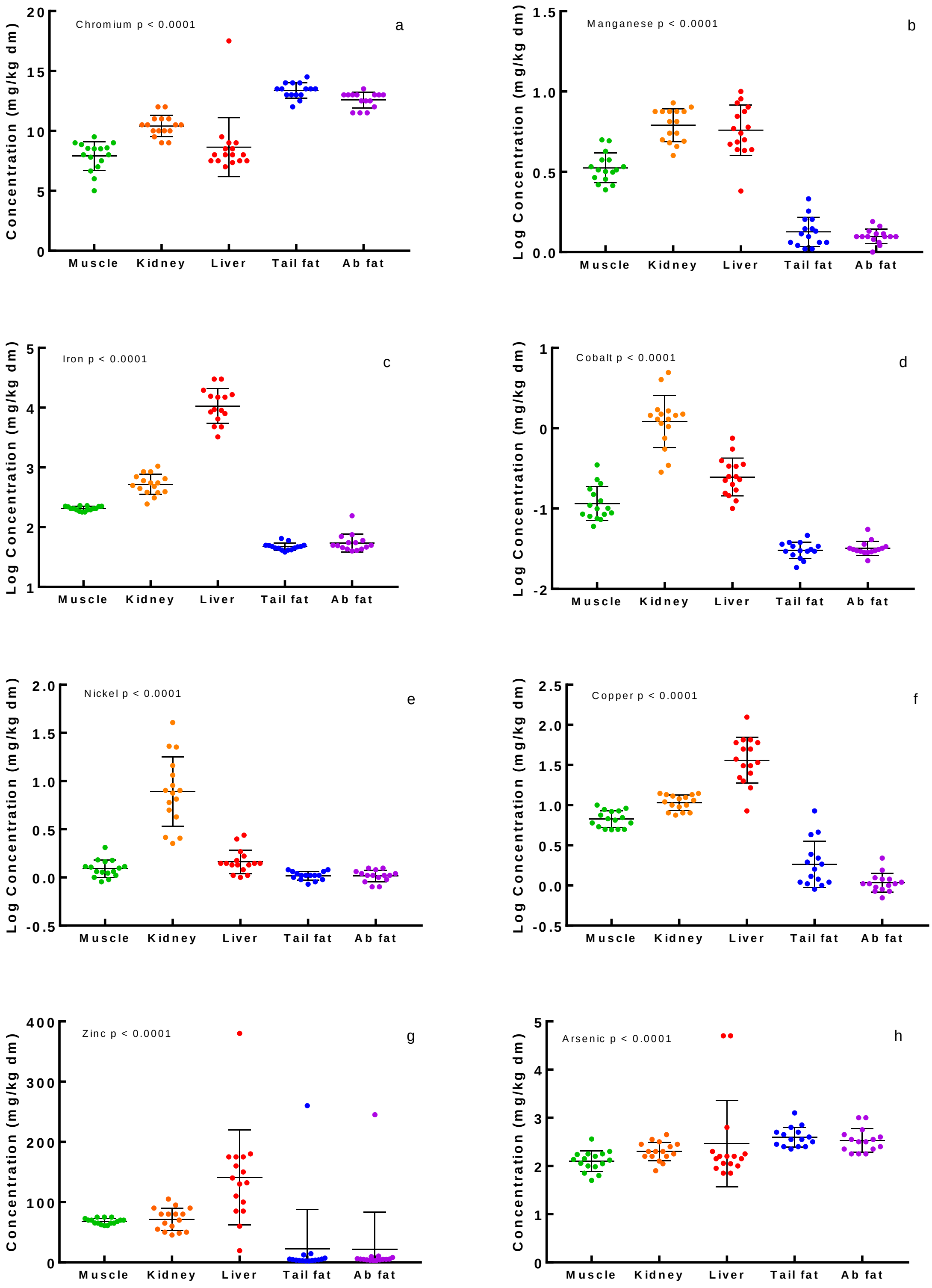

Figure 3.2-2: Scatterplots of elemental concentrations in five crocodile tissues. (a) Chromium, (b) manganese, (c) iron, (d) cobalt, (e) nickel, (f) copper, (g) zinc, (h) arsenic. The overall ANOVA p-values, means, and standard deviations are indicated in each plot. When data were not normally distributed, log-transformed data were used.

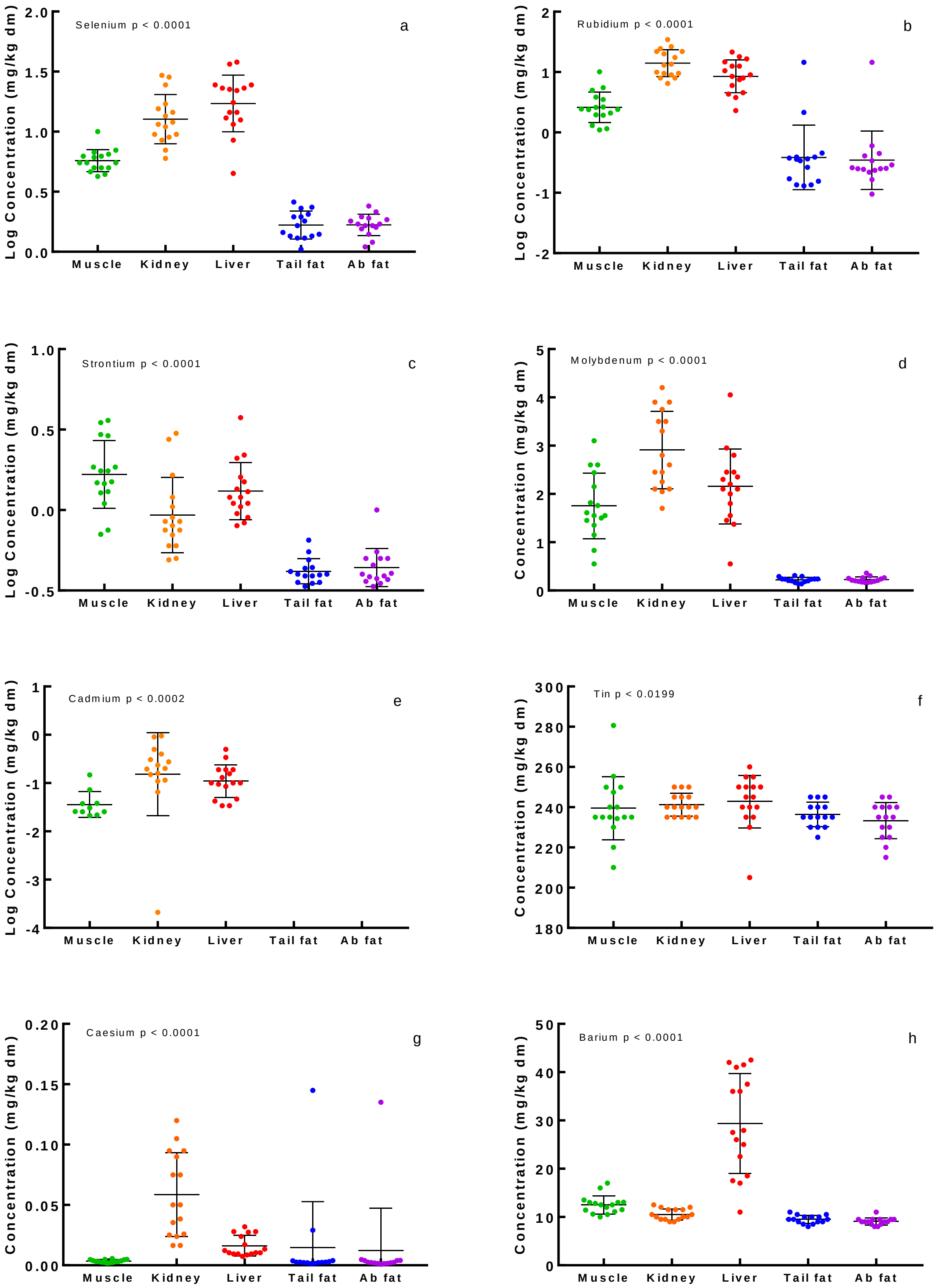

Figure 3.2-3: Scatterplots of elemental concentrations in five crocodile tissues. (a) Selenium, (b) rubidium, (c) strontium, (d) molybdenum, (e) cadmium, (f) tin, (g) caesium, (h) barium. The overall ANOVA p-values, means, and standard deviations are indicated in each plot. When data were not normally distributed, log-transformed data were used.

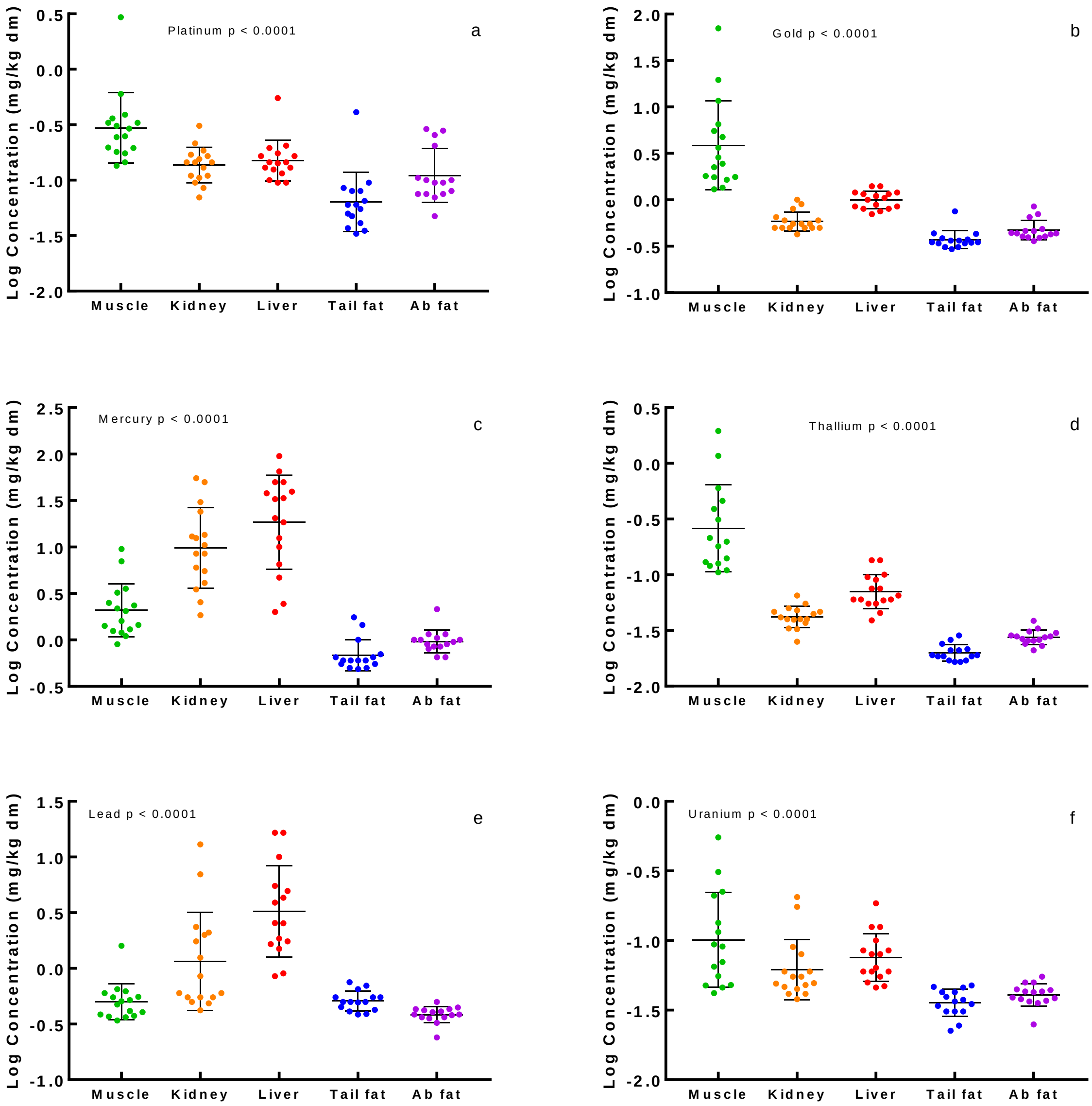

Figure 3.2-4: Scatterplots of elemental concentrations in five crocodile tissues. (a) Platinum, (b) gold, (c) mercury, (d) thallium, (e) lead, (f) uranium. The overall ANOVA p-values, means, and standard deviations are indicated in each plot. When data were not normally distributed, log-transformed data were used.

The concentrations of Be (Fig. 3.2-1b), B (Fig. 3.2-1c), Ti (Fig. 3.2-1g), Mn (Fig. 3.2-2b), Co (Fig. 3.2-2d), Zn (Fig. 3.2-2g), Se (Fig. 3.2-3a), Sr (Fig. 3.2-3c), Mo (Fig. 3.2-3d), Cd (Fig. 3.2-3e), and Tl (Fig. 3.2-4d) were significantly lower in both lipid tissues when compared with the other tissues (Table 3.3-1).

The concentrations of Li (Fig. 3.2-1a), Be (Fig. 3.2-1b), B (Fig. 3.2-1c), Al (Fig. 3.2-1d), Si (Fig. 3.2-1e), Ti (Fig. 3.2-1g), Mn (Fig. 3.2-2b), Fe (Fig. 3.2-2c), Co (Fig. 3.2-2d), Cu (Fig. 3.2-2f), Zn (Fig. 3.2-2g), Se (Fig. 3.2-3a), Rb (Fig. 3.2-2b), Sr (Fig.3.2-3c), Mo (Fig. 3.2-3d), Cd (Fig. 3.2-3e), Cs (Fig. 3.2-3g), Hg (Fig. 3.2-4c) and Tl (Fig. 3.2-4d) were significantly lower in both lipid tissues when compared with liver and kidney tissues (Table 3.3-1). It is therefore evident that elemental concentrations of abdominal and tail fat indicated the greatest significant differences when compared to liver and kidney tissues (Table 3.3-2).

The concentrations of Sn (Fig. 3.2-3f) had the smallest number of significant differences between all the groups, showing only a significant difference in the concentrations of liver tissue versus abdominal fat.

The concentrations of only Be (Fig. 3.2-1b), B (Fig. 3.2-1c), Sc (Fig. 3.2-1f), Ni (Fig. 3.2-2e), Se (Fig. 3.2-3a), Mo (Fig. 3.2-3d), Ba (Fig. 3.2-3h), Tl (Fig. 3.2-4d) had significant differences between liver and kidney tissues. This makes kidney and liver tissues very similar compared to each other, after abdominal and tail fat (Table 3.3-2).

Elemental concentrations of Sc (Fig. 3.2-1f), Ti (Fig. 3.2-1g), V (Fig. 3.2-1h), Se (Fig. 3.2-3a), Sr (Fig. 3.2-3c), Mo (Fig. 3.2-3d), Ba (Fig. 3.2-3h), Hg (Fig. 3.2-4c), Pb (Fig. 3.2-4e) in liver tissue showed large ranges compared with other elemental concentrations. Their relative %CVs were also much larger (Table 1, Appendix).

Be (Fig. 3.2-1b) and Cd (Fig. 3.2-3e) concentrations were below detection limits in the lipid tissues of the crocodiles and therefore not included in the scatterplots.

For most elements, the concentrations in the lipid tissues were lower, and for many of them significantly lower, than for the other three tissues.

Some outliers were removed to provide a better display of the graphs but were retained for statistical purposes. One data point (CS 01) from muscle tissue was excluded form the Tl graph (Fig. 3.2-4d, 8.5 mg/kg dm) and from the U graph (Fig.3.2-4f, 2.9 mg/kg dm). One data point (CS 14) from liver tissue was excluded from the Pb graph (Fig. 3.2-4e, 110 mg/kg dm) due to being much larger than other concentrations.

Six muscle tissues of Cd (Fig. 3.2-3e) were below detection limits and therefore not represented on the scatterplots. Cd in one kidney tissue sample (Fig.3.2-3e) was also below detection limits.

3.3 REGRESSIONS: ASSOCIATIONS BETWEEN ELEMENTAL CONCENTRATIONS WITH CROCODILE LENGTH

As mentioned in the previous chapters, crocodile age cannot be accurately determined and therefore it cannot be assumed that a longer or heavier animal implies older animals. The ages of the sampled wild crocodiles cannot therefore be accurately determined. There is not yet an exact aging method for crocodiles. The length of a crocodile also depends on whether it is male or female, as females tend to be smaller and more slender than males. A study from Zimbabwe of Nile crocodiles indicated that the female crocodiles matured sexually when they reached lengths of 2.6 to 2.9 m and the males when they had lengths of 2.7 to 3.0 m (Kofron, 1990). However, it can safely be assumed that longer crocodiles will generally be older crocodiles. All crocodiles sampled during this study were considered as adult. In the absence of a reliable method for age determination of the sampled crocodiles, I conducted regressions between the different tissue elemental concentrations and body length. Here, I only present regressions of tissue concentrations versus length and mass that were significantly different ($p < 0.05$) from zero (Figs. 3.3-1 to 3.3-3).

3.3.1 Abdominal fat

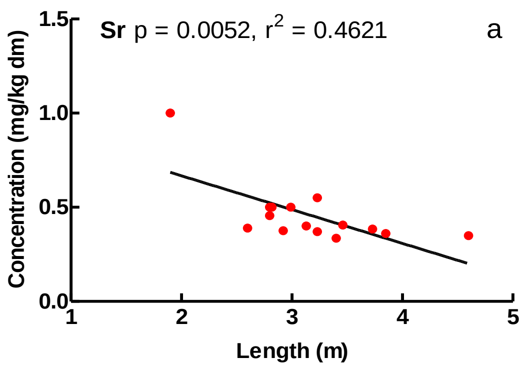

Figure 3.3-1: Regression of normally distributed strontium (a) concentrations in abdominal fat with crocodile length.

Strontium was the only element that showed a significant, but negative, association with crocodile length. The significance of the regression line (p-value) and coefficient of determination (r^2) are shown inside the graph.

3.3.2 Muscle tissue

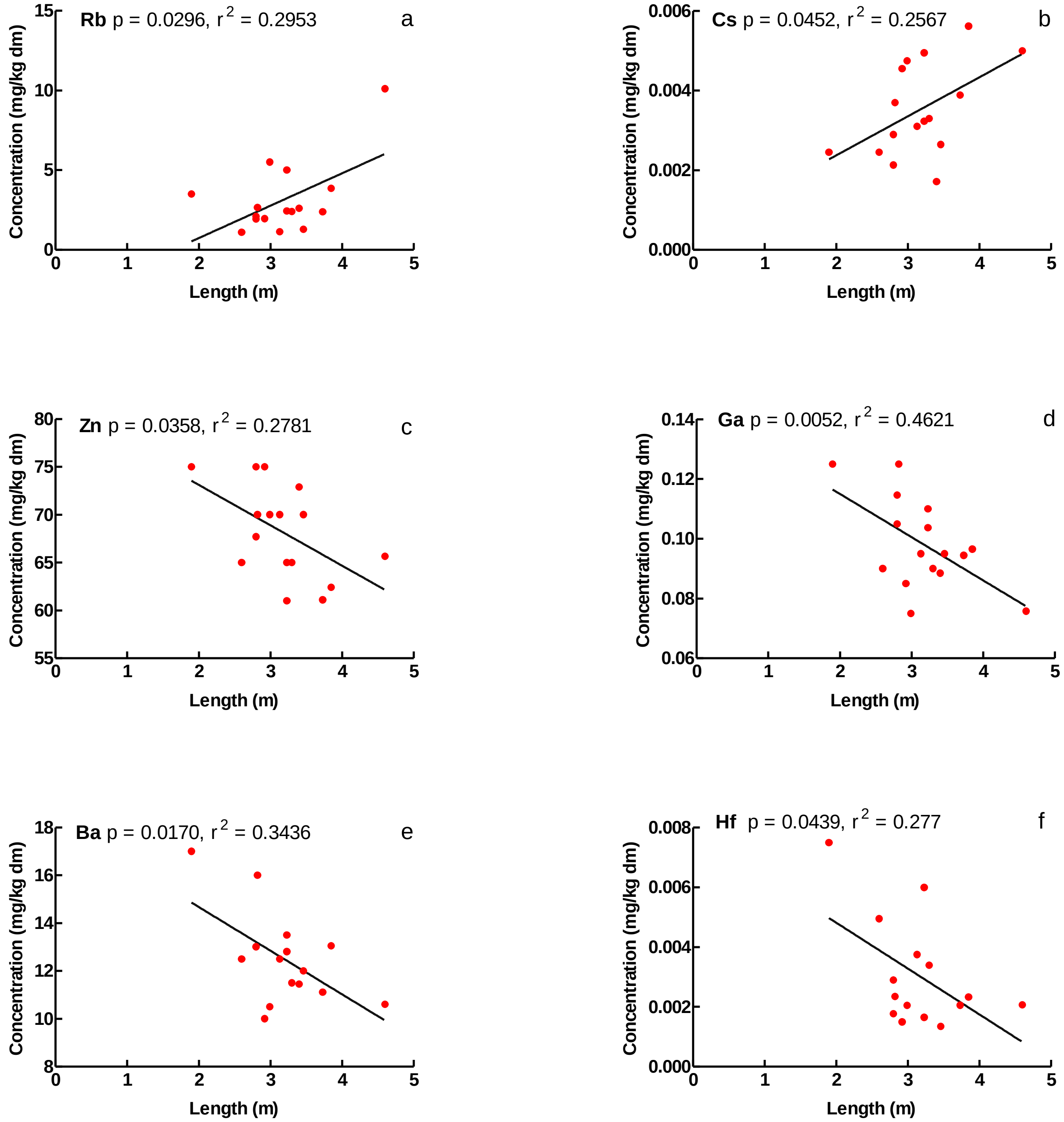

Figure 3.3-2: Regressions of normally distributed elemental concentrations in muscle tissue with crocodile length. (a) Rubidium, (b) caesium, (c) zinc, (d) gallium, (e) barium, (f) hafnium. Only elemental concentrations that showed significant associations ($p < 0.05$) with crocodile length are shown. The significance of the regression line (p-value) and coefficient of determination (r^2) are shown inside each graph.

3.3.3 Kidney tissue

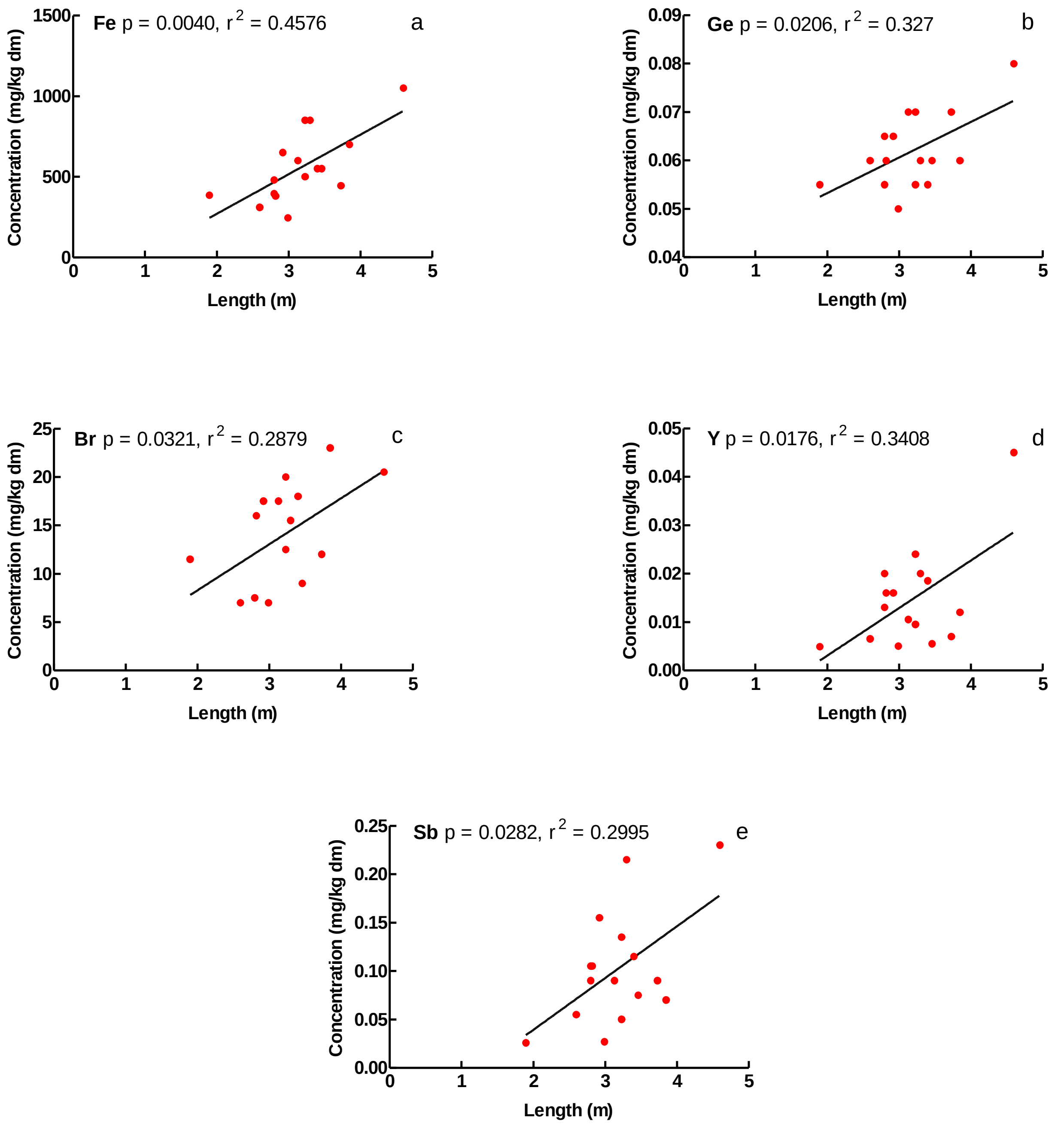

Figure 3.3-3: Regressions of normally distributed elemental concentrations in kidney tissue with crocodile length. (a) Iron, (b) germanium, (c) bromine, (d) yttrium, (e) antimony.

3.3.4 Liver tissue

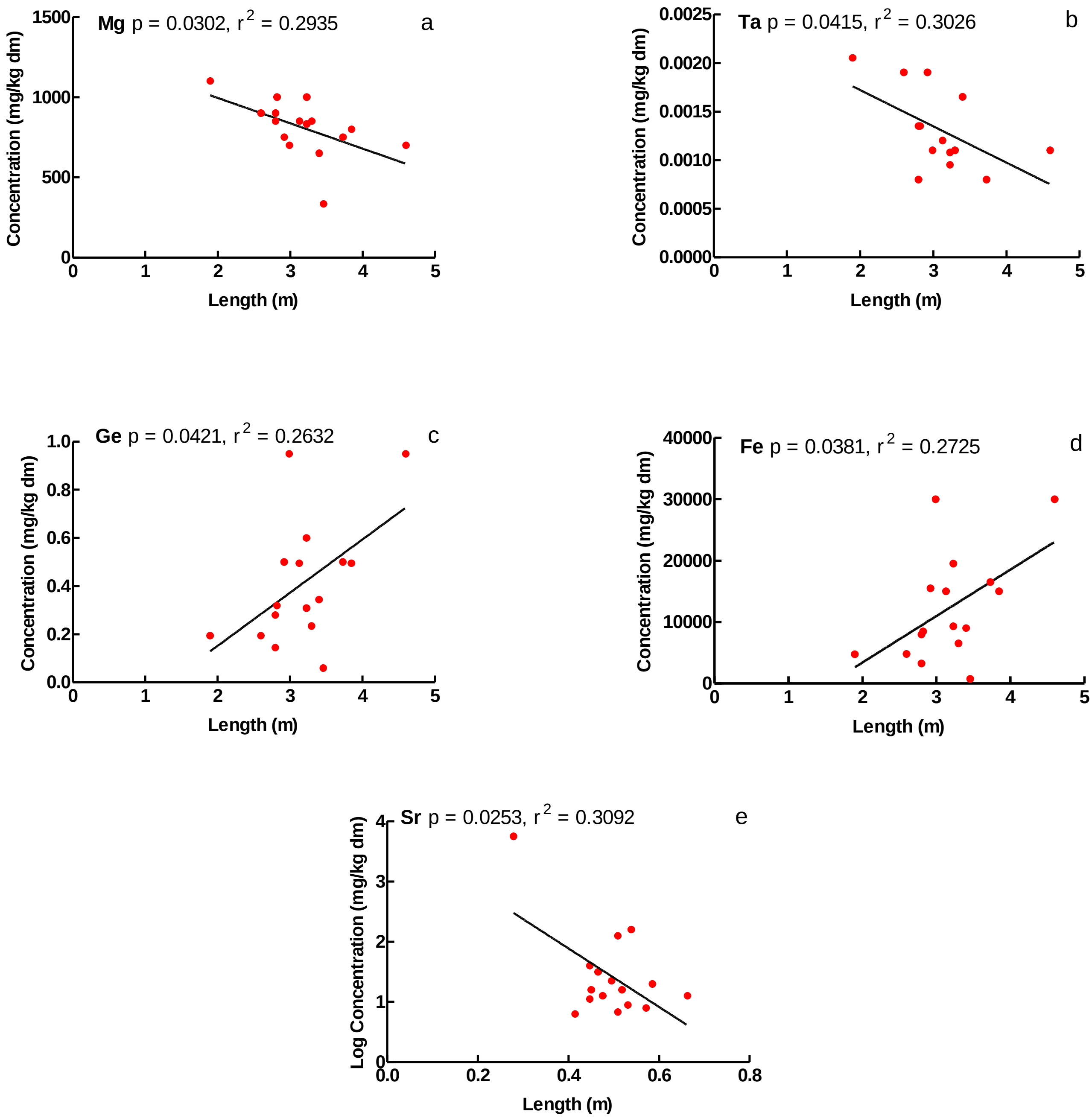

Figure 3.3-4: Regressions of normally distributed elemental concentrations in liver tissue with crocodile length. (a) Magnesium, (b) tantalum, (c) germanium, (d) iron, (e) strontium. Please note that the x-axes for strontium are on a log-scale.

3.3.5 Tail fat

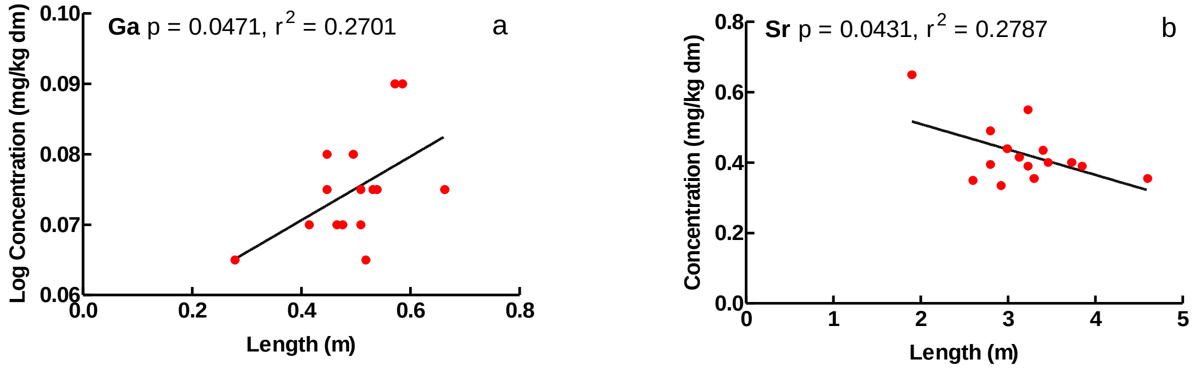

Figure 3.3-5: Regressions of normally distributed elemental concentrations in tail fat with crocodile length. (a) Gallium, (b) strontium. Please note that the x-axes for Ga are on a log-scale.

Abdominal fat:

There was only one element (Sr) that showed a significant, but negative association ($p = 0.0052$) between length and concentration in abdominal fat (Fig. 3.3-1a). The shortest crocodile had more than double the Sr concentration than the longest crocodile.

Muscle tissue:

Two elements, namely Rb (Fig. 3.3-2a, $p = 0.0296$) and Cs (Fig. 3.3-2b, $p = 0.0452$), showed significant positive associations between length and concentrations in muscle tissue. The longest crocodile had high Rb concentrations in comparison with the other crocodiles. Four elements, namely Zn (Fig. 3.3-2c, $p = 0.0358$), Ga (Fig. 3.3-2d, $p = 0.0052$), Ba (Fig. 3.3-2e, $p = 0.0170$), and Hf (Fig. 3.3-2f, $p = 0.0439$) showed negative associations between length and concentrations in muscle tissue. In comparison with all regressions of length with elemental concentrations in muscle tissue, Ga data points (Fig. 3.3-2d) showed the best coefficient of determination ($r^2 = 0.4621$). The shortest crocodile had more than double the Ga concentrations than the longest crocodile.

Kidney tissue:

All (five) statistically significant elements, namely Fe (Fig. 3.3-3a, $p = 0.0040$), Ge (Fig. 3.3-3b, $p = 0.0206$), Br (Fig. 3.3-3c, $p = 0.0321$), Y (Fig. 3.3-1d, $p = 0.0176$), and Sb (Fig. 3.3-3e, $p = 0.0282$) showed negative associations between length and concentrations in kidney tissues. In comparison with all the regressions of length and concentrations in kidney tissue, Fe data showed the best coefficient of determination ($r^2 = 0.4576$). The longest crocodile had more than double the Fe concentrations than the shortest crocodile.

Liver tissue:

Three elements, namely Mg (Fig. 3.3-4a, p = 0.0302), Ta (Fig. 3.3-4b, p = 0.0415) and Sr (Fig. 3.3-4e, p = 0.0253) showed a negative association between length and concentrations in liver tissue. Only two elements, Ge (Fig. 3.3-4c, p = 0.0421) and Fe (Fig. 3.3-4d, p = 0.0381) showed a positive association between length and concentrations in liver. Compared with all the regressions, Sr data had the best coefficient of determination (r^2 = 0.3092). The shortest crocodile had almost four times the Sr concentration of the longest crocodile.

Tail fat:

Only two significant associations of concentrations between length and tail fat were found; one positive, namely Ga (Fig. 3.3-5a, p = 0.0471) and one negative, namely Sr (Fig. 3.3-5b, p = 0.0431). Of these two regressions, Sr had the best coefficient of determination (r^2 = 0.2787). The shortest crocodile had almost double the Sr concentration of the longest crocodile. Compared to Sr concentrations in abdominal fat, these statistics indicate notably the same results.

Summary of associations:

Negative associations of length and concentration regressions were found in abdominal fat – Sr (Fig. 3.3-1a); muscle tissue – Zn (Fig. 3.3-2c), Ga (Fig. 3.3-2d), Ba (Fig. 3.3-2e), Hf (Fig. 3.3-2f); liver tissue – Mg (Fig. 3.3-4a), Ta (Fig. 3.3-4b), Sr (Fig. 3.3-4e); and tail fat – Sr (Fig. 3.3-5). Strontium concentrations showed significant negative regressions in both abdominal and tail fat, as well as liver tissue, with length. No positive regressions of Sr concentrations and length were found.

Positive regressions between length and concentrations were found for muscle tissue – Rb (Fig. 3.3-2a), Cs (Fig. 3.3-2b); kidney tissue – Fe (Fig. 3.3-3a), Ge (Fig. 3.3-3b), Br (Fig. 3.3-3c), Y (Fig. 3.3-3d), Sb (Fig. 3.3-3e); liver tissue – Ge (Fig. 3.3-4c), Fe (Fig. 3.3-4d); and tail fat – Ga (Fig. 3.3-5a). Iron (Fe) concentrations showed significant positive regressions for both kidney and liver tissues versus length (Fig. 3.3-3a, p = 0.0040; Fig. 3.3-4d, p = 0.0381). No negative associations of Fe concentrations and length were found.

3.4 REGRESSIONS: ELEMENTAL CONCENTRATIONS WITH CROCODILE MASS

3.4.1 Abdominal fat

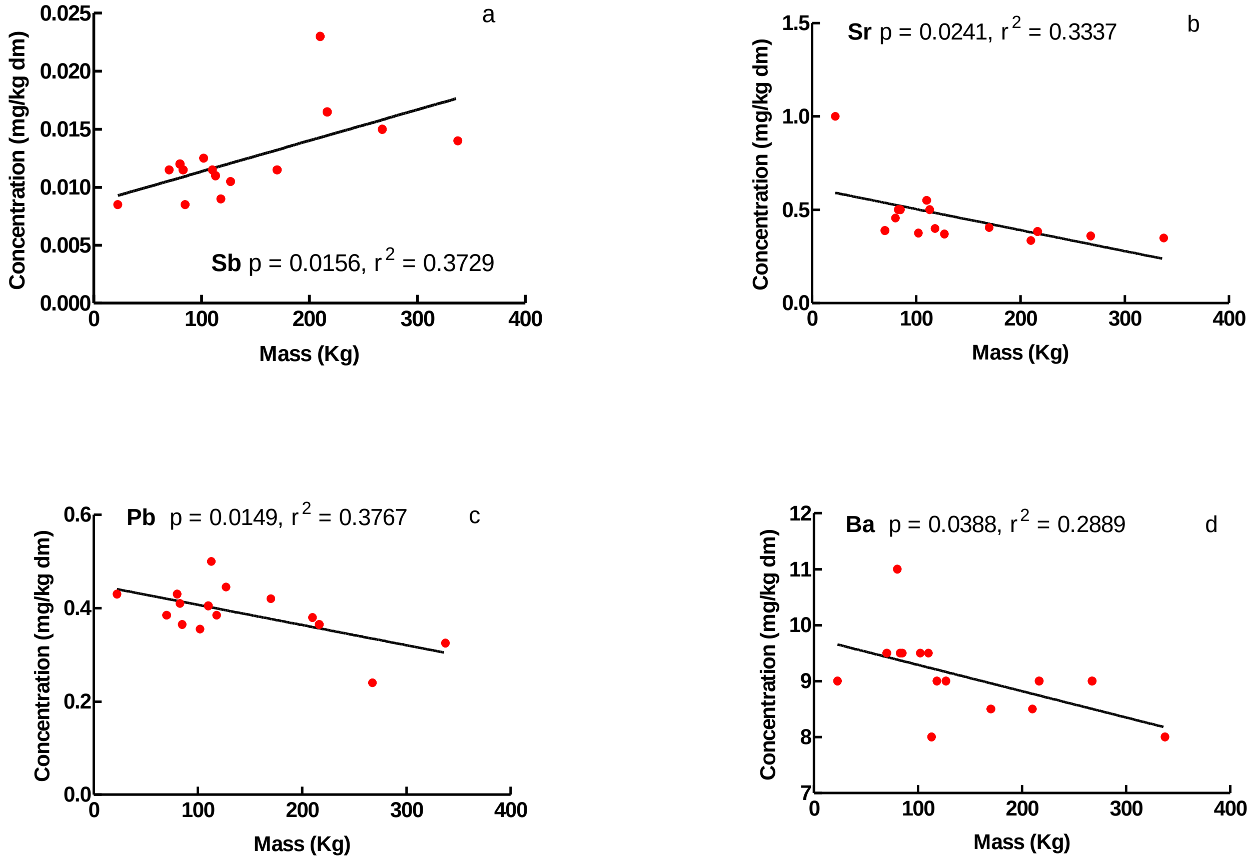

Figure 3.4-1: Regressions of normally distributed elemental concentrations in abdominal fat with crocodile mass. (a) Antimony, (b) strontium, (c) lead, (d) barium.

3.4.2 Muscle tissue

Log

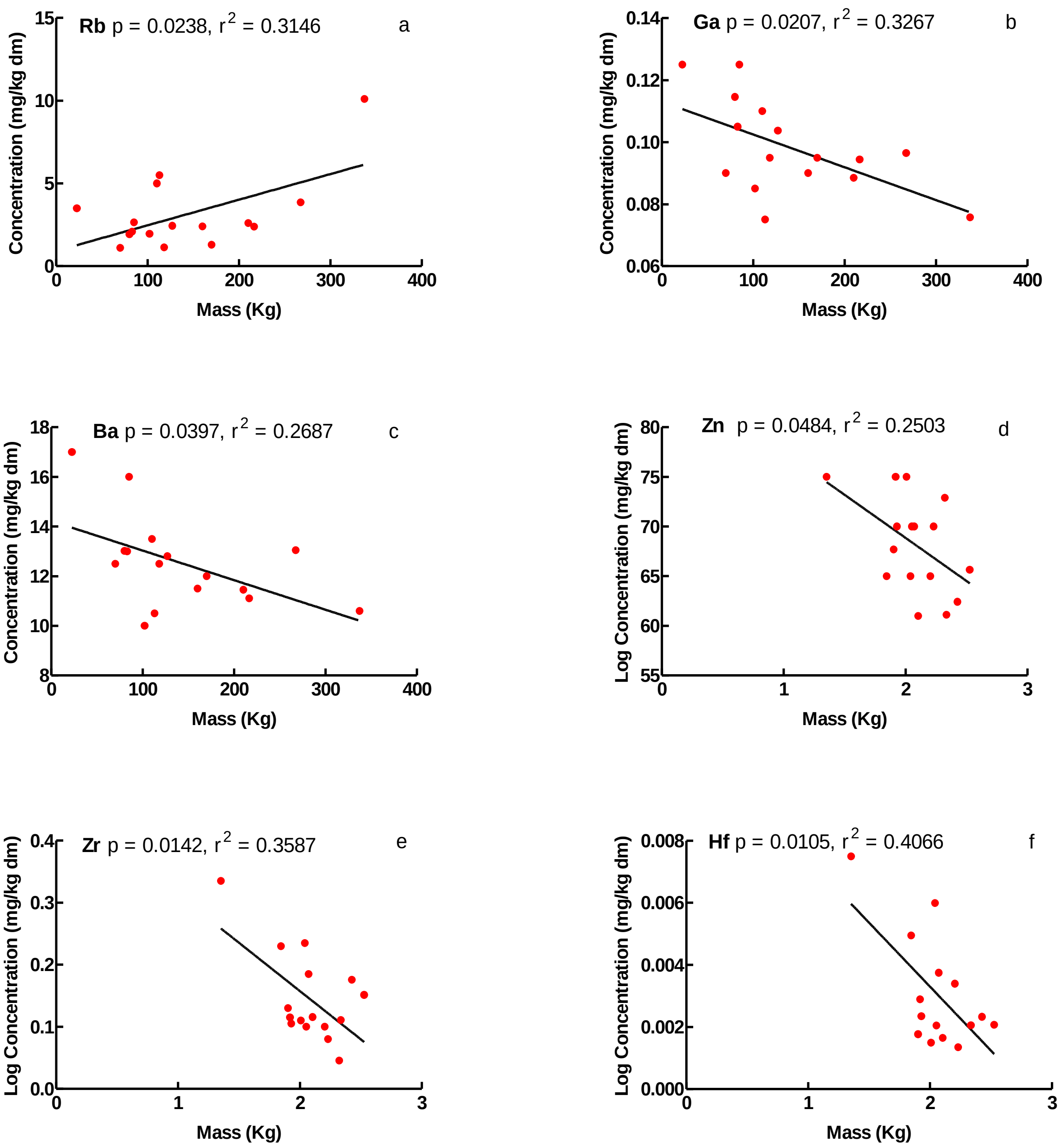

Figure 3.4-2: Regressions of normally distributed elemental concentrations in muscle tissue with crocodile mass. (a) Rubidium, (b) gallium, (c) barium, (d) zinc, (e) zirconium, (f) hafnium. Please note that the x-axes of Zn, Zr, and Hf are on a log-scale.

3.4.3 Kidney tissue

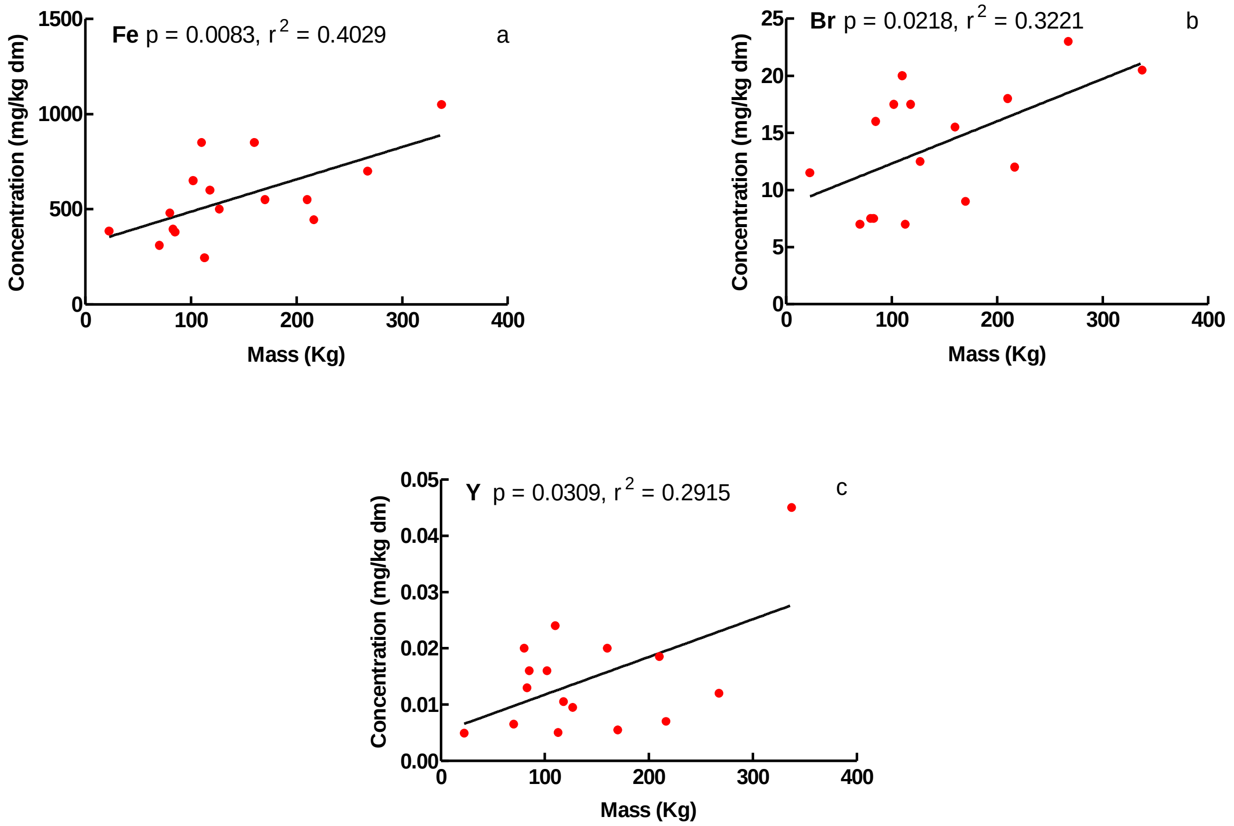

Figure 3.4-3: Regressions of normally distributed elemental concentrations in kidney tissue with crocodile mass. (a) Iron, (b) bromine, (c) yttrium.

3.4.4 Liver tissue

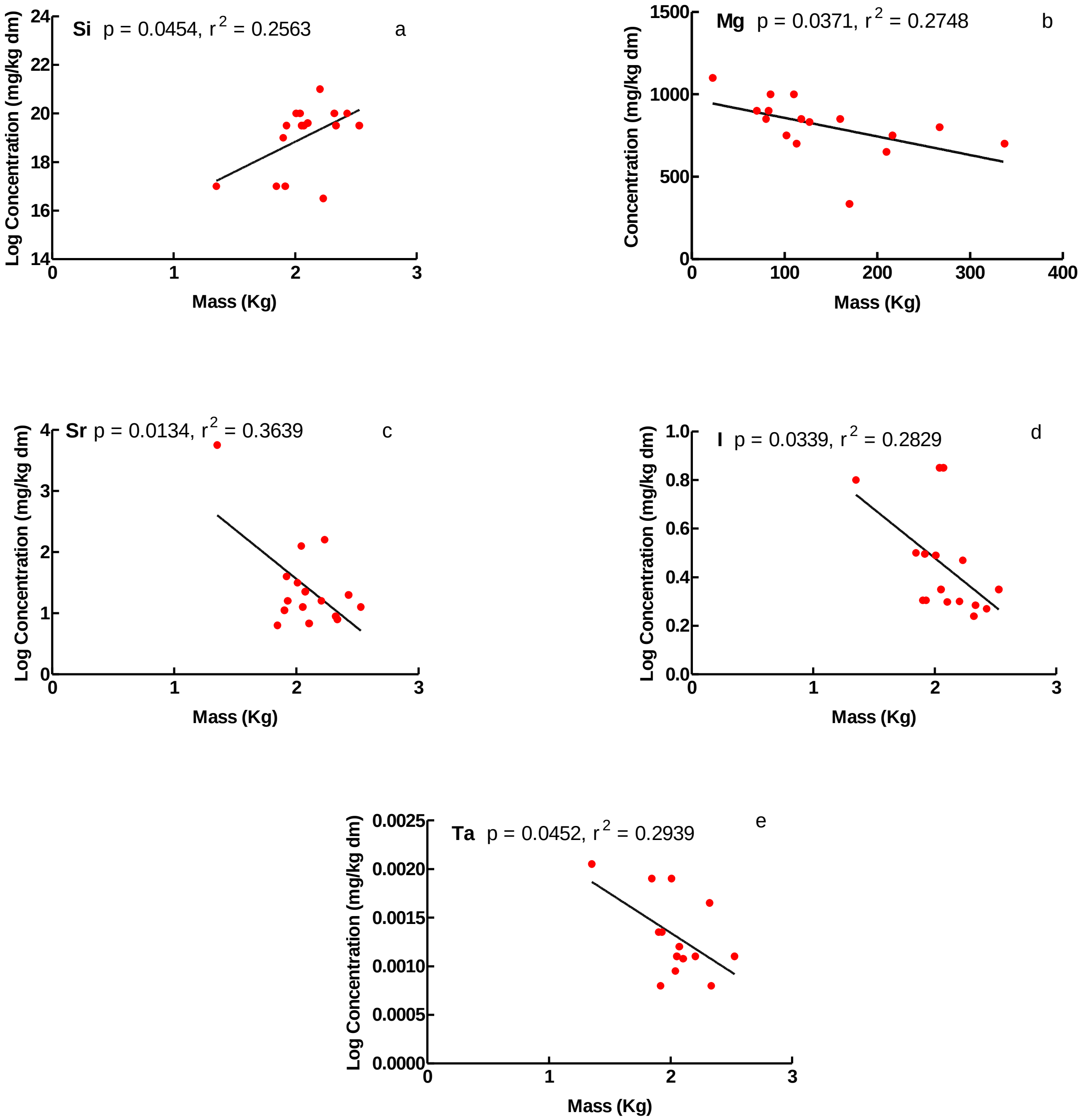

Figure 3.4-4: Regressions of normally distributed elemental concentrations in liver tissue with crocodile mass. (a) Silicon, (b) magnesium, (c) strontium, (d) iodine, (e) tantalum. Please note that the x-axes for silicon, strontium, iodine, and tantalum are on a log-scale.

3.4.5 Tail fat

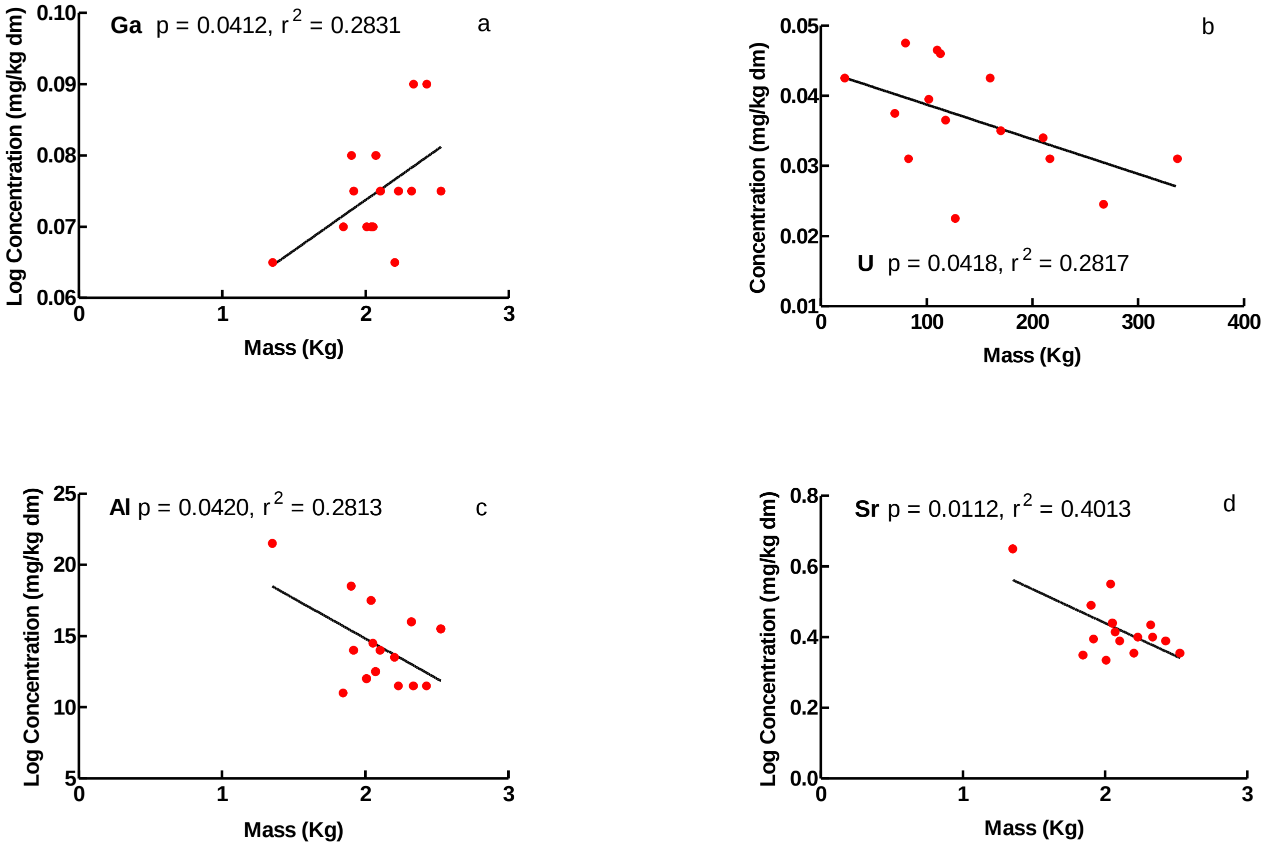

Figure 3.4-5: Regressions of normally distributed elemental concentrations in tail fat with crocodile mass. (a) Gallium, (b) uranium, (c) aluminium, (d) strontium. Please note that the x-axes for gallium, aluminium, and strontium are on a log-scale.

Abdominal fat:

Four elements, namely Sb (Fig. 3.4-1a, $p = 0.0156$), Sr (Fig. 3.4-1b, $p = 0.0241$), Pb (Fig. 3.4-1c, $p = 0.0149$), and Ba (Fig. 3.4-1d, $p = 0.0388$) had significant regressions between mass and elemental concentrations. Of these four regressions, only one, namely Sb (Fig. 3.4-1a) had a positive association between mass and concentrations. The remaining three, namely Sr, Pb and Ba (Figs.3.4-1b-d) had a negative association between mass and concentrations. Compared to all regressions of abdominal fat elemental concentrations and mass, Pb indicated the best coefficient of determination ($r^2 = 0.3767$), narrowly better than Sr ($r^2 = 0.3337$).

Muscle tissue:

Six elements, namely Rb (Fig. 3.4-2a, $p = 0.0238$), Ga (Fig. 3.4-2b, $p = 0.0207$), Ba (Fig. 3.4-2c, $p = 0.0397$), Zn (Fig. 3.4-2d, $p = 0.0484$), Zr (Fig. 3.4-2e, $p = 0.0142$), and Hf (Fig. 3.4-2f, $p = 0.0105$) showed significant regressions between crocodile mass and elemental concentrations in muscle tissue. Out of all regressions, only one, namely Rb (Fig 3.4-2a) had a positive association between crocodile mass and concentrations. The remainder of the elements, namely Ga, Ba, Zn, Zr, and Hf (Fig. 3.4-2b-f), had negative associations between crocodile mass and concentrations. Although all the regressions of elemental concentrations in muscle tissue and crocodile mass had relatively good coefficients of determination, Hf (Fig. 3.4-2f) had the best coefficient of determination ($r^2 = 0.4066$) of all statistically significant regressions. Hafnium also displayed the steepest gradient showing that the lightest crocodile had an almost four times higher concentration of Hf than the heaviest crocodile.

Kidney tissue:

Only three elements, namely Fe (Fig. 3.4-3a, $p = 0.0083$), Br (Fig. 3.4-3b, $p = 0.0218$), and Y (Fig. 3.4-3c, $p = 0.0309$) showed significant regressions between elemental concentrations in kidney tissue and crocodile mass. All three these elements indicated a positive association with crocodile mass and however all indicated relatively good coefficients of determination, Fe (Fig. 3.4-3a) indicated the best ($r^2 = 0.4029$). The heaviest crocodile had twice the Fe concentration than the lightest. Yttrium (Fig. 3.4-3c) also had a steep gradient with the heaviest crocodile having almost five times the Y concentration than the lightest crocodile.

Liver tissue:

Five elements, namely Si (Fig. 3.4-4a, $p = 0.0454$), Mg (Fig. 3.4-4b, $p = 0.0371$), Sr (Fig. 3.4-4c, $p = 0.0134$), I (Fig. 3.4-4d, $p = 0.0339$), and Ta (Fig. 3.4-4e, $p = 0.0452$) showed significant regressions between elemental concentrations in liver tissue and crocodile mass. Of all above-mentioned regressions, only one, namely Si (Fig. 3.4-4a) indicated a positive association between elemental concentration and crocodile mass. The remaining four elements, namely Mg, Sr, I, and

Ta (Figs. 3.3-4b-e) indicated negative associations between concentrations and crocodile mass. Although all elements showed relatively the same coefficients of determination, Sr had the best fit of the points to the regression line with an r^2 of 0.3639 (Fig. 3.4-4c). Sr, I, and Ta (Fig. 3.4-4c-e) indicated strong negative gradients. The lightest crocodile had almost four times the strontium, iodine and tantalum concentrations than the heaviest crocodile.

Tail fat:

Four elements, namely Ga (Fig. 3.4-5a, $p = 0.0412$), U (Fig. 3.4-5b, $p = 0.0418$), Al (Fig. 3.4-5c, $p = 0.0420$), and Sr (Fig. 3.4-5d, $p = 0.0112$) showed significant regressions between elemental concentrations in tail fat and crocodile mass. One of these regressions, namely Ga (Fig. 3.4-5a) indicated a positive association between concentrations and crocodile mass. The rest of the elements, namely U, Al, and Sr (Figs. 3.4-5b-d) showed negative associations between concentrations and crocodile mass. Although most the elements indicated a relatively similar coefficient of determination, strontium (Fig. 3.4-5d) showed the best fit of the points to the regression line with an r^2 of 0.4013. The lightest crocodile had almost double the Sr levels of the heaviest crocodile. Al also indicated a strong gradient with the lightest crocodile which had almost double the Al concentration in comparison with the heaviest crocodile.

Summary of associations:

All negative associations: Negative associations of crocodile mass with elemental concentrations were found in abdominal fat – Sr, Pb, and Ba (Figs. 3.4-1b-d); muscle tissue – Ga, Ba, Zn, Zr, Hf (Figs. 3.4-2b-f); liver tissue – Mg, Sr, I, and Ta (Figs. 3.4-4b-e); and tail fat – U, Al, and Sr (Figs. 3.4-5b-d). Sr shows once again significant negative associations with mass in abdominal and tail fat as well as in liver tissue. No positive associations of Sr concentrations and mass were found.

All positive associations: A grouping of all the positive associations of crocodile mass and elemental compositions were found in abdominal fat – Sb (Fig. 3.4-1a); kidney tissue – Fe, Br, and Y (Figs. 3.4-2a-c); liver tissue – Si (Fig. 3.4-4a); and tail fat – Ga (Fig. 3.4-5a).

Of all the negative associations (15 counted), three, namely Mg, Sr, and Ba are categorised in the same group (Group 2) namely the alkaline earth metals; four elements, namely Zn, Zr, Hf and Ta are categorised as transitional metals; three elements, namely Al, Ga, and Pb are categorised as post-transitional metals; one element namely I in the halogens category; and one element namely U in the actinoides category. Of all the positive associations (six), only two elements namely, Fe and Y are in the Transitional Metals category; two elements namely Si and Sb are categorised as Metalloids; one element namely Ga categorised as Post-Transitional Metals; and one element, Br categorised as a Halogen. The highest coefficient of determination of the entire group was found for Hf (Fig. 3.4-2f) with an r^2 of 0.4066).

3.5 DIFFERENCES IN ELEMENTAL CONCENTRATIONS BETWEEN MALES AND FEMALES

P-values of the Mann-Whitney tests of elemental concentrations compared between male and female crocodiles are given in Table 3-5. As mentioned previously, of the sixteen crocodiles sampled, eight were female and eight were male. During this analysis of elemental concentrations and male and female comparisons, the Mann-Whitney unpaired test was used to compare the means. There were very few significant differences between the sexes. Only the log-transformed data of Sn ($p = 0.0329$) in muscle tissue; the untransformed data of As ($p = 0.7518$) and Cu ($p = 0.0306$) in kidney tissue; and the untransformed data of Zn ($p = 0.0014$) in liver tissue were different.

Table 3-5: Male vs Female Mann-Whitney p-values summary

Ab Fat: Male vs Female	Log Li	B	Log Al	Si	Sc	Log Ti	V
Mann-Whitney test p-value	0.0798	0.8150	0.4871	0.7032	0.2180	0.9537	0.8145
	As	**Se**	**Rb**	**Log Sr**	**Mo**	**Sn**	**Cs**
	0.4150	0.1828	0.3969	0.5230	0.6019	0.8142	0.3056
	Cr	**Mn**	**Log Fe**	**Co**	**Ni**	**Cu**	**Zn**
	0.9510	0.9523	0.5621	0.9537	1.0000	0.3227	0.4855
	Ba	**Log Pt**	**Log Au**	**Hg**	**Tl**	**Pb**	**Log U**
	0.4341	0.4851	0.6126	0.7709	1.0000	0.2463	0.4871

Muscle: Male vs Female	Log Li	B	Al	Si	Sc	Ti	V
Mann-Whitney test p-value	0.9580	0.5283	1.0000	0.5606	0.5632	0.7909	0.3703
	As	**Se**	**Log Rb**	**Sr**	**Mo**	**Log Sn**	**Cs**
	0.6363	0.2244	0.5054	1.0000	0.9163	**0.0329**	0.0659
	Cr	**Mn**	**Fe**	**Log Co**	**Ni**	**Cu**	**Zn**
	0.9581	0.5630	0.1139	0.4418	0.3439	0.7518	0.0554
	Ba	**Pt**	**Au**	**Hg**	**Tl**	**Pb**	**Log U**
	0.5283	0.5054	0.5737	0.4418	0.7209	0.3823	1.0000

Kidney: Male vs Female	**Li**	**B**	**Log Al**	**Si**	**Sc**	**Ti**	**V**
Mann-Whitney test p-value	0.7518	0.0662	0.9591	0.7061	0.6693	0.1386	0.1605
	As	**Log Se**	**Log Rb**	**Log Sr**	**Mo**	**Sn**	**Cs**
	0.7518	1.0000	0.9581	0.5990	0.8332	0.1733	0.9162
	Cr	**Mn**	**Fe**	**Log Co**	**Log Ni**	**Cu**	**Zn**
	0.9573	0.7489	0.1886	0.0238	0.5054	**0.0306**	0.3681
	Ba	**Pt**	**Log Au**	**Log Hg**	**Tl**	**Log Pb**	**Log U**
	0.9155	0.7518	0.2135	1.0000	0.5982	0.9161	0.5630

Liver: Male vs Female	[1]**Log Li**	**Log B**	**Log Al**	**Si**	**Sc**	**Ti**	**V**
Mann-Whitney test p-value	0.2680	0.4926	0.9581	0.7876	0.3414	0.6731	0.5627
	As	**Se**	**Log Rb**	**Log Sr**	**Log Mo**	**Sn**	**Log Cs**
	0.3699[2]	1.0000	0.4619	0.8746	0.7525	1.0000	0.1013
	Log Cr	**Mn**	**Fe**	**Log Co**	**Ni**	**Cu**	**Zn**
	0.4877	0.5992	0.2072	0.0585	0.5954	0.6355	**0.0014**
	Ba	**Log Pt**	**Log Au**	**Hg**	**Tl**	**Log Pb**	**Log U**
	0.8335	0.5274	0.4295	0.5283	1.0000	0.3442	0.4926

[1] Log indicates that normal untransformed data was not significant and therefore log-transformed data were used instead.
[2] Items indicated in red refer to no statistical difference of any kind.

Tail Fat: Male vs Female

Mann-Whitney test p-value

Li	B	Al	Si	Sc	Ti	V
0.9536	1.0000	0.3541	0.7773	0.3554	0.3493	0.3120
As	**Se**	**Log Rb**	**Sr**	**Mo**	**Sn**	**Cs**
0.6838	0.3244	0.8168	1.0000	0.8158	0.0657	0.5163
Cr	**Mn**	**Log Fe**	**Co**	**Ni**	**Log Cu**	**Log Zn**
0.5113	0.6836	1.0000	0.5611	0.5949	0.8168	0.6019
Ba	**Pt**	**Au**	**Log Hg**	**Tl**	**Pb**	**U**
0.3489	0.8168	0.6846	0.5582	0.0708	0.3820	0.0716

3.6 MULTIVARIATE ANALYSES

Nonmetric Multidimensional Scaling (NMDS) was used to illustrate the results. This indirect gradient analysis produced ordinations based on a distance and dissimilarity matrix. The illustrated ordinations are for two groupings, namely crocodiles with indications of pansteatitis, and between the sexes. Pansteatitis ordinations illustrate differences between suspected pansteatitis affected (see Table 3.1) and healthy crocodiles. Sex ordinations illustrate differences between male and female crocodiles. Mass and length were used as co-variants. Heart / fatbody ratios and heart / spleen ratios are included for completeness, but will not be further discussed.

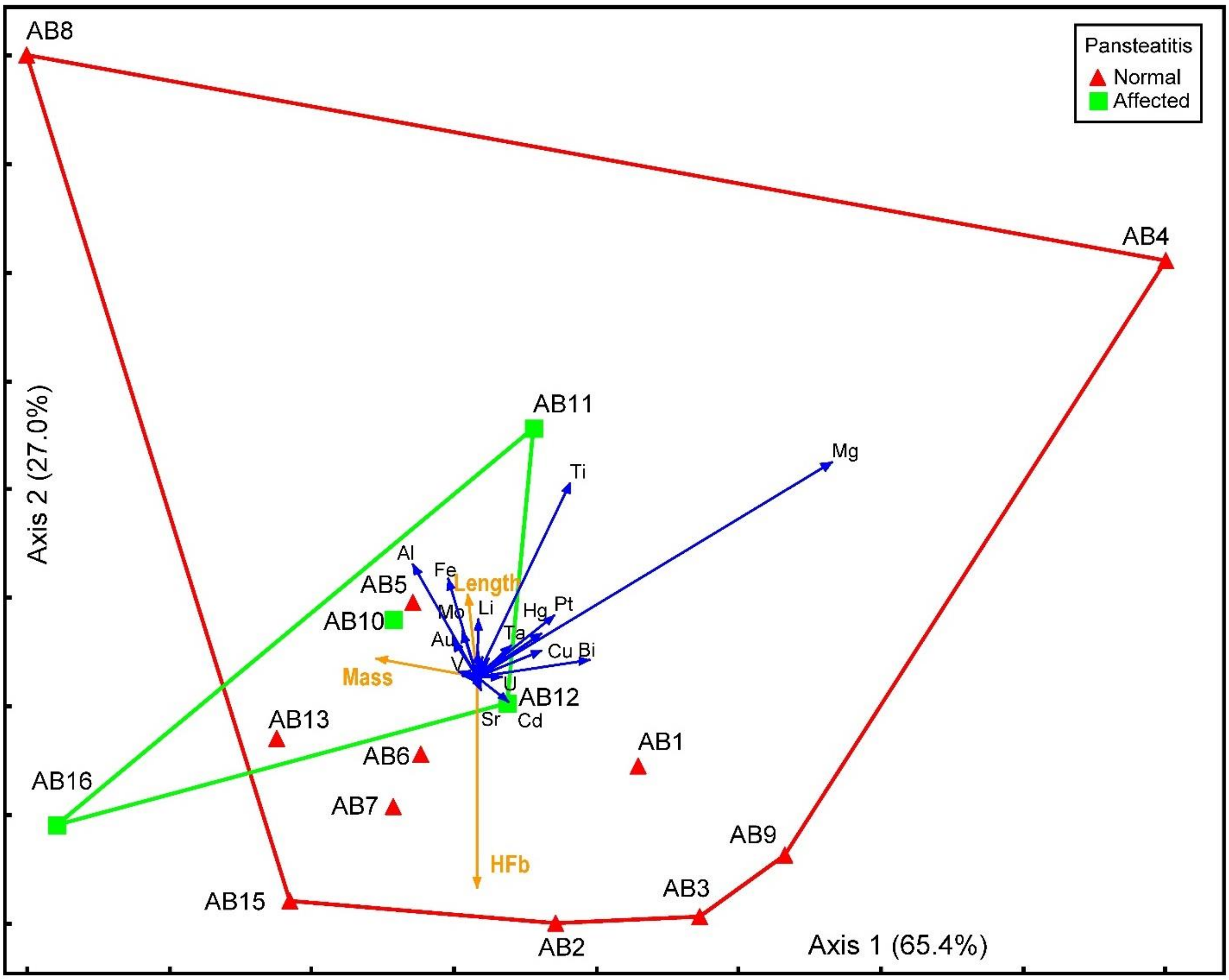

Figure 3.5: Nonmetric multidimensional scaling (NMS) of revitalised (by element) concentration data of abdominal fat compared between pansteatitis affected and normal animals. HFb = Heart / Fat body ratio

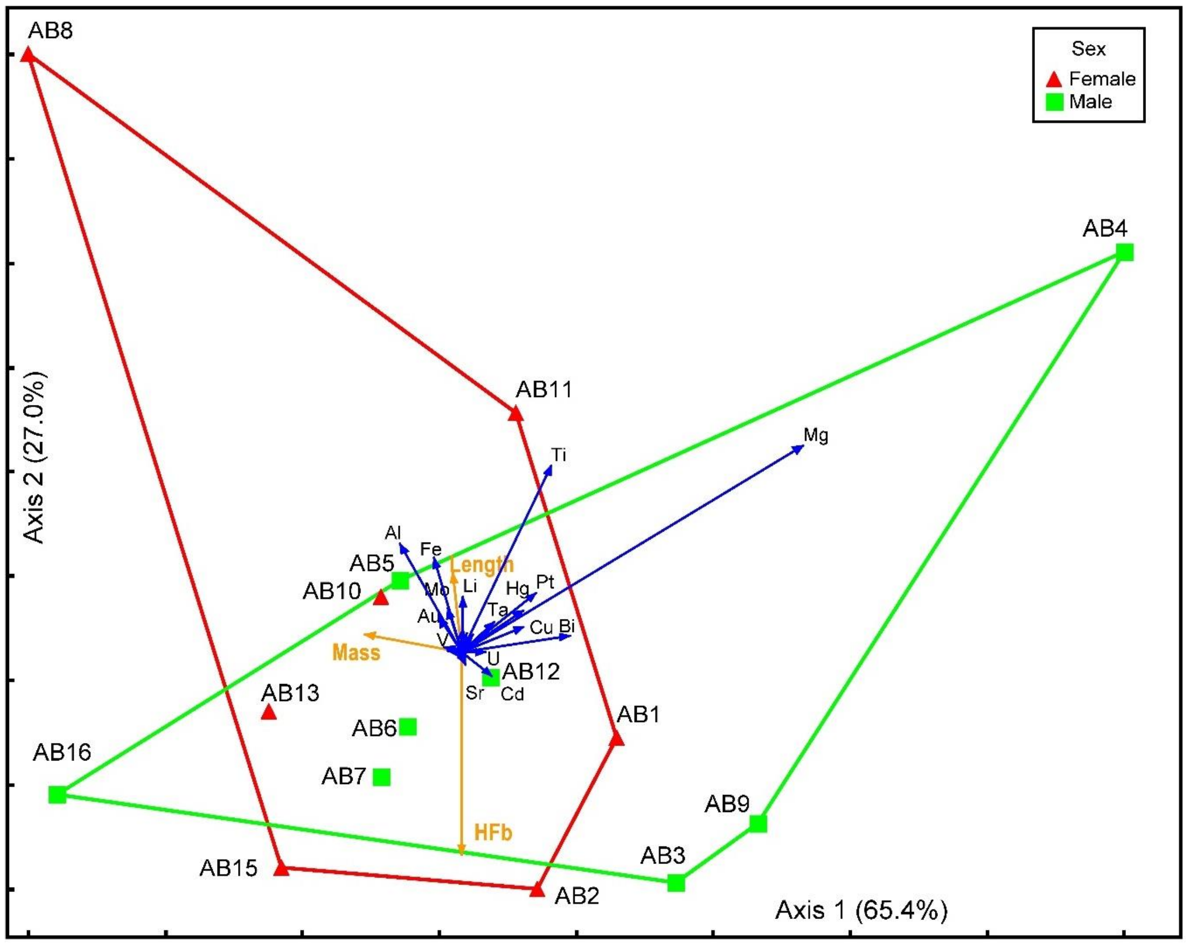

Figure 3.6: Nonmetric multidimensional scaling (NMS) of revitalised (by element) concentration data of abdominal fat compared between male and female animals. HFb = Heart / Fat body ratio

Only two dimensions were needed to ordinate elements in abdominal fat and gender (Figs. 3.5, 3.6). Final stress was 10.49, and the final instability was 0.00000. Axis 1 represents 65.4% of the variation, and axis 2 represents 27.0%. The descriptive variables are shown as orange vectors. Convex hulls for the two different groupings are shown. The convex hulls for the pansteatitis affected and non-affected individuals (Fig. 3.5), and between the sexes (Fig.3.6) overlapped.

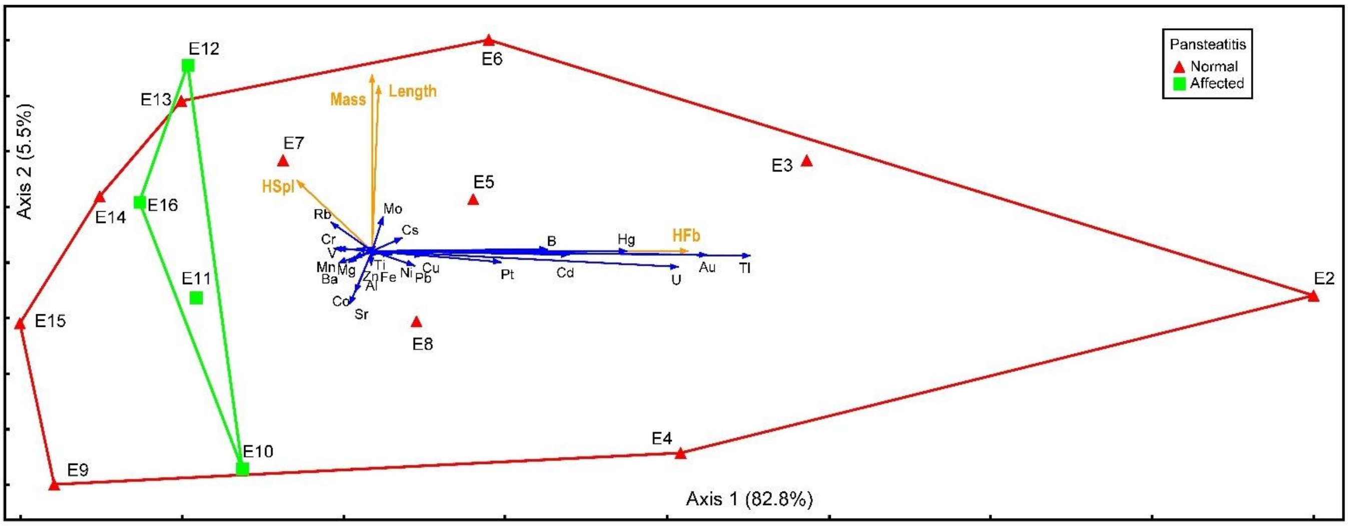

Figure 3.7: Nonmetric multidimensional scaling (NMS) of revitalised (by element) concentration data of muscle tissue compared between pansteatitis affected and normal animals. HFb = Heart / Fat body ratio; HSpl = Heart / Spleen ratio.

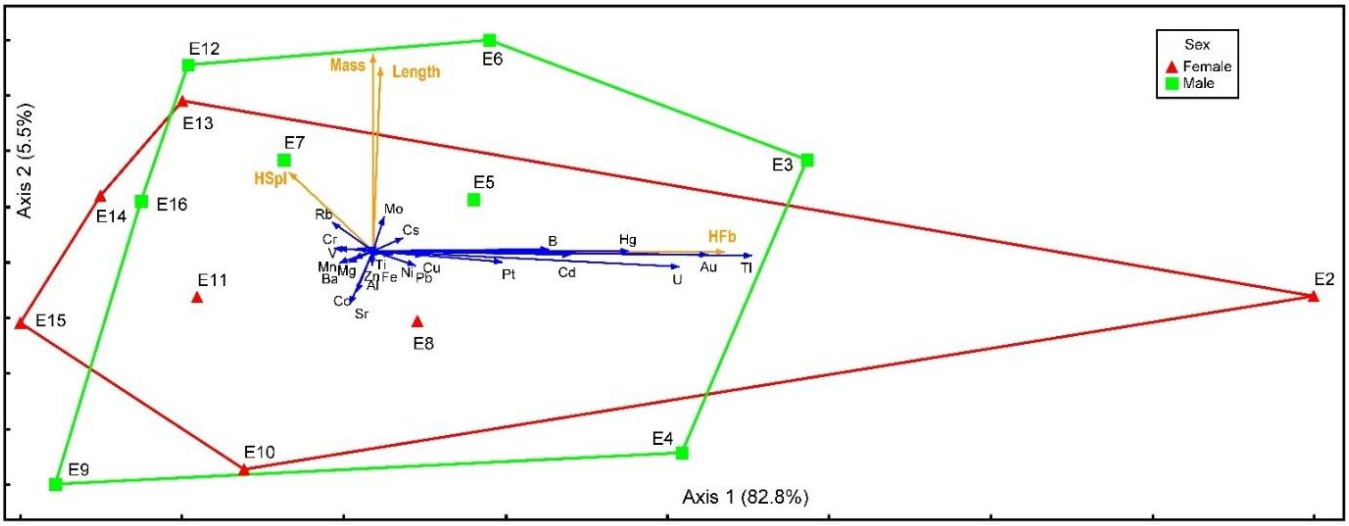

Figure 3.8: Nonmetric multidimensional scaling (NMS) of revitalised (by element) concentration data of muscle tissue compared between male and female animals. HFb = Heart / Fat body ratio; HSpl = Heart / Spleen ratio

Only two dimensions were needed to ordinate elements in muscle tissue and gender (Figs. 3.7, 3.8). Final stress was 12.07, and the final instability was < 0.00001. Axis 1 represents 82.8% of the variation, and axis 2 represents 5.5%. The descriptive variables are shown as orange vectors. Convex hulls for the two different groupings are shown. The convex hulls for the pansteatitis affected and non-affected individuals (Fig. 3.7), and between the sexes (Fig.3.8) overlapped.

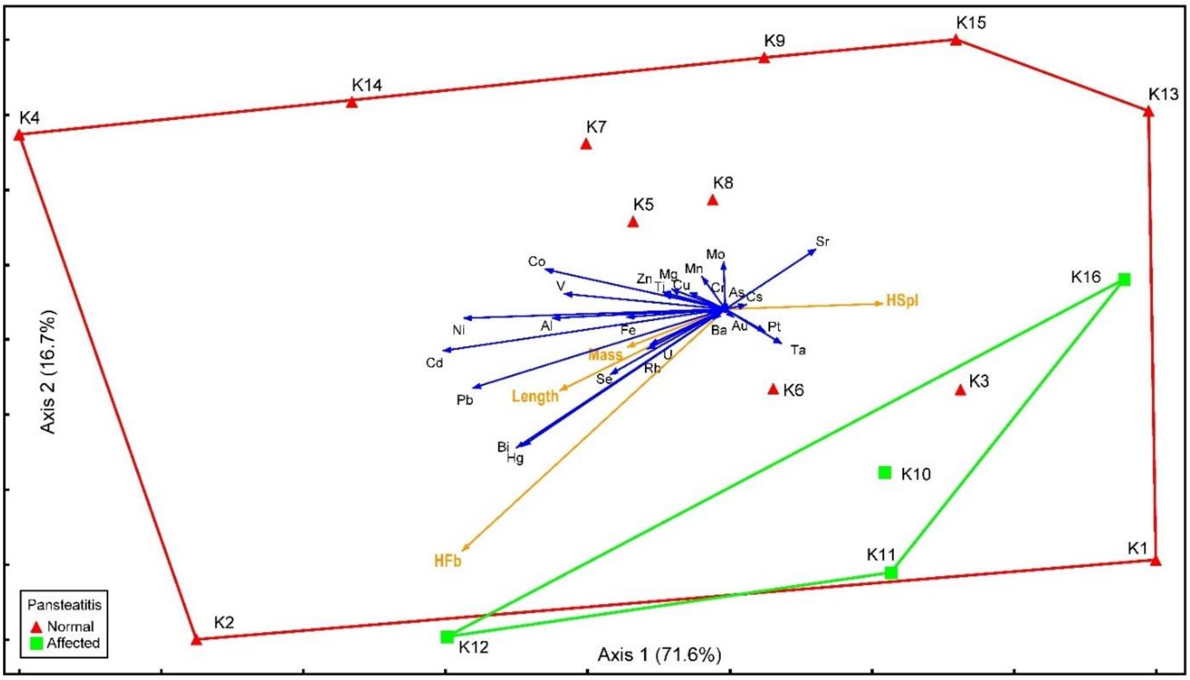

Figure 3.9: Nonmetric multidimensional scaling (NMS) of revitalised (by element) concentration data of muscle tissue compared between pansteatitis affected and normal animals. HFb = Heart / Fat body ratio; HSpl = Heart / Spleen ratio.

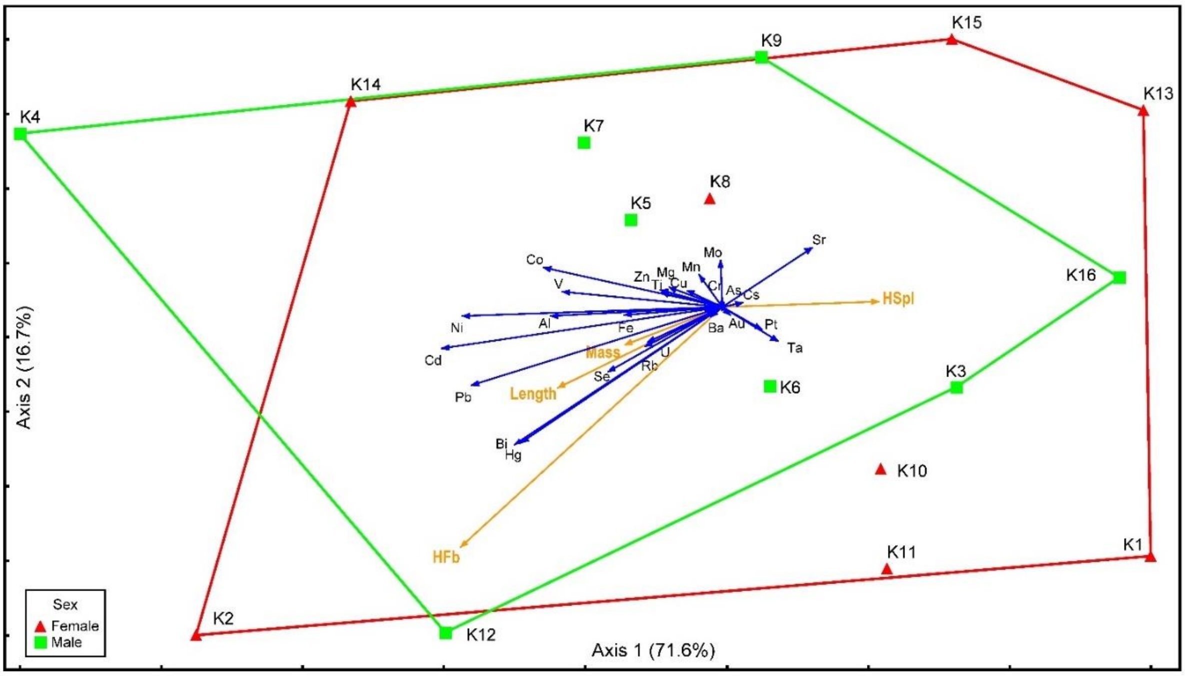

Figure 3.10: Nonmetric multidimensional scaling (NMS) of revitalised (by element) concentration data of kidney tissue compared between male and female animals. HFb = Heart / Fat body ratio; HSpl = Heart / Spleen ratio

Only two dimensions were needed to ordinate elements in kidney tissue and gender (Figs. 3.9, 3.10). Final stress was 12.24, and the final instability was < 0.00001. Axis 1 represents 71.6% of the variation, and axis 2 represents 16.7%. The descriptive variables are shown as orange vectors. Convex hulls for the two different groupings are shown. The convex hulls for the pansteatitis affected and non-affected individuals (Fig. 3.9), and between the sexes (Fig. 3.10) overlapped.

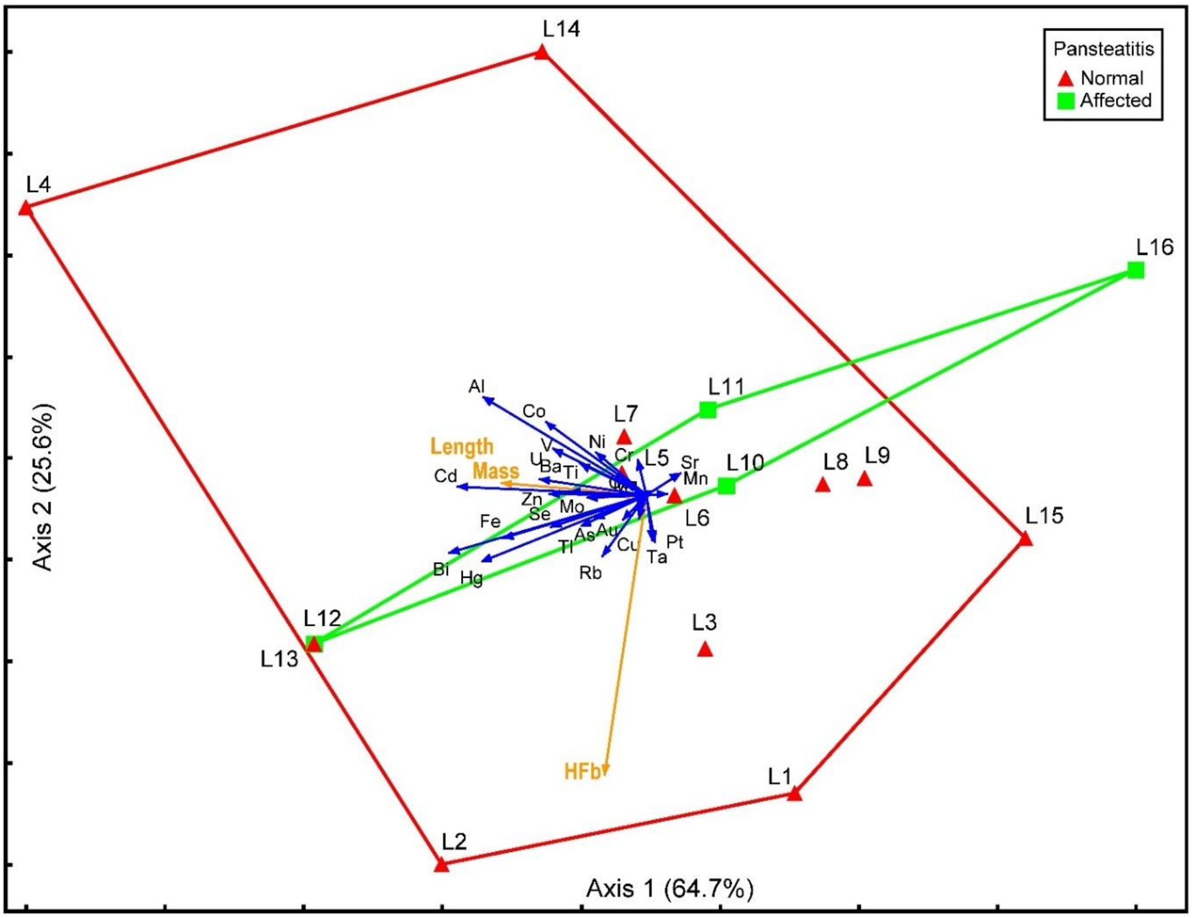

Figure 3.11: Nonmetric multidimensional scaling (NMS) of revitalised (by element) concentration data of liver tissue compared between pansteatitis affected and normal animals. HFb = Heart / Fat body ratio; HSpl = Heart / Spleen ratio.

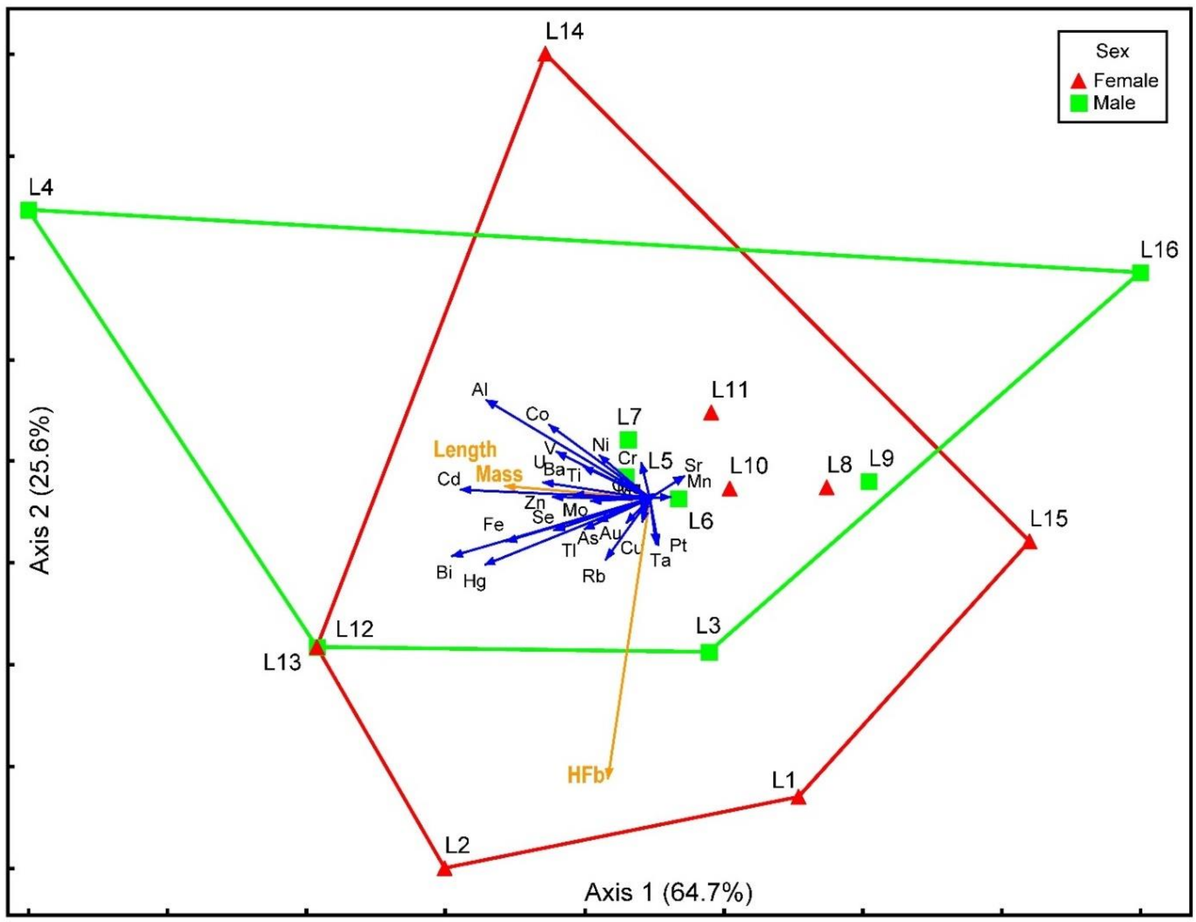

Figure 3.12: Nonmetric multidimensional scaling (NMS) of revitalised (by element) concentration data of liver tissue compared between male and female animals. HFb = Heart / Fat body ratio; HSpl = Heart / Spleen ratio

Only two dimensions were needed to ordinate elements in liver tissue and gender (Figs. 3.11, 3.12). Final stress was 10.59, and the final instability was < 0.00001. Axis 1 represents 64.7% of the variation, and axis 2 represents 25.6%. The descriptive variables are shown as orange vectors. Convex hulls for the two different groupings are shown. The convex hulls for the pansteatitis affected and non-affected individuals (Fig. 3.11), and between the sexes (Fig. 3.12) overlapped.

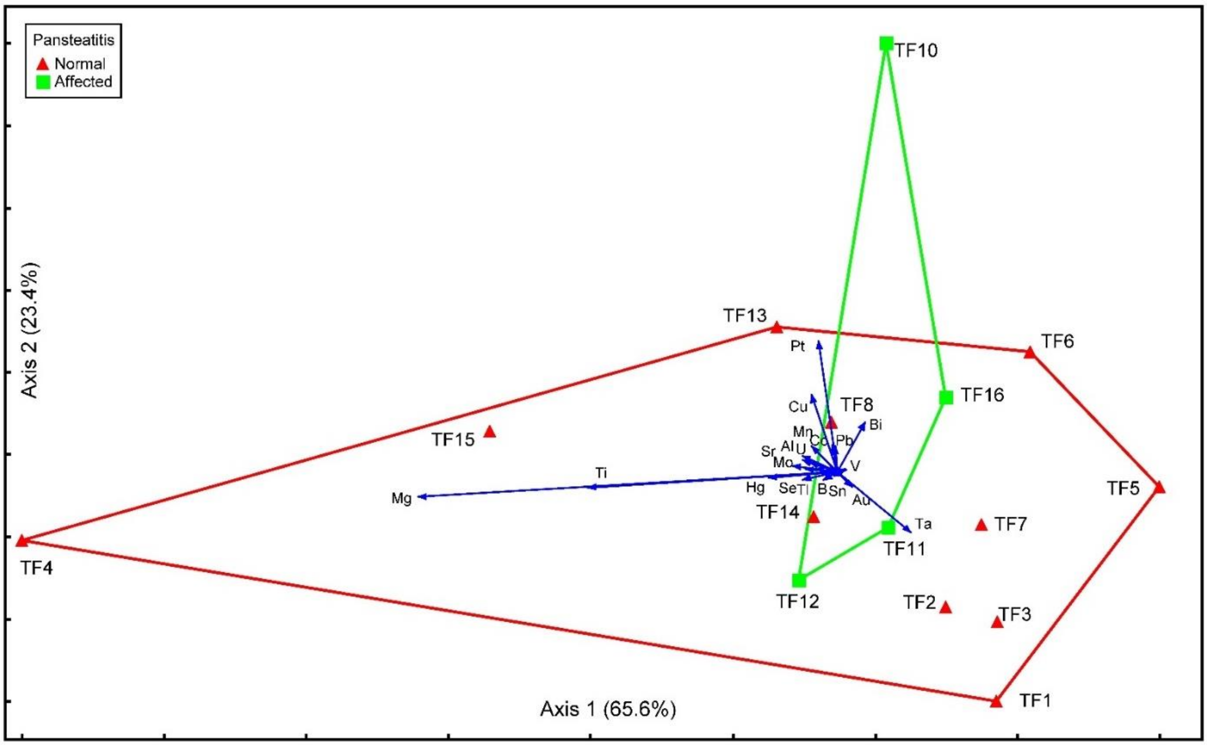

Figure 3.13: Nonmetric multidimensional scaling (NMS) of revitalised (by element) concentration data of tail fat compared between pansteatitis affected and normal animals. HFb = Heart / Fat body ratio; HSpl = Heart / Spleen ratio.

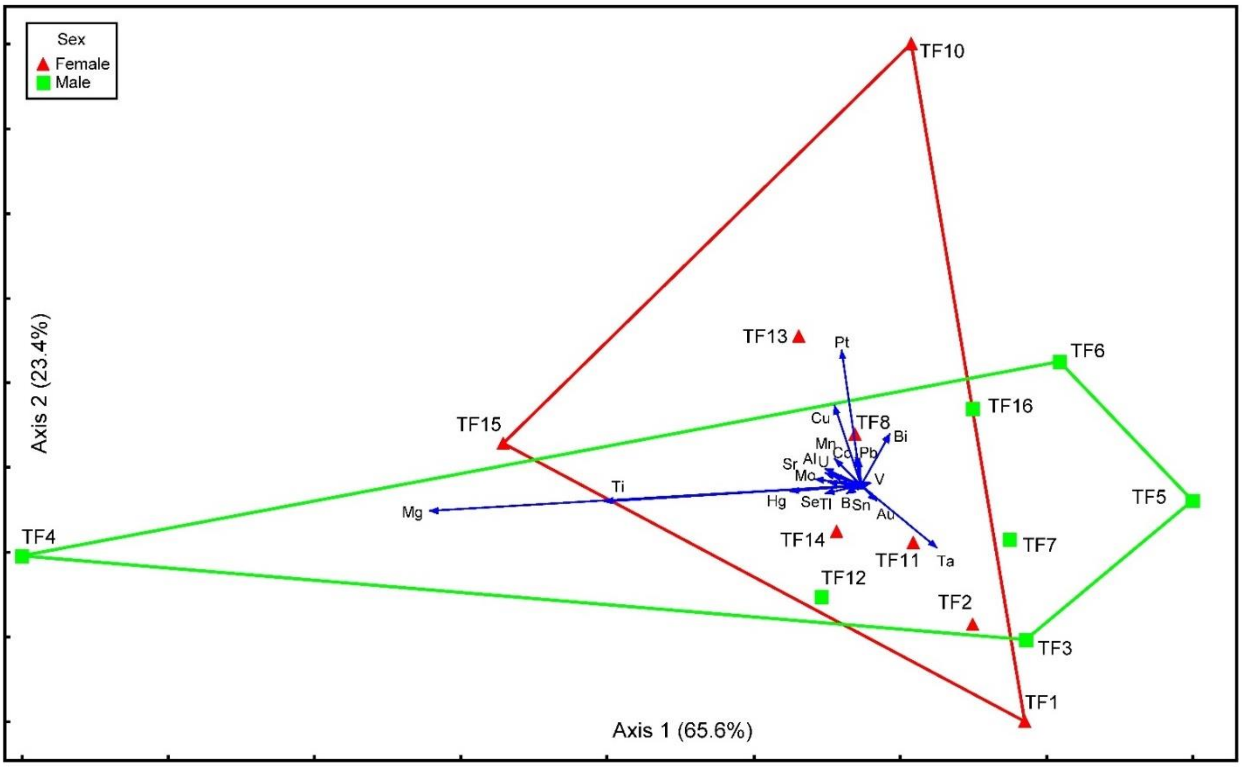

Figure 3.14: Nonmetric multidimensional scaling (NMS) of revitalised (by element) concentration data of tail fat compared between male and female animals. HFb = Heart / Fat body ratio; HSpl = Heart / Spleen ratio.

Only two dimensions were needed to ordinate elements in tail fat and gender (Figs. 3.13, 3.14). Final stress was 10.45, and the final instability was < 0.00001. Axis 1 represents 65.6% of the variation, and axis 2 represents 23.4%. The descriptive variables are shown as orange vectors. Convex hulls for the two different groupings are shown. The convex hulls for the pansteatitis affected and non-affected individuals (Fig. 3.13), and between the sexes (Fig. 3.14) overlapped.

3.7 COMPARISONS WITH LITERATURE

Some work has been done by other workers on elemental concentrations in crocodile tissues. This is provided in Table 3.6 and will be discussed in Chapter 4.

Table 3.6. Summary of comparative data published by others.

Croc. Spp	Tissue	Location	Year	Sample	Al	Ti	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Pd	Cd	Hg	Pb	References
Nile crocodile	Muscle	Crocodile farm, South Africa	2000	Wet	7				3				11							Hoffman, Fisher & Sales,2000
Nile crocodile	Muscle	Olifants River, KNP	2000	Dry	147.2		9.8	0.1	399.6		10.3	10.5	39.4			20.3			20.3	Swanepoel, Boomker & Kriek, 2000;
Nile crocodile	Muscle	Sabie River, KNP	2000	Dry	73.5		18.4	0.1	615.4		9.1	12.6	44.7			0			0	Swanepoel, Boomker & Kriek, 2000;
Nile crocodile	Muscle	Silvervis Dam, KNP	2000	Dry	376.8		90.5	17.8	156		24.9	7.9	109.7			3.7			3.7	Swanepoel, Boomker & Kriek, 2000;
Nile crocodile	Liver	Kafue River, Zambia	2004	Wet				1.4		0.02		5.7	18	0.008	1.8		0.04	3.5	8.7	Almli et al., 2005;
Nile crocodile	Liver	Luangwa River, Zambia	2004	Wet				1.1		0.05		4	31	0.049	2.3		0.04	3.7	3.3	Almli et al., 2005;
Alligator	Muscle	Louisiana, USA	2011	Dry					41.9			8.8	52.8							Gullory et al, 2011;
Chinese alligator	Muscle	Anhui Province, China	2006	Dry			0.155	2.68	67			6.4	120.97	0.306			0.155	0.193	0.73	Xu et al., 2006;
Estuarine crocodile	Muscle	Australia	2011	Dry	89.9	6.22	0.413	0.706	88.7c		0.507	1.14	81.4		0.993				0.308	Jeffree, Markich & Twining, 2011;
Alligator	Muscle	Lake Rodman, USA	1985	Dry			0.05		7.42			0.28	19				0.02	0.51	0.07	Delany, Bell & Sundlof, 1988;
Alligator	Muscle	Lake Hancock, USA	1985	Dry			0.05		8.45			0.34	25.5				0.01	0.1	0.1	Delany, Bell & Sundlof, 1988;
Alligator	Muscle	Lake Orange, USA	1985	Dry			0.05		17.7			0.39	27.88				0.06	0.37	0.04	Delany, Bell & Sundlof, 1988;
Alligator	Muscle	Lake Newnans, USA	1985	Dry			0.05		15.38			0.41	36				0.02	0.27	0.12	Delany, Bell & Sundlof, 1988;
Alligator	Muscle	Lake Apopka, USA	1985	Dry			0.03		4.56			0.4	14.2				0.03	0.11	0.07	Delany, Bell & Sundlof, 1988;
Alligator	Muscle	Lake George, USA	1985	Dry			0.06		22.76			6.03	2.17				0.03	0.04	0.09	Delany, Bell & Sundlof, 1988;
Alligator	Liver	Lake Apopka, USA	2014	Dry	13	0.0022	0.261	2.38	1770	0.0285		25.2	84.5	0.111	5.46		0.0869	1.76	2	Horai et al., 2014;
Alligator	Liver	Lake Woodruff, USA	2014	Dry	18.6	0.00221	0.385	2.97	3690	0.0334		26.7	86.5	0.122	11.2		0.0955	7.77	0.349	Horai et al., 2014

CHAPTER 4: DISCUSSION

4.1 INTRODUCTION

As discussed in Chapter 1, excess of metallic elements in tissues can be harmful to organisms. It can cause a series of problems including health issues for the organisms as well as people consuming these animals, cause disease, cause a decline in survival and fitness, and therefore raise mortality rates. This study was initiated to provide a better understanding of elemental concentrations within the tissues of healthy crocodiles.

Following are the discussions on the associations between elemental concentrations *vis a vis* the different tissues (Section 4.2), the associations of the elements with length and mass (Section 4.3), the association of elemental concentrations between the sexes (Section 4.4), the interpretation of the multivariate analyses (Section 4.5), comparisons of my results with the findings of others (Section 4.6), and the assessment of biopsies as a sampling method (Section 4.7),

4.2 ASSOCIATIONS BETWEEN ELEMENTAL CONCENTRATIONS AND ORGANS

The means of all the analysed elements are provided in Table 3.2. All elements are discussed in Chapter 1 and details regarding each element are provided in Tables 1.1. and 1.2.

The concentration data in Chapter 3, sections 3.1, and 3.2, for brevity and ease of interpretation has been summarised in Table 4.1. This table summarises those elements that did not differ significantly between tissues (bottom row).

- Most of the elements that were not statistically different between muscle and the other tissues were with kidney (31) and liver (34). Muscle tissue had no differences with 21 elements for both tail fat and abdominal fat.
- Kidney and liver shared 38 elements with no significant different concentrations, but only 10 and 16 with abdominal fat and tail fat, respectively.
- Only three elements were comparable between liver and tail fat, but had no significant different concentrations for 16 elements with abdominal fat.
- Tail fat and abdominal fat, on the other hand, had no differences whatsoever for any of the 47 elements thus compared.

It is clear that there is little pattern of prediction or consistency of concentrations between tissues, except between the two fatty tissues, and kidney and liver. To a lesser extent, muscle and liver, and muscle and kidney had corresponding concentrations. These tissues may therefore be useful when taking biopsies from live animals to determine pollutant loads in the crocodiles.

Table 4.1 also sums the elements with the least number of significant differences between any of the organs (right hand column).

- Of the 47 elements that had the least differences in concentrations between tissues were those with very low concentrations: Zr (18), Ta (17), Y, (14), and Ga (14). This may probably due to their concentrations being close to their detection limits.
- Elements with the most differences were Be (7), Na (7), and Ti (7).

There was little pattern between concentrations of elements between organs. Those with low concentrations may probably be accorded less importance due to analytical restrictions and a naturally low environmental occurrence that is superseded by differences in dietary preferences and life histories.

Combined, Table 4.1 can be used to select the required tissue sample to best assess elemental concentrations for specific elements, but there is no single organ that will be useful to extrapolate all elements to all other organs.

This was also the finding of Nilsen *et al.* (2017), the only other comprehensive study I could find for crocodilians. Unfortunately, they presented the data on a wet mass basis and they had a very different approach, so concentrations and findings cannot be directly compared. While I did not look at correlations between different elemental concentrations (thereby predicting the concentration of one element from another of which they found but a few), Nilsen *et al.* (2017) also found variable concentrations between organs for the elements they measured. The proportions of the elemental distributions seem to differ as much as what was found for the present study.

Table 4.1. The concentrations of elements that did not differ significantly (p > 0.05, Dunn's test from Table 3.3-1) between tissues.

	Muscle				Kidney				Liver				Tail fat				Abdominal fat				
	K	L	TF	AF	M	L	TF	AB	M	K	TF	AF	M	L	K	AF	M	K	L	TF	**Count**
Li	x	x			x	x			x	x						x				x	**4**
Be		x				x	x	x	x							x		x		x	**7**
B		x				x		x	x	x						x		x		x	**8**
Na	x				x							x				x			x	x	**6**
Mg	x	x			x	x			x	x						x				x	**8**
Al			x	x		x				x			x			x	x			x	**8**
Si	x			x	x	x				x						x	x			x	**8**
K	x	x			x	x			x	x						x				x	**8**
Ca		x				x			x	x						x				x	**6**
Sc	x	x			x	x		x	x	x						x		x	x	x	**11**
Ti	x			x	x	x				x						x				x	**7**
V		x				x	x		x	x	x			x	x	x	x			x	**11**
Cr	x	x			x	x		x	x	x		x				x		x	x	x	**12**
Mn	x	x		x	x	x			x	x						x				x	**9**
Fe	x	x			x	x			x	x						x	x			x	**9**
Co		x	x	x		x			x	x			x			x				x	**9**
Ni	x	x	x		x				x				x			x	x			x	**9**
Cu	x	x	x		x	x			x	x			x			x				x	**10**
Zn	x	x			x	x			x	x					x	x				x	**9**
Ga	x	x	x	x	x		x	x	x			x	x			x		x	x	x	**14**
Ge	x	x			x	x			x	x						x	x			x	**9**
As	x	x			x	x		x	x	x		x				x		x	x	x	**12**
Se	x	x			x	x			x	x						x				x	**8**
Br	x	x	x	x	x	x			x	x			x			x				x	**11**
Rb	x	x	x		x	x			x	x			x			x	x			x	**11**
Sr	x	x	x	x	x	x			x	x			x			x				x	**11**
Y	x	x	x	x	x	x			x	x		x	x			x	x		x	x	**14**
Zr		x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	**18**
Nb	x	x	x			x			x	x			x			x	x			x	**10**
Mo	x	x			x	x			x	x					x	x				x	**9**
Pd			x	x			x	x			x	x	x			x		x	x	x	**11**
Cd			x			x				x			x	x	x	x	x			x	**9**
Sn			x	x								x	x			x	x		x	x	**8**
Sb			x	x		x				x			x		x	x	x			x	**9**
I			x	x									x			x				x	**5**
Cs	x	x			x	x	x	x	x	x					x	x	x	x		x	**13**
Ba		x	x	x	x		x	x	x			x	x			x		x	x	x	**13**
Ce	x	x		x	x	x			x	x					x	x	x			x	**11**
Ta	x	x	x	x	x	x		x	x	x		x	x		x	x	x	x	x	x	**17**
W	x		x	x	x		x	x				x	x			x	x	x	x	x	**13**
Pt	x	x			x	x		x	x	x		x				x		x	x	x	**12**
Au		x				x		x	x	x		x				x		x	x	x	**10**
Hg	x			x	x	x				x		x				x	x			x	**9**
Tl		x				x	x	x	x	x		x				x		x	x	x	**11**
Pb	x		x	x	x	x	x			x			x		x	x	x			x	**12**
Bi			x	x		x				x			x			x	x			x	**8**
U	x	x			x	x		x	x	x		x				x		x	x	x	**12**
Count	**31**	**34**	**21**	**21**	**31**	**38**	**10**	**16**	**34**	**38**	**3**	**16**	**21**	**3**	**10**	**47**	**20**	**16**	**16**	**47**	

4.3 ASSOCIATIONS BETWEEN ELEMENTAL CONCENTRATIONS AND CROCODILE MASS AND LENGTH

Based on Section 3.3, there were surprisingly few significant regressions between elements and the two somatic metrics, crocodile mass and length. Table 4.2 summarises the results.

- Magnesium was significantly negatively associated with mass and length in liver tissue. So were Sr, and Ta.
- Strontium also decreased significantly in concentrations in abdominal fat with mass and length.
- Iron concentrations in the kidney increased significantly with mass and length, as did Y.
- Zink, Ga, Ba, and Hf decreased significantly with mass and length in muscle tissue, while Rb increased significantly.
- There were other elements that decreased or increased significantly with either length or mass in some organs, but not both.

Decreased concentrations may be due to dilution by growth (Newman and Heagler, 1991), while increased concentrations might be due to accumulation. Mercury, surprisingly, did not show any association between mass and length, both of which are indirect indicators of age. With increased age, higher concentrations would be expected.

Overall, there were surprisingly little direct associations of concentrations between mass and length. This may be due to differences in individual life histories of long-lived animals (Rowe, 2008) and feeding preferences.

Although Nilsen *et al.* (2017) found some indications of differences between age classes, these were not compelling since the interpretations were confounded by uneven sampling from very different locations.

There are factors that should be considered that might affect the results for this study. These include the sample size (although 16 crocodiles seems enough to get a good idea), differences in food preferences and behaviour, natural differences in background concentrations in the area where the crocodiles were shot, as well as pollution where the organisms live and feed (Schroeder *et al*., 1970; Friedland, 1990; Sarkar, 2002; Paustenbach and Galbraith, 2006; Bouwman *et al*., 2014; van der Shyff *et al*., 2016). However, the physiological regulation of elements should also be taken into consideration. Essential metals are more effectively regulated by the animals' physiology to maintain good body condition and allow growth and reproduction. Therefore, these elements would show less variation in concentrations when compared with nonessential metals (Linder and Grillitsch, 2000; Kenyon *et al.,* 2001).

Table 4.2. Results of significant regressions between elements with mass and crocodile length.

	Abdominal Fat		Muscle		Kidney		Liver		Tail Fat	
	Length	**Mass**	**Length**	**Mass**	**Length**	**Mass**	**Length**	**Mass**	**Length**	**Mass**
Li										
Be										
B										
Na										
Mg							N	N		
Al										N
Si								P		
K										
Ca										
Sc										
Ti										
V										
Cr										
Mn										
Fe					P	P	P			
Co										
Ni										
Cu										
Zn			N	N						
Ga			N	N					P	P
Ge					P		P			
As										
Se										
Br						P				
Rb			P	P						
Sr	N	N					N	N	N	N
Y					P	P				
Zr				N						
Nb										
Mo										
Pd										
Cd										
In										
Sn										
Sb		P			P					
I								N		
Cs			P							
Ba		N	N	N						
Ce										
Hf			N	N						
Ta							N	N		
W										
Pt										
Au										
Hg										
Tl										
Pb		N								
Bi										
U										N

4.4 ASSOCIATIONS OF ELEMENTAL CONCENTRATIONS WITH SEXES

Only the log-transformed concentrations of Sn (p = 0.0329) in muscle tissue, the untransformed As (p = 0.7518) and Cu concentrations (p = 0.0306) in kidney tissue; and the untransformed concentrations of Zn (p = 0.0014) in liver tissue were different between the sexes (Table 3.5). The reasons for these differences are not known. These differences may therefore be used to determine sexes when supplied with samples form unknown origin, but with capture, sexing can be done. Nilsen *et al*. (2017) did not have a satisfactory distribution between male and female samples to derive any conclusions.

4.5 INTERPRETATION OF MULTIVARIATE ANALYSES

The graphs in Section 3.6 all show overlap of the convex hulls, supporting the findings above of little differences between length, mass, and sex, but also from presumed pansteatic conditions. From an elemental composition point of view (at least for those that I measured), healthy crocodiles sampled from the KNP seem to be remarkably homogenous. This seems to have been the case from the Nilsen *et al*. (2017) study as well (except for Hg), although this was not specifically concluded.

4.6 COMPARISONS WITH DATA FROM ELSEWHERE

The following discussion is based on Tables 3.2 and 3.6, and is based on available and comparable data. I measured many more elements, but these have not been reported yet by other studies.

Al: The mean Al concentration in KNP crocodile muscle tissue (13 mg/kg dm) was lower than in most other studies. Compared with previous data from the KNP, the concentrations were also much lower. The mean Al concentrations in liver (330 mg/kg) for the present study was an order of magnitude higher than for alligators from the USA (Horai *et al*., 2014), but they did not analyse muscle tissue to compare with.

Ti: The mean Ti concentration in KNP muscle tissue (5.8 mg/kg dm) was lower than in other studies like those from Australia (Jeffree *et al*., 2011). The mean Ti concentrations in liver (11 mg/kg) for the present study was higher than for alligators from the USA (0.0022 mg/kg dm and 0.00221 mg/kg dm) (Horai *et al*., 2014), but they did not analyse muscle tissue to compare with.

Cr: The mean Cr concentrations in KNP muscle tissue (7.9 mg/kg dm) was higher than in most other studies, especially those from the USA (Delany *et al.*, 1988; Jeffree *et al*., 2011). However, mean Cr concentrations of muscle tissue in the present study was lower than those documented in crocodile muscle other parts of the KNP during a previous study (Swanepoel *et al*., 2000). The mean Cr concentrations in liver (8.6 mg/kg) for the present study was an order of magnitude higher than for alligators from the USA (Horai *et al*., 2014).

Mn: The mean Mn concentration in KNP muscle tissue (3.4 mg/kg dm) was higher than in most other studies, especially those from Australia and some from a previous study in the KNP (Swanepoel *et al.*, 2000; Jeffree *et al.,* 2011). The mean Mn concentration in liver (6.1 mg/kg) for the present study was higher than for other crocodiles from Zambia (Almli *et al*., 2005) and the USA (Horai *et al*., 2014).

Fe: The mean Fe concentration in KNP muscle tissue (210 mg/kg dm) was higher than in most other studies, especially those from the USA and Australia (Delany *et al*., 1988; Jeffree *et al*., 2011). However, mean Fe concentrations of muscle tissue in the present study was lower than two documented in the Olifants River and Sabie River of the KNP during a previous study (Swanepoel *et al*., 2000) The mean Fe concentrations in liver (12000 mg/kg) for the present study was higher than for alligators from the USA (Horai *et al*., 2014).

Ni: The mean Ni concentration in KNP muscle tissue (1.3 mg/kg dm) was lower than in most other studies, especially those from the previous study in the KNP (Swanepoel *et al.,* 2000). However, Ni concentrations in KNP muscle tissue were higher than the concentrations documented in Australia (Jeffree *et al.*, 2011). No Ni liver concentrations could be found in other studies.

Co: The mean Co concentration in KNP liver tissue (0.29 mg/kg dm) was higher than in most other studies, especially those from the USA and Zambia (Almli *et al*., 2005; Horai *et al*., 2014). No muscle tissue data was found on Co concentrations in other studies.

Cu: The mean Cu concentrations in KNP muscle tissue (6.9 mg/kg dm) was lower than in most other studies, especially those from the previous KNP study and Australia (Swanepoel *et al.*, 2000; Jeffree *et al.*, 2011). However, mean Cu concentrations of muscle tissue in the present study was higher than some documented in the USA (Delany *et al*., 1988). The mean Cu concentrations in liver (44 mg/kg) for the present study was an order of magnitude higher than for alligators from the USA (Horai *et al.*, 2014) and crocodiles in Zambia (Almli *et al.*, 2005).

Zn: The mean Zn concentration in KNP muscle tissue (68 mg/kg dm) was higher than in most other studies, especially those from the previous KNP study and from the USA (Delany *et al.*, 1988; Swanepoel *et al*., 2000). However, the mean Zn concentration of muscle tissue in the present study was lower than some documented from Australia and China (Xu *et al*., 2006; Jeffree *et al*., 2011). The mean Zn concentrations in liver (140 mg/kg) for the present study was an order of magnitude higher than for alligators from the USA (Horai *et al*., 2014) and crocodiles in Zambia (Almli *et al*., 2005).

As: The mean As concentration in KNP muscle tissue (2.1 mg/kg dm) was higher than in all other studies, specifically those from China (Xu *et al*., 2006). The mean As concentration in liver (2.5 mg/kg) for the present study was an order of magnitude higher than for alligators from the USA (Horai *et al*., 2014) and crocodiles from Zambia (Almli *et al*., 2005).

Se: The mean Se concentration in KNP muscle tissue (5.58 mg/kg dm) was higher than in all other studies, specifically those from Australia (Jeffree *et al*., 2011). The mean Se concentrations in liver (19 mg/kg) for the present study was an order of magnitude higher than for alligators from the USA (Horai *et al*., 2014) and crocodiles in Zambia (Almli *et al*., 2005).

Pd: The mean Pd concentration in KNP muscle tissue (1.2 mg/kg dm) was lower than in most other studies, especially those from the previous KNP study (Swanepoel *et al*., 2000). No concentrations for Pd in liver tissues were found in other studies.

Cd: The mean Cd concentration in KNP muscle tissue (0.045 mg/kg dm) was higher than in most other studies, especially those from the USA (Delany *et al*., 1988). However, the mean Cd concentration of muscle tissue in the present study was lower than some documented in China (Xu *et al.,* 2006). The mean Cd concentrations in liver (0.15 mg/kg) for the present study was higher than for crocodiles in Zambia (Almli *et al*., 2005), but lower than for alligators from the USA (Horai *et al*., 2014).

Hg: The mean Hg concentration in KNP muscle tissue (2.7 mg/kg dm) was markedly higher than in all other studies, especially those from the USA (Delany *et al*., 1988) and China (Xu *et al*., 2006). The mean Hg concentrations in liver (30 mg/kg) for the present study was two orders of magnitude higher than for alligators from the USA (Horai *et al*., 2014) and Nile crocodiles in Zambia (Almli *et al*., 2005).

Hg is poisonous in all forms and is therefore one of the most poisonous metals (Emsley, 2003) especially to reptiles. Hg concentrations in liver tissues were very high in comparison with the other tissues (Fig.3.2-4c) ($p<0.0001$). According to the mean concentrations, liver tissue (30 mg/kg dm) had extremely high concentrations of mercury while tail fat (0.75 mg/kg dm) and abdominal fat (1 mg/kg dm) had the lowest concentrations of Hg (Table 3.2). Hg levels in tail fat and abdominal fat were very similar but lower than the other tissues. This might indicate that tail fat can be a good indicator of Hg levels in abdominal fat and vice versa, but not for the other tissues. Liver and kidney tissues appear to be the best accumulating organs of Hg and therefore might be good indicators of Hg levels in the environment. There were no specific sex differences in Hg concentrations. Information on Hg concentrations in Nile crocodiles from this area is important because of potential health risks to humans and other animals that consume them (Tchounwou *et al*., 2003), as well as the potential to use these apex predators and bio-indicators. It is documented that the concentration of Hg in food sources is increasing and that it is a worrying outcome (Guallar *et al*., 2002; Clarkson *et al*., 2003).

Pb: The mean Pb concentration in KNP muscle tissue (0.55 mg/kg dm) was significantly higher than in all other studies, especially those from the USA (Delany *et al*., 1988) and China (Xu *et al*., 2006). The mean Pb concentrations in liver (12 mg/kg) for the present study were much higher than for alligators from the USA (Horai *et al*., 2014) and crocodiles in Zambia (Almli *et al*., 2005).

For many toxic elements therefore, the relatively higher concentrations of the KNP crocodiles compared with historic data and with data from other regions are of concern and should be further investigated. An ecotoxicological assessment was not part of this study, but the data clearly indicates cause for concern.

The ecotoxicology of reptiles from Africa are under-represented in the literature (Grillitsch and Schiesari, 2010). To date, I could only find five publications on the concentrations of metallic elements in Nile Crocodile tissues (Hoffman *et al*., 2000; Swanepoel *et al*., 2000; Almli *et al*., 2005; du Preez *et al*., 2016; Warner *et al.*, 2016). The other freshwater, marine, and terrestrial reptiles from Africa are also rather thinly represented in literature. This represents a large gap in our understanding of the threats of metallic elements in an important group of animals in Africa.

4.7 BIOPSIES OF TAIL FAT USED TO INDICATE CONCENTRATIONS IN ABDOMINAL FAT

The results clearly showed that the two fatty tissues, namely abdominal fat and tail fat had very similar concentrations (Table 3.2). These were the only fatty tissues sampled and analysed compared to the other three tissues, namely kidney, liver and muscle tissues. As displayed in the mean concentrations table (Table 3.2) and subsequent graphs, it is clear that the two fatty tissues in most cases had much lower concentrations of most elements compared with the other tissues. It can therefore be assumed that fatty tissues do not retain elements at the same concentrations as the other tissues. However, considering that pansteatitis occur in abdominal and tail fat tissues (refer Chapter 1), these results might indicate possible signs and symptoms of pansteatitis.

As it is difficult to extract a biopsy of abdominal fat without killing the crocodile or causing serious harm to the animal, these results might indicate the possibility of using tail fat biopsies, which are easier to retrieve, to indicate elemental composition of abdominal fat tissues. The multiple comparisons indicate that there were no differences in concentrations between abdominal and tail fat (Table 3.3-3) (Table 3.3-2) (Table 3.4). The data also indicated that no significant differences were found between male and female crocodiles, and abdominal fat and tail fat tissues (Table 3.5).

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

The hypothesis of this study was: “Selected elemental concentrations in crocodile muscle and/or tail fat biopsied tissues will allow inference of concentrations in other tissues.”

It is clear from the analyses that there is no single tissue that can be used to predict the concentrations of all elements from one tissue to another. Instead, Tables 4.1 and 4.2 can be used to select the relevant and easiest tissue to biopsy in order to predict the concentrations in other organs of interest.

There were few patterns to discern, but mass, length, and sex did not discriminate between elemental concentrations with any confidence in any tissue. Due to the large variations in concentrations, proper scientific studies using live-captured animals would need a balanced sampling design. The numbers of individuals needed for such a scientific study for the targeted elements and organs can be calculated from my data. However, it seems that for regular and general surveys, the capture and biopsy of fewer animals with less consideration for mass, length and sex, may be more appropriate.

My study provides the largest such dataset and baseline for any African crocodile. The data and interpretations will assist in monitoring changes and comparisons with other regions and contribute to a better understanding of the biology, ecology, and threats faced by these apex predators, the largest in Africa.

Recommendations:

- Future study objectives should carefully select the best organ for biopsy (muscle of tail fat since the other organs are located to deep) for a small range of elements of interest.
- An ecotoxicological assessment of the data, which fell outside the scope of this study, should be conducted.
- The data can also be analysed to calculate risks associated with human consumption (that is, humans eating crocodiles, not the other way around).
- The high concentrations of certain elements that I detected are of concern and should be investigated. Some potential sources are listed in Chapter 1.
- A specimen bank of crocodile samples should be established so that long-term monitoring and changes can be followed.

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ANNEXURES

Abdominal Fat							
Element	**n**	**Min.**	**Median**	**Max.**	**Mean**	**SD**	**CV %**
Li	15	0.0060	0.0075	0.015	0.0082	0.0024	29.2%
Be	2	0.0098	0.011	0.012	0.011	0.0014	13.2%
B	15	3.3	4.5	6.0	4.4	0.70	16.0%
Na	15	750	1100	4300	1500	1000	64.7%
Mg	15	90	110	2000	240	475	195.8%
Al	15	10	15	55	17	11	64.6%
Si	15	16	16	17	16	0.42	2.6%
K	15	220	380	23000	2000	5800	292.4%
Ca	15	220	250	470	270	64	24.0%
Sc	15	0.060	0.075	0.095	0.075	0.0096	12.9%
Ti	15	0.85	1.2	7.0	1.8	1.7	97.7%
V	15	3.6	4.2	4.5	4.1	0.27	6.6%
Cr	15	12	13.0	14	13	0.65	5.2%
Mn	15	1.0	1.3	1.6	1.3	0.13	10.4%
Fe	15	40	50.0	160	59	29	48.8%
Co	15	0.023	0.031	0.055	0.033	0.0074	22.6%
Ni	15	0.80	1.1	1.3	1.0	0.14	13.5%
Cu	15	0.70	1.1	2.2	1.1	0.36	31.9%
Zn	15	3.0	5.0	250	22	62	287.5%
Ga	15	0.065	0.070	0.095	0.071	0.0081	11.4%
Ge	15	0.035	0.043	0.055	0.044	0.0052	12.0%
As	15	2.3	2.5	3.0	2.5	0.24	9.6%
Se	15	1.1	1.7	2.4	1.7	0.33	19.6%
Br	15	2.2	2.7	8.0	3.1	1.4	46.6%
Rb	15	0.095	0.26	14.5	1.2	3.7	296.6%
Sr	15	0.34	0.40	1.0	0.46	0.16	35.7%
Y	15	0.0026	0.0048	0.0075	0.0048	0.0016	34.0%
Zr	15	0.060	0.12	0.35	0.16	0.091	55.6%
Nb	15	0.0010	0.0021	0.0065	0.0023	0.0013	58.5%
Mo	15	0.17	0.21	0.36	0.23	0.055	24.5%
Pd	15	0.12	0.17	0.80	0.26	0.21	80.0%
Cd	2	0.011	0.011	0.011	0.011	0.0	0.0%
In	15	0.095	0.11	0.12	0.11	0.0069	6.2%
Sn	15	220	240	250	230	9.0	3.9%
Sb	15	0.0085	0.012	0.023	0.012	0.0037	29.6%
I	15	0.17	0.65	1.9	0.68	0.46	67.3%
Cs	14	0.0010	0.0024	0.14	0.012	0.035	293.9%
Ba	15	8.0	9.0	11	9.1	0.74	8.1%
Ce	15	0.0090	0.012	0.023	0.014	0.0047	34.4%
Hf	15	0.00085	0.0024	0.0075	0.0030	0.0020	67.3%
Ta	9	0.00065	0.00095	0.0018	0.0011	0.00045	42.0%
W	7	0.00070	0.0011	0.0030	0.0014	0.00079	57.9%
Pt	15	0.048	0.095	0.29	0.13	0.083	63.9%
Au	15	0.36	0.44	0.85	0.49	0.14	28.2%
Hg	15	0.65	0.95	2.2	1.0	0.35	35.0%
Tl	15	0.021	0.028	0.039	0.028	0.0043	15.4%
Pb	15	0.24	0.39	0.50	0.39	0.059	15.2%
Bi	14	0.0012	0.0021	0.0044	0.0022	0.00097	44.2%
U	15	0.025	0.043	0.055	0.041	0.0072	17.4%

Kidney							
Element	**n**	**Min.**	**Median**	**Max.**	**Mean**	**SD**	**CV %**
Li	16	0.0075	0.013	0.032	0.014	0.0065	44.78%
Be	9	0.0098	0.012	0.016	0.012	0.0020	17.02%
B	16	5.0	6.0	7.0	5.9	0.63	10.60%
Na	16	6500	11000	19000	11000	3900	34.05%
Mg	16	500	1100	1300	960	230	23.99%
Al	16	18	50	170	65	43	66.24%
Si	16	17	18	20	18	0.99	5.51%
K	16	3500	8500	11000	7900	2000	25.24%
Ca	16	430	580	900	590	108	18.30%
Sc	16	0.080	0.090	0.13	0.094	0.013	13.75%
Ti	16	4.1	6.3	9.0	6.5	1.6	24.19%
V	16	4.1	22	50	23	14	62.10%
Cr	16	9.0	11	12	10.4	0.88	8.45%
Mn	16	4.0	6.5	8.5	6.3	1.4	22.54%
Fe	16	250	530	1100	560	220	39.02%
Co	16	0.29	1.38	5.0	1.6	1.2	79.47%
Ni	16	2.3	7.8	41	11	10	93.31%
Cu	16	7.5	11	14	11	2.3	21.19%
Zn	16	46	75	110	72	19	26.11%
Ga	16	0.065	0.085	0.10	0.086	0.011	13.12%
Ge	16	0.050	0.060	0.080	0.062	0.0077	12.48%
As	16	1.9	2.3	2.7	2.3	0.20	8.51%
Se	16	6.0	12	30	14	7.3	51.47%
Br	16	7.0	14	23	14	5.3	38.40%
Rb	16	6.5	13	35	16	8.2	51.89%
Sr	16	0.49	0.83	3.0	1.1	0.75	69.22%
Y	16	0.0050	0.013	0.045	0.015	0.010	69.30%
Zr	16	0.11	0.27	1.9	0.36	0.41	112.26%
Nb	16	0.0010	0.0083	0.33	0.028	0.081	287.99%
Mo	16	1.7	2.7	4.2	2.9	0.80	27.58%
Pd	16	0.14	0.18	0.35	0.19	0.059	30.91%
Cd	15	0.030	0.20	0.95	0.31	0.280	91.54%
In	16	0.095	0.12	0.12	0.11	0.0068	6.08%
Sn	16	240	240	250	240	5.6	2.33%
Sb	16	0.026	0.090	0.23	0.10	0.059	57.54%
I	16	0.31	0.60	0.75	0.55	0.16	28.59%
Cs	16	0.017	0.050	0.12	0.059	0.035	59.34%
Ba	16	9.0	10	13	11	1.1	10.89%
Ce	16	0.0275	0.060	0.15	0.067	0.032	48.39%
Hf	16	0.0017	0.0045	0.038	0.006503	0.0084	129.01%
Ta	15	0.00065	0.0011	0.0034	0.0012	0.00071	57.46%
W	15	0.00085	0.0013	0.0050	0.0018	0.0013	72.38%
Pt	16	0.07	0.15	0.31	0.15	0.058	39.80%
Au	16	0.43	0.55	1.0	0.60	0.16	26.82%
Hg	16	1.9	9.5	55	16	16	104.83%
Tl	16	0.025	0.041	0.065	0.043	0.0095	22.10%
Pb	16	0.42	0.73	13	2.2	3.3	153.54%
Bi	16	0.0033	0.013	0.24	0.030	0.056	184.03%
U	16	0.038	0.052	0.21	0.071	0.049	68.39%

Liver							
Element	**n**	**Min.**	**Median**	**Max.**	**Mean**	**SD**	**CV %**
Li	16	0.011	0.015	0.033	0.016	0.0058	36.38%
Be	14	0.024	0.048	0.12	0.052	0.021	40.23%
B	16	7.0	8.9	13	9.0	1.7	18.51%
Na	16	3200	5000	9500	5100	1400	27.57%
Mg	16	340	840	1100	810	180	21.62%
Al	16	29	270	1100	330	300	89.62%
Si	16	17	20	21	19.0	1.4	7.16%
K	16	2300	10000	14000	10000	2500	24.57%
Ca	16	500	730	1200	790	220	28.38%
Sc	16	0.075	0.15	0.23	0.15	0.040	26.97%
Ti	16	2.1	11	22	11	4.3	37.71%
V	16	3.6	21	41	21	11	51.43%
Cr	16	7.0	8.0	18	8.6	2.5	28.44%
Mn	16	2.4	5.7	10	6.1	2.1	33.95%
Fe	16	750	9200	30000	12000	8700	70.91%
Co	16	0.10	0.24	0.75	0.29	0.17	59.55%
Ni	16	1.0	1.4	2.8	1.5	0.49	32.20%
Cu	16	8.5	36	130	44	28	64.44%
Zn	16	20	140	380	140	79	55.82%
Ga	16	0.090	0.29	0.50	0.31	0.13	40.96%
Ge	16	0.060	0.33	0.95	0.41	0.26	63.01%
As	16	1.9	2.2	4.7	2.5	0.90	36.56%
Se	16	4.5	20	38	19	9.2	47.53%
Br	16	5.5	17	29	17	6.0	35.02%
Rb	16	2.3	8.8	22	10	5.6	55.49%
Sr	16	0.80	1.2	3.8	1.4	0.74	51.63%
Y	16	0.017	0.19	0.80	0.21	0.18	87.62%
Zr	16	0.080	0.25	0.43	0.24	0.095	39.84%
Nb	16	0.0019	0.011	0.042	0.013	0.0094	72.04%
Mo	16	0.55	2.2	4.05	2.2	0.78	36.12%
Pd	16	0.22	0.37	1.05	0.41	0.19	45.69%
Cd	16	0.034	0.10	0.5	0.15	0.12	84.43%
In	16	0.095	0.12	0.125	0.12	0.0077	6.68%
Sn	16	210	250	260	240	13	5.37%
Sb	16	0.080	0.26	6	0.80	1.4	180.99%
I	16	0.24	0.35	0.85	0.45	0.21	46.88%
Cs	16	0.0075	0.011	0.032	0.016	0.0086	53.23%
Ba	16	11	28	42.5	29	10	35.21%
Ce	16	0.10	0.75	2.15	0.85	0.54	64.41%
Hf	16	0.0014	0.0048	0.009	0.004397	0.0021	47.45%
Ta	14	0.00080	0.0012	0.00205	0.0013	0.00041	31.50%
W	15	0.0037	0.0050	0.04	0.011	0.010	95.91%
Pt	16	0.095	0.14	0.55	0.17	0.11	64.12%
Au	16	0.70	1.0	1.4	1.0	0.22	21.88%
Hg	16	2.0	27	95	30	26	86.27%
Tl	16	0.039	0.063	0.135	0.075	0.029	38.31%
Pb	16	0.85	3.2	110	12	27	230.83%
Bi	16	0.0028	0.0095	0.065	0.016	0.020	120.39%
U	16	0.046	0.072	0.185	0.082	0.037	45.50%

Muscle							
Element	**n**	**Min.**	**Median**	**Max.**	**Mean**	**SD**	**CV %**
Li	16	0.010	0.014	0.035	0.016	0.0065	41.11%
Be	15	0.014	0.041	0.15	0.052	0.040	77.20%
B	16	13	24	120	34	27	78.68%
Na	16	8500	11000	13000	11000	1400	12.51%
Mg	16	900	1000	1200	1000	100	9.70%
Al	16	9.5	12	21	13	3.2	24.41%
Si	16	13.5	17	20	17	1.7	9.90%
K	16	6000	7500	9300	7600	750	9.93%
Ca	16	2500	2800	3100	2800	160	5.79%
Sc	16	0.095	0.13	0.24	0.15	0.049	33.12%
Ti	16	5.0	5.5	8.5	5.8	0.95	16.43%
V	16	1.8	2.7	3.0	2.6	0.34	13.31%
Cr	16	5.0	8.3	9.5	7.9	1.2	15.34%
Mn	16	2.4	3.3	5	3.4	0.76	22.30%
Fe	16	180	210	230	210	17	8.31%
Co	16	0.060	0.10	0.4	0.13	0.077	58.56%
Ni	16	0.90	1.2	2.1	1.3	0.28	22.49%
Cu	16	4.9	6.6	10	6.9	1.7	24.40%
Zn	16	61	69	75	68	4.8	7.05%
Ga	16	0.075	0.095	0.13	0.098	0.015	15.36%
Ge	16	0.030	0.038	0.045	0.037	0.0041	10.99%
As	16	1.7	2.1	2.6	2.1	0.21	10.06%
Se	16	4.2	5.5	10	5.85	1.4	23.68%
Br	16	6.0	15	37	17.05	9.4	55.08%
Rb	16	1.1	2.4	10	3.1	2.2	71.94%
Sr	16	0.71	1.6	3.6	1.86	0.90	48.59%
Y	16	0.0019	0.0038	0.029	0.005496	0.0065	118.37%
Zr	16	0.046	0.12	0.34	0.15	0.072	49.64%
Nb	16	0.0012	0.0019	0.0060	0.0025	0.0015	60.88%
Mo	16	0.55	1.6	3.1	1.8	0.68	38.92%
Pd	16	0.25	0.63	6.5	1.2	1.5	131.40%
Cd	10	0.021	0.028	0.15	0.045	0.039	87.84%
In	16	0.095	0.11	0.14	0.11	0.010	9.10%
Sn	16	210	240	280	240	16	6.55%
Sb	16	0.0046	0.0075	0.014	0.0077	0.0023	29.56%
I	16	0.31	0.53	2.0	0.65	0.39	60.71%
Cs	16	0.0017	0.0033	0.0056	0.0035	0.0012	32.99%
Ba	16	10	13	17	13	1.9	14.93%
Ce	16	0.0075	0.013	0.090	0.018	0.020	110.35%
Hf	15	0.0014	0.0023	0.0075	0.0030	0.0018	59.34%
Ta	13	0.0010	0.0014	0.94	0.073	0.26	353.86%
W	14	0.00080	0.0012	0.0026	0.0014	0.00052	37.93%
Pt	16	0.14	0.27	3.0	0.44	0.68	153.35%
Au	16	1.3	2.7	70	8.7	17	196.56%
Hg	16	0.90	1.8	9.5	2.7	2.4	88.42%
Tl	16	0.11	0.21	8.5	0.92	2.1	226.43%
Pb	16	0.34	0.49	1.6	0.55	0.30	54.68%
Bi	14	0.0012	0.0020	0.0044	0.0021	0.00087	41.22%
U	16	0.042	0.092	2.9	0.31	0.70	224.76%

Tail Fat							
Element	**n**	**Min.**	**Median**	**Max.**	**Mean**	**SD**	**CV %**
Li	15	0.0050	0.0090	0.012	0.0086	0.0018	20.44%
Be	1	0.020	0.020	0.020	0.020	0.0	0.00%
B	15	3.2	3.8	5.5	3.9	0.67	17.04%
Na	15	700	950	3900	1300	790	62.53%
Mg	15	95	110	1900	250	460	185.41%
Al	15	11	14	22	14	3.0	21.08%
Si	15	16	16	16	16	0.24	1.56%
K	15	210	350	23000	2100	5700	275.41%
Ca	15	210	250	490	270	67	24.99%
Sc	15	0.055	0.070	0.085	0.071	0.0072	10.08%
Ti	15	0.75	1.0	7.0	1.4	1.6	110.01%
V	15	3.9	4.4	4.7	4.4	0.19	4.44%
Cr	15	12	14	15	13	0.64	4.79%
Mn	15	1.1	1.3	2.2	1.4	0.31	22.67%
Fe	15	39	47	65	48	7.0	14.67%
Co	15	0.019	0.030	0.047	0.031	0.0070	22.63%
Ni	15	0.85	1.1	1.2	1.0	0.10	9.97%
Cu	15	0.90	1.6	8.5	2.3	2.1	87.62%
Zn	15	2.4	4.0	260	22	66	294.83%
Ga	15	0.065	0.075	0.09	0.075	0.0076	10.08%
Ge	15	0.037	0.042	0.055	0.044	0.0046	10.60%
As	15	2.4	2.6	3.1	2.6	0.21	7.93%
Se	15	1.1	1.7	2.6	1.7	0.46	26.79%
Br	15	1.8	2.2	5.5	2.4	0.91	37.62%
Rb	15	0.13	0.36	15	1.4	3.7	270.98%
Sr	15	0.34	0.40	0.65	0.42	0.084	19.86%
Y	15	0.0018	0.0036	0.013	0.0042	0.0027	65.74%
Zr	15	0.065	0.12	1.6	0.30	0.42	142.93%
Nb	13	0.0012	0.0019	0.0031	0.0021	0.00063	30.11%
Mo	15	0.14	0.24	0.31	0.22	0.052	23.56%
Pd	15	0.11	0.13	0.20	0.15	0.033	22.72%
Cd	1	0.011	0.011	0.011	0.011	0.0	0.00%
In	15	0.11	0.11	0.13	0.11	0.0059	5.28%
Sn	15	230	240	250	240	6.1	2.59%
Sb	15	0.0065	0.011	0.025	0.012	0.0049	41.89%
I	15	0.18	0.80	4.3	1.0	1.0	95.01%
Cs	14	0.00145	0.0027	0.15	0.015	0.038	261.10%
Ba	15	8.0	9.5	11	9.5	0.85	8.90%
Ce	15	0.0085	0.013	0.028	0.016	0.0061	38.26%
Hf	14	0.0014	0.0030	0.019	0.0048	0.0050	103.08%
Ta	6	0.00065	0.0010	0.0014	0.0010	0.00032	31.43%
W	10	0.00070	0.0010	0.0031	0.0012	0.00072	60.37%
Pt	15	0.033	0.060	0.41	0.082	0.093	112.72%
Au	15	0.30	0.35	0.75	0.38	0.11	28.43%
Hg	15	0.49	0.60	1.8	0.75	0.37	49.92%
Tl	15	0.017	0.019	0.029	0.020	0.0036	17.75%
Pb	15	0.39	0.50	0.75	0.52	0.11	21.04%
Bi	13	0.0014	0.0026	0.0095	0.0030	0.0022	73.63%
U	15	0.023	0.037	0.048	0.037	0.0077	21.13%