Effects of gender, age, season on the mineral profiles of impala on the Experimental Farm in Messena

(Aepyceros *melampus)* (Lichtenstein, 1812)

By

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Declaration

I, Nchaupe Bright Laaka, declare that this research has been done by me.

Signature : ______________________

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ABSTRACT

The purpose of this study was to provide baseline data on the mineral status of impala and to investigate the possibility of using impala as sentinel species for monitoring important elements that may affect livestock and wildlife production in southern Africa. Approximately 27 impala were sampled at the Messina Experimental Farm. Animals of different sexes, age groups and anatomical locations were sampled. Specific tissues sampled were: liver, thyroid gland, kidney fat and kidney and approximately 100g of each tissue was collected.

The study examines direct cause and effect by taking a holistic approach, which includes examining exposures according to age, sex and body burden. In order to review and summarize the health state of sampled areas in terms of exposure to environmental heavy metal contaminants. Impala (*Aepyceros melampus*) were used as indicator species in this study.

CHAPTER 1

1.1 Introduction

Africa is the second largest continent on earth, encompassing 20% of its surface (Cooke, 1978). Largely tropical, Africa extends into temperate zones up to 37 degrees north and 35 degrees south. Eastern and southern Africa display steep elevation gradients due to the prevalence of volcanic orogeny and rifting (Cooke, 1978). Local landscapes are distinguished by substantial geological heterogeneity, dissected landforms, and resultant steep gradients of precipitation and vegetation. The consequent pronounced fragmentation of habitats and sharp combination of distinct vegetation types, combined with climatic oscillations in geological time, contributed to major adaptive radiations of the mammalian fauna (Keast *et al*, 1972 and Maglio *et al*, 1978).

Early zoological expeditions recorded that habitat fragmentation, and wide spatial variation of animal densities and diversities, were distinctive features of the African ecosystem (Jaeger, 1911; Mobius, 1895; Roosevelt, Heller, 1914; White, 1915). Early records provided the basis of natural history information on animal distributions, habitat preferences, feeding habits, and general ecology. The mammalian fauna has been increasingly isolated and fragmented within game reserves of varying size, habitat diversity and animal species diversity.

Studies of impala, *Aepyceros melampus* Lichstenstein 1812, in South Rhodesia during 1959-60, suggested that this species is a successional animal, favoured by factors which open up forests, dense woodland or tall grassland. The highest numbers of impala have been recorded in areas which would be rated as poor cattle range. Impala population densities compare with those of deer, *Odocoileus,* in North America. Deer share their ranges with few other ungulates, whereas impala may share their ground with as many as 16 other species of ungulates (Dasmann and Mossman, 1962).

Impala were found to have a clearly marked breeding season, with the young being born during a brief period in the early part of the wet season. Ewes produced their first lamb at two years of age and although no twinning was observed, other studies do report twinning in impala. The rut occurs early in the dry season, at which time territorial behaviour was observed (Dasmann and Mossman, 1962).

Age classes of impala were determined by tooth replacement and wear. Yearlings comprised about 25% of the populations. A shortage of young adults was noted, and a high percentage of older animals were found in the population. Females predominate among the adults, which may be explained by differential sex mortality among lambs (Dasmann and Mossman, 1962).

Records on movements suggest that impala herds occupy relatively small home ranges, where the distribution of food, cover and water permits. In the absence of one of these factors, impala will move considerable distance to obtain resources. In one area, movements of at least 15 miles to and from water during the dry season were observed

(Dasmann and Mossman, 1962). Impala are widely distributed throughout southern Africa, making it an ideal sentinel species to monitor environmental effects in this region.

Relations between the geofigureical nature of soils and their parent material, and the occurrence of nutritional deficiencies and excesses in grazing livestock, have been documented since the 1960s and earlier. Exposure to heavy metals, in particular lead, zinc, cadmium and the metalloid arsenic, have also received attention in areas contaminated by past mining and smelting activities. Soil and plant factors influencing the dietary supply of both essential trace elements and toxic metals have been studied, including their speciation and bioavailability. Soil ingestion has been recognized as an important exposure pathway of heavy metal contamination to grazing cattle, and as an antagonist of copper supply and source of dietary cobalt in sheep. (Bowell, and Ansah, 1993)

The concentrations of selected trace elements, cobalt, copper, iron, molybdenum, selenium and zinc were analyzed in soils, grass, and bush and tree samples from the Mole National Park, Ghana. The distribution of essential nutrients, cobalt, copper, manganese and selenium is controlled by bedrock geology, whereas, iron, molybdenum and zinc distribution is controlled by soil and hydrological processes. In the soils, iron, manganese and cobalt are largely fixed in the mineral fraction, while most of the copper, molybdenum and selenium in the soils can be extracted by disodium ethylenediaminetetracetate. Copper, cobalt and manganese appear to be preferentially concentrated in grass species, while molybdenum and selenium are concentrated in browse plants (Bowell, and Ansah, 1993).

Variations in uptake exist between wet and dry seasons with all trace elements studied, except iron and manganese, which showed a marked increased availability in the wet season and an increased concentration in the residual fraction of the mineral and organic soils in the dry season. In the dry season, the plant concentration of molybdenum and selenium decreased, while copper and zinc showed increased concentrations. This may be related to a lower pH of the ground water during the dry season (Bowell and Ansah, 1993).

A budget of metal input and output in the ecosystem at Mole National Park has been computed. Potential dietary deficiencies in cobalt could be observed, however, other metals in the soil and plant concentrations were sufficient to prevent straight forward deficiencies, and the concentrations of molybdenum and selenium were sufficiently low to be considered safe (Bowell and Ansah, 1993).

Concentrations of major trace elements in soils and grass were determined at Shimba Hills National Reserve in Kenya using geochemical mapping techniques (Sutton, Maskall and Thornton, 2002). The study investigated the influence of soil and vegetation type on the concentrations of sodium, potassium, magnesium, calcium, manganese, phosphorus, cobalt, copper, zinc, molybdenum, nickel and selenium in soil and grass. These concentrations were measured with regards to the nutrition of the sable antelope. Low concentrations in surface soils of a number of major and minor elements were related to the geochemical nature of the fundamental parent materials of sands, sandstones and grits. High element concentrations in surface soils in natural forest areas were attributed to the influence of litter fall, whilst in grassland areas, soil element significance is controlled by soil type and decreases in the order: ferrasols >acrisols > arenosols. The lessening of major and minor elements in soils were not reflected as fully in grasses, in which nutrient concentrations were of similar extent, to those reported from other Kenyan conservation areas.

Burning of grassland areas leads to higher concentrations of potassium, phosphorus, cobalt, copper and molybdenum in grasses, high soil-plant uptake ratios for phosphorus and potassium, and elevated soil pH. It is suggested that increased availability of phosphorus in soils at elevated soil pH levels contributes to its improved uptake into grass. A review of the mineral status of grass at the reserve using guidelines developed for domesticated ruminants, indicated deficiencies of sodium, potassium, phosphorus and zinc, and that the Ca:P ratio exceeded the tolerable range for animals (Sutton, Maskall and Thornton, 2002).

Increased industrialization has led to high levels of metals and other toxic contaminants within the environment. More than 100 years of extreme mining and smelting activity have resulted in the release and area-wide deposition of airborne metals to the extent that both the terrestrial and aquatic environments are now substantially contaminated (Hazelett *et al*., 1984).

Species diversity and human health are constantly threatened by the production and release of toxic chemicals into the atmosphere, water or soil (Ecobichon 1996; Hill 1999; Jorgenson 2001; Loizeau *et al.*, 2001). The awareness of the potential or real danger posed by heavy metals to the ecosystems, and in particular to human health, has grown tremendously in the past decades. Heavy metals such as cadmium, lead and mercury are known toxic metals with no beneficial effects to man and wildlife (Tyler, 1981 and Borgman, 1983). Certain metals, such as copper and cobalt, are classified as essential to life due to their involvement in certain physiological processes. Elevated levels of copper have however been established as toxic (Spear, 1981), where they are known to cause brain damage in mammals (DWAF, 1996).

Polychlorinated biphenyls (PCBs) and chlorinated pesticides belong to a class of ubiquitous pollutants that are highly lipophillic and refractory to chemical degradation. Due to these physiochemical properties, and their slow rate of enzymatic biotransformation in all organisms, organochlorine pollutants tend to accumulate to toxic levels in both aquatic and terrestrial food chains (Ecobichon 1996; Hill 1999; Jorgenson 2001; Loizeau *et al*., 2000). Chronic exposure to persistent organochlorine chemicals has been associated with deleterious effects in fish and wildlife, leading to poor fertility, impaired immune function and population declines in the contaminated areas (Colborn *et al*., 1993; Hunter *et al*., 1981; Fry and Toone, 1981; Guillette *et al*., 1996; Tyler *et al*., 1998).

Monitoring programs and research for metals in the environmental samples have become widely established because of concerns of over accumulation and toxic effects, particularly in aquatic organisms and to humans consuming the organisms. The criteria by which organisms are accepted as biological indicators for the assessment of contamination were proposed more than 25 years ago, and remain unchanged (Phillips, 1976; Fowler and Oregioni, 1976). A potentially hazardous chemical becomes toxic only when it reaches an active site in the target species at sufficiently high concentrations to generate adverse reactions. Therefore, exposure levels in combination with toxicokinetic indices such as uptake, metabolism, and elimination, are important parameters that determine the health risk resulting from a particular toxic substance.

Before establishing reliable risk assessments, it is necessary to determine the biologically available level of toxic chemicals and their potential accumulation, that is, the efficiency by which such chemicals are transported from environmental matrices to relevant biologic receptors (Eaton and Klaassen, 1996; Hutton, 1982; Mackay, 1991; Thibodeaux, 1979). Typically, the bioavailability and bioaccumulation of environmental pollutants are assessed by measuring chemical residues in tissues and fluids of animals or humans. Many different species can be used to estimate the impact of toxic chemicals on aquatic habitats (Kendall *et al*., 1996; Wells, 1999). Bivalves are widely used as bioindicators of pollution by heavy metals in coastal areas because they are known to concentrate these elements, providing a tight integrated indication of environmental contamination. In comparison to fish and crustaceans, bivalves have a very low level of activity of enzyme systems capable of metabolizing persistent organic pollutants (POPs), such as aromatic hydrocarbons and polychlorinated biphenyls (Phillips, 1977, 1980, 1990).

Factors known to influence metal concentrations and accumulation in these organisms include metal bioavailability, season of sampling, hydrodynamics, the environment, size, sex, changes in tissue composition and reproductive cycle (Boyden and Phillips, 1981). Seasonal variations have, to a great extent, been related to seasonal changes in flesh weight during development of gonadic tissues (Joiris *et al*., 1998, 2000). Element concentrations in molluscs at the same location differ between different species and individuals due to species-specific ability to regulate or accumulate trace metals (Reinfelder *et al*., 1997; Otchere *et al*., 2003). Different animals, in the same community, at the same trophic level, could accumulate pollutants differently due to differences in either the habitats' or niches' physical and chemical properties.

1.2 Problem statement

Increased industrialization has led to elevated levels of metals and other toxic contaminants within the environment in certain areas, particularly those affected by high volume point source emissions, reaching contamination levels which may seriously threaten habitat quality and/ or health of resident wildlife species (Hazellet *et al*., 1984). Monitoring levels of heavy metals in wildlife species can be achieved by using indicator species such as impala.

1.3 Goals of the study

1.3.1 The goal of the study was to assess the extent of exposure of wildlife in Messina Experimental Farm in the Limpopo Province to bioaccumulating environmental contaminants, and the associated risk to their health and well-being.

1.3.2 The goal builds on direct cause and effect studies by taking a holistic approach, which includes examining exposures according to age, sex and body burden. The study aims to review and summarize the state of sampled areas, in terms of the wildlife health impacts due to exposure to environmental contaminants.

1.4 Hypothesis

Impala is an indicator species for gauging the presence of heavy metals and other elements that are not readily dissipated by microbial decontamination in living organisms and that may cause adverse health effects in wildlife and domestic livestock.

CHAPTER 2

LITERATURE REVIEW

2.1 Description of impala

Impala, *Aepyceros melampus* (Lichtenstein, 1812), the only representative of the subfamily, *Aepycerotinae*, are medium sized antelope. Adult males stand about 0.9m at the shoulder and have a mean mass of approximately 50kg; the females are slightly smaller with a mean mass of approximately 40kg. The body is reddish-brown with white hair inside the ears, over each eye and on the chin, upper throat, under parts and buttocks. A narrow black line runs along the middle of the lower rear of the long tail, and a vertical black stripe appears on the back of each thigh. Impala have large, brush-like tufts of long, coarse black hair that cover a scent gland located just above the heel on each hind leg. Only male impala have horns, and these horns reach their full length and thickness at about four years of age (Skinner and Smithers, 1990).

2.2 Distribution

Impala are distributed widely from southern Africa to the Eastern woodland parts of Africa, extending westwards in the more southerly parts of their range to the extreme southern parts of Angola. In the sub region, they have been introduced widely, and reintroduced to privately owned lands and game reserves in Zimbabwe, the Transvaal and Natal, where they are distributed widely throughout (Skinner and Smithers, 1990).

2.3 Habitat

Impala are particularly prevalent in transition zones or ecotypes, typically between open grassland and closed bush land or woodland (Murray 1982). According to Du Toit (1990), they are rather inactive ungulates with year-round home ranges varying from 50ha to 581ha. Despite their limited mobility, impala respond to the climatic variability of semi-arid environments by grazing during wet seasons (Dunham 1982, Hansen *et al*. 1985, Klein and Fairall 1986, Meissner *et al*. 1996). In the northern parts of the sub region, they are associated particularly with acacia and mopane, *Colophospermum mopane*, where cover and the availability of surface water are essential habitat requirements.

2.4 Habits

Impala are sociable and are seen occurring in small herds of 6 to 15 or 20, as well as larger gatherings of 50 to 100 from the wet to the early dry season. Young rams are ousted from the herd by the dominant ram to form small bachelor herds. The dominant ram maintains a group of ewes throughout the rutting and lambing season.

2.5 Diet

Body size and morphology limit the range of food that ruminants can efficiently eat (Hofman, 1989), while food selection affects the social structure patterns of dispersal and predator avoidance (Jarman, 1974). Ruminants can be divided into three classes, namely bulk feeders (grazers), selective feeders (browsers) and intermediate feeders (eating both grass and browse) (Bothma, 1989). Impala are classified as intermediate, mixed feeders among wild ruminants (Hofmann, 1973). According to Bothma (1989), the impala's main feeding spectrum is grazing and browsing, i.e. they eat tender young grass shoots in the wet season, herbs and shrubs, and are area selective. Through the year, impala eat about 35% grass, 54% trees and/or shrubs and 11% herbaceous plants. They seem to prefer the so called decreaser species and increaser 2 herbaceous species, decreaser species grass and other herbaceous species, the abundance of which decreases with over–or underutilization. (Examples include *Digitaria eriantha, Panicum maximum*, *Themeda triandra*.)

Increaser 2 species are grass and other herbaceous species, the abundance of which increases during overutilization. These species can be divided into three groups, depending on the rate of over-utilization. (Examples include *Digitaria, argyrograpta, Aristida species,* and *Brachiaria eruciformis.)* (Wentzel *et al.,* 1991). In the late winter, the toxic leaves of the *Spirostachys africana* are an important food source for the animals (Bothma, 1989). Impala are dependent on the availability of drinking water, during the dry season they must drink daily. Young (1972a) reported that, in the Kruger National Park, they remained within 8km of water; however, half his records indicated that herds remained within 1.6km of a water source.

2.6 Feeding Behaviour

Impala are commonly classified as intermediate feeders among wild ruminants because they graze exclusively on green grasses during the summer months, and shift to an essentially browse habit during the winter, when a diet of leaves, pods and twigs is supplemented with dried grasses. Impala are grazers by choice and browse woody vegetation during drought periods when availability of grasses and forbs are low (Ables and Ables, 1969, Kenya). Impala prefer browsing to grazing, although they frequently graze, especially on riverside grasses and on flush growth after a burn (Gardens, 1965).

2.7 Toxicological overview of heavy metal pollutants

Heavy metal toxicity is frequently the result of long term, low level exposure to pollutants common in our environment: air, water, food. The degree to which a system, organ, tissue, or cell is affected by a heavy metal toxin depends on the toxin itself and the degree of exposure to the toxin. Exposure to toxic metals is associated with many chronic diseases. Recent research has found that even low levels of lead, mercury, cadmium, arsenic and aluminium can cause a wide variety of health problems. Heavy metal toxins exert a dramatic effect on health, and contribute to the progression of many different debilitating conditions (ASTDR, 1989).

They also have the ability to impair not just a single cell or tissue, but many of the body's systems that are responsible for normal behaviour and proper physiological functioning (ATSDR, 1989). Exposure to heavy metals has been linked with developmental retardation, various cancers, kidney damage, and even death in some instances of exposure to very high concentrations. Exposure to high levels of mercury and lead has also been associated with the development of autoimmunity, in which the immune system starts to attack its own cells, mistaking them for foreign invaders (Janet Glover-Kerkvliet, 1995).

It is clear from the literature on animals, and epidemiological studies on humans, that the developing organism is more sensitive to behavioural deficits resulting from polychlorinated biphenyl exposure than the adult. Two well-designed prospective epidemiological studies in humans provide evidence of behavioural deficits associated with low level (*in utero*) exposure to PCB's. In one study, exposure was via contaminated Great Lakes fish (Fein *et al*., 1984; Jacobson *et al*., 1984, 1985, 1989, 1990; Schwartz *et al*., 1983).

2.8 Critical Pollutants

2.8.1 PCBs (polychlorinated biphenyls)

PCBs are organic chemicals consisting of 209 individual congeners. They have been used as coolants and lubricants in transformers, capacitors, and other electrical equipment. PCBs manufacture ceased in 1977 due to accumulation of PCBs in the environment and potential adverse health effects. Today PCBs, can be discharged into the environment from hazardous waste sites containing PCBs, illegal dumping, and leaks from transformers. PCBs can travel in air. In water PCBs, adhere to organic particles and sediments, with a small amount dissolving in the water. Fish and marine mammals may bioaccumulate PCBs in excess to the amounts found in water. Human exposure may result from eating food containing PCBs (e.g., fish, meat, dairy products) or drinking well water contaminated with PCBs from appliances. PCBs have been associated with birth defects and reproductive problems in human studies. They cause liver cancer in animals, but it is not known if PCBs are human carcinogens (ATSDR, 1997).

2.8.2 Mercury

Mercury is a heavy metal of unusual geological occurrence found naturally, although concentrations in the environment are normally low (S.A Water quality, 1996). It can be found in the metallic liquid form; as inorganic mercury compounds (salts) with chlorine, sulphur, or oxygen; and as organic mercury in combination with carbon. Inorganic mercury and elemental mercury are both toxins that can produce a wide range of adverse health effects. Methyl mercury is the most common form of organic mercury. Metallic mercury is utilized in the production of chlorine gas and caustic soda, and is used in thermometers, dental fillings, and batteries. Inorganic mercury is found in air from mining, burning of coal and waste, and from industry, and can also be found in water or soil as natural deposits, or from waste disposal and volcanic activity. Methyl mercury is produced by the action of microscopic organisms in water and soil. Organic mercury is known to bioaccumulate or pass up the food chain due to an organism's inability to process and eliminate the metal. It is found primarily in marine life, and can often be found in produce and farm animals, processed grains and dairy products. (ATSDR, 1989; Brenner and Snyder, 1980).

The absorption and distribution of mercury depends largely upon its chemical state. Organic mercury is absorbed through the gastrointestinal tract more readily than inorganic mercury compounds, with the latter being very poorly absorbed. After absorption in the gastrointestinal tract, organic mercury is readily distributed through the body, but tends to concentrate in the brain and kidneys (Goyer, 1991). Inorganic and organic forms of mercury have also been found in the red blood cells, liver, muscle tissue and gall bladder (Peterson *et al*., 1991, Dutczak *et al.,* 1991, ATSDR, 1989). Mercury exposure can result in a wide variety of health conditions.

The degree of impairment, and the clinical manifestations that accompany mercury exposure, largely depend upon its chemical state and the route of exposure. While inorganic mercury compounds are considered to be less toxic than organic mercury compounds (primarily due to difficulties in absorption), inorganic mercury that is absorbed is readily converted into the organic form by physiological processes in the liver.

2.8.3 Arsenic

Arsenic is a greyish semi-metal and occurs in three oxidation states: - (0), (III) and (V) (S.A Water Quality, 1996). The use of this toxic element in numerous industrial processes has resulted in its presence in many biological and ecological systems. Arsenic is used mostly in the production of wood preservatives, insecticides, and weed killers. Arsenic is present in the air as a result of burning of arsenic containing materials such as wood, coal, metal alloys, and arsenic waste (ATSDR, 1989; Morton and Caron, 1989). Arsenic concentrations can also be found in marine life (primarily fish and shellfish) and riot control gas (Hine *et al*., 1977). Many arsenic compounds are absorbed through the gastrointestinal tract. Absorption within the lungs is dependent upon the size of the arsenic compound, and much of the inhaled arsenic is later absorbed in the stomach after (respiratory) mucocillary clearance (ATSDR, 1989). After the absorption of organic compounds, the primary areas of distribution are the liver, kidney, lung, spleen, aorta, and the skin.

Metabolically, arsenic compounds are methylated to form monomethylarsenic and dimethylarsenic acid. Arsenic does not accumulate to an immense degree in animal tissues, and generally tends to be involved in the redistribution of copper, ultimately leading to a decline in renal copper excretion. Arsenic increases renal copper retention levels by 500%, although decreasing hepatic copper levels by 5% (SA Water Quality, 1996).

Arsenic is a highly toxic element. Its health effects are well known and multiform. Acute exposure to arsenic compounds can cause nausea, anorexia, vomiting, abdominal pain, muscle cramps (ATSDR, 1989). Garlic – like breath, malaise and fatigue have been observed in individuals exposed to an acute dose of arsenic (Feldman *et al*., 1979). Animal studies have shown similar acute effects when arsenic compounds were delivered orally to Rhesus monkeys (Heywood and Sortwell, 1979).

Repeated exposure to organic compounds have been shown to lead to the development of peripheral neuropathy, encephalopathy, cardiovascular distress, peripheral vascular disease, gangrene of the lower legs ('Black foot disease'), kidney and liver damage, myocardial infarction, anaemia and leucopenia (ATSDR, 1989; Blom *et al*., 1985; Feldman *et al*., 1979; Heyman *et al*., 1956; Hine *et al*., 1977; Langerskvist *et al*., 1986; Morton and Caron, 1989). Other chronic effects of arsenic intoxication are neurotoxic effects, chronic respiratory diseases, neurological disorders and cardiovascular disease (Blom *et al*., 1985; Kyle and Pease, 1965; Morton and Caron, 1989). Studies have shown close associations between both inhaled and ingested arsenic and cancer rates. Cancers of the skin, liver, respiratory tract and gastrointestinal tract are well documented with regards to arsenic exposure (IARC, 1980; Lee-Feldstein, 1989).

2.8.4 Copper

Copper occurs naturally in elemental form and as a component of many different compounds. This common environmental element is essential for all living organisms. Copper occurs in three oxidation states: - metallic copper (0), cupous copper (I) and cupric copper (II). Copper is used extensively in the manufacturing of electrical equipment and different metallic alloys. Copper is released into the environment primarily through mining, sewage treatment plants, solid waste disposal and agricultural processes (ATSDR, 1990). It is present in the air and water due to natural discharges like volcanic eruptions and windblown dust. It is a common compound of fungicides and algaecides, and agricultural use of copper for these purposes can result in its presence in the soil, ground water, animals and many forms of produce (ATSDR, 1990). Although copper is essential for good health, it may be harmful at high levels.

Absorption of copper occurs through the lungs, gastrointestinal tract and skin (U.S EPA, 1987). The degree to which copper is absorbed in the gastrointestinal tract largely depends on its chemical state and the presence of other compounds, like zinc (U.S.A.F., 1990). Once absorbed, copper is distributed primarily to the liver, kidneys, spleen, heart, lungs, stomach and intestines.

Copper is an essential element required in the vascular and skeletal systems, the central nervous system, and in reproductive system developments. It is mainly used as a dietary supplement due to its important role in nutrition (SA Water Quality, 1996). Copper toxicity is dependent on linked molybdenum and sulphate concentrations. Continued ingestion of copper compounds can cause cirrhosis and other debilitating liver conditions (Mueller-Hoecker *et al*., 1989). Vineyard workers exposed to copper fumes for a long period of time developed pulmonary fibrosis and granulomus of the lungs, liver impairment and liver disease. Similar results were obtained in animals chronically exposed to copper - containing dust and fumes (Johansson *et al*., 1984; Stockinger, 1981). Further animal studies on copper toxicity have shown varying degrees of liver and kidney damage such as necrosis of the kidney, and sclerosis and cirrhosis of the liver; decreased total weight, brain weight and red blood cell count; increased platelet counts; and the presence of gastric ulcers (Kline *et al*., 1977; Rana and Kumar, 1978). Copper also appears to affect reproduction and development in humans and animals. Offspring of hamsters that received copper sulphate injections while pregnant exhibited increased incidences of hernias, encephalopathy, abnormal spinal curvature and spina bifida (Ferm and Hanlon, 1974).

2.8.5 Lead

Lead is a bluish-white soft metal which is highly sensitive and spongy and very resistant to decay. Lead is a cumulative poison and may accrue in the roots of some plants, e.g. hay, potatoes and lettuce ,to concentrations poisonous to humans and animals (SA Water Quality, 1996). Lead is a metal found occurring naturally in the earth's crust, entering the environment through mining, the burning of fossil fuels and the manufacturing of batteries, ammunition, metal products and roofing (ATSDR, 1993). Several industrial processes create lead dust and fumes, resulting in its presence in the air. Mining, smelting and manufacturing processes, the burning of fossil fuels and municipal waste and incorrect removal of lead based paint results in airborne lead concentrations. Lead from water and airborne sources have been shown to accumulate in agricultural areas, leading to increased concentrations in agricultural produce and farm animals (ATSDR, 1993). Lead is absorbed into the body following inhalation or ingestion. After lead is absorbed into the body, it circulates in the blood stream and distributes primarily in the soft tissues such as kidneys, brain, muscle, and bone.

Lead is one of the most toxic elements occurring naturally on earth. Lead poisoning occurs mostly in cattle and sheep and is usually acute. The onset of clinical symptoms is more rapid in young animals. High concentrations of lead can cause irreversible brain damage, seizures, coma and death if not treated immediately (U.S. EPA, 1986). The central nervous system becomes severely damaged at blood concentrations starting at 40mcg/dl, causing a reduction in nerve conduction velocities and neuritis (ATSDR, 1993).

Evidence suggests that lead may cause fatigue, information processing difficulties and a reduction in motor and sensory reaction times (Ehle and McKee, 1990). At blood concentrations above 70mcg/dl, lead has been shown to cause anaemia, characterized by a reduction in haemoglobin levels, and erythropoeisis (Goyer, 1988; U.S. EPA, 1986). The kidneys are targets of lead toxicity and prone to impairment at moderate to high levels of lead concentrations. Kidney disease, both acute and chronic nephropathy, is a characteristic of lead toxicity. Other signs or symptoms of lead toxicity include gastrointestinal disturbances such as abdominal pain, cramps, constipation, anorexia and weight loss, immunosuppression, and slight liver impairment (ATSDR, 1993; US EPA, 1986).

2.8.6 Cadmium

Cadmium is a soft, bluish-white metal, chemically similar to zinc and highly toxic to living organisms. The presence of cadmium in livestock drinking water is of concern simply because it bioaccumulates. The natural half life of cadmium is approximately 200 days. It can persist in animal tissue longer than antagonistic trace metals, causing delayed toxicity. The speed at which cadmium is absorbed is higher when ingested through water as compared to dietary intake. Cadmium accumulates in the liver and kidney (75%), however, it may also be found in muscle tissue and milk (SA Water Quality, 1996).

Cadmium may be found naturally in the earth's crust, usually combined with other elements. It can be found in water and soil from waste disposal, or from spills from hazardous waste sites. Fish, plants, and animals take in cadmium from the environment. Human exposure may result from inhalation of cadmium from municipal waste sites. It can also result from ingestion of contaminated water, fish, plants and animals.

Toxicity as a result of cadmium is not frequently observed even though it is highly toxic. The fact is attributable to low cadmium absorption. Cattle have no homeostatic control over cadmium tissue concentrations. After absorption cadmium usually combines with a protein which reduces poisoning. Low levels of cadmium concentrations are excreted in milk, 0.0008% of ingested cadmium as compared to 8 – 12 % zinc (SA Water Quality, 1996).

Low level, long term exposures from air, food, or water may result in kidney disease. Ingestion of very high levels in food and drinking water may result in vomiting and diarrhoea. Based upon weak human and strong animal evidence, the U.S. Department of Health and Human Services has determined that cadmium and its compounds may be carcinogenic (ATSDR, 1993).

The risk of cadmium toxicity is increased by low dietary levels of protein, as a result of increased intestinal absorption and retention of cadmium in the kidneys and liver. Adequate protein levels have been indicated to reduce hepato- and nephrotoxicity associated with cadmium poisoning. The dietary protein type is, however, also a determining factor. A 45% decrease in fertility has been observed in cases of protein dietary deficiency coupled with toxicity of cadmium (SA Water Quality, 1996).

2.8.7 Chloride

Chloride is the anion of the element chlorine, which does not occur free in nature, but is only found as chloride. It is said that chloride is a normal constituent of water, is highly soluble and, once in solution, tends to accumulate. The SA Water Quality (1996) states that chloride is of concern in water supplies because higher concentrations impart an unpalatable taste to water, and accelerate the corrosion rate of metals. Typical concentrations of chloride in fresh water range from a few to hundreds of milligrams.

Chloride is found within cells, in body fluids, in gastric secretions and in the form of salt. Metabolically, chloride is said to play a vital role in regulating osmotic pressure and the acid base balance. Chloride is efficiently conserved, and its requirement is small, although an increase in muscular activity increases the need for chloride. Chloride requirement in growing ruminants is low, hence deficiencies are unlikely under normal production conditions. At higher concentrations, normally much lower than those which are toxic, chloride renders drinking water unpalatable to quite a number of livestock.

2.8.8 Chromium

Chromium (VI) is a highly oxidised state of metal chromium. It appears as a yellow-coloured dichromate salt under neutral or alkaline conditions, or as the orange-coloured chromate salt under acidic conditions. The SA Water Quality (1996) indicated that chromium can also function as a vital element, by being a component of a hormone and a vitamin. It is also found to function as a co-factor with insulin, needed for normal glucose utilisation, and growth. The liver is the main storage site, whereas excretion, if absorbed, is generally via the kidneys (SA Water Quality, 1996).

The most common ore of chromium is chromite, in which chromium occurs in the trivalent state. It is also stated that increased concentrations of chromium found in the environment are due to industrial pollution. As a result of chromium's high water solubility, it is very mobile in the environment and readily moves through the soil profile, contaminating ground water supplies (SA Water Quality, 1996).

The SA Water Quality (1996) states that excess chromium rarely manifests itself, since only small amounts are normally present in water and diets, body utilisation is very poor, and there is a wide margin between beneficial and harmful doses. Due to these reasons, bioaccumulation does not normally occur to any significant degree. Toxic effects of chromium include diarrhoea, and dehydration. There is also evidence that chromium is carcinogenic (SA Water Quality, 1996).

2.8.9 Cobalt

Cobalt is a hard, grey magnetic metal. The name cobalt is derived from the German 'kobelt', a term for gnomes and goblins, as the toxic effects from inhalation of dusts in cobalt mines was originally thought to be the work of goblins. Cobalt is an important trace element in nutrition and forms part of vitamin B12, which contains 4% of cobalt and is required for red blood cell synthesis (SA Water Quality, 1996).

Increased levels of cobalt may occur in the vicinity of mines where the ores that are processed contain cobalt. The report also states that cobalt tends to be concentrated in particles of manganese oxide in soils as a result the low levels of cobalt in natural waters may be due to an adsorption reaction of this type. Also, sufficient dietary intake of cobalt is important to prevent anaemia (SA Water Quality, 1996).

Cobalt is stored in the liver, kidneys, adrenal glands and bones, and is poorly retained in body tissues. Excess cobalt is rapidly excreted, primarily in urine, although cobalt is secreted in the bile and thus reabsorption is possible. The status of cobalt in ruminants is determined by serum vitamin B12, plasma methyl malonate or liver cobalt/vitamin B12 concentrations. Ruminants are not dependent on a dietary source of vitamin B12 due to the synthesizing of vitamin B12 by rumen micro organisms, coupled with that fact that absorption is regulated by a fundamental factor. The SA Water Quality (1996), reports that, even under practical conditions, the occurrence of cobalt toxicosis is highly unlikely, and there is a wide margin of safety between toxic concentrations and nutritional requirement level, with retention being poor.

2.8.10 Fluoride

Fluoride is the most electronegative member of the halogens and having a strong affinity for positive ions and readily forming complexes with many metals. In its elemental form, fluorine is a greenish yellow gas which readily dissolves in water to form hydrofluoric acid.

Water is not the only source of fluoride, it is also present in many foods and, as a result of very pronounced electron affinity of the fluoride atom, fluoride interacts with almost every element in the periodic table. It is thought to be one of the major ions that allows for the solubilisation of beryllium, scandium, niobium, tantalum, and tin in natural waters. Fluoride is said to react readily with calcium to form the relatively insoluble calcium fluoride (SA Water Quality, 1996).

The SA Water Quality (1996) states that excessive amounts of fluoride causes tooth damage in young animals and bone lesions that cause crippling in older animals. Fluoride is, however, also beneficial to animals, reducing osteoclast activity and increasing esteoblast activity. Signs of fluorosis are normally observed in the second and third year of exposure. The report also states that adverse affects due to fluorosis are indirect and include lameness, and decreased feed and water intake, which result in a decline in growth and health.

2.8.11 Iron

Pure iron is silvery in colour, however, it appears as greyish-black or brown deposits due to oxidation. It is found in three oxidation states, namely 0, II and III, of which the III oxidation state is the most common. Iron is biologically an important micronutrient required by all living organisms (SA Water Quality, 1996). It is the fourth richest element and comprises 5 % of the earth's crust. It is found in several minerals, the most common of which is haematite, extensively used as an iron ore for metallurgical purposes. The natural cycling of iron can also result in the co-precipitation of trace metals such as, arsenic, copper, cadmium and lead (SA Water Quality, 1996).

Iron is an important constituent of animal diets and has a low order of toxicity, however; it can be harmful in higher concentrations. Iron is an essential component of several proteins and enzymes involved in oxygen transportation and utilisation. Metabolically, iron is efficiently conserved and thus dietary requirements are low. Also, SA Water Quality (1996) reports that high levels of copper, manganese, lead and cadmium increase the iron requirement by competing for absorption sites (SA Water Quality, 1996).

2.8.12 Magnesium

Magnesium is an alkaline earth metal which reacts with oxygen and water to form magnesium oxide and magnesium hydroxide, respectively. Magnesium is a common constituent of water and occurs as a doubly positively charged magnesium ion. The solubility of magnesium in water is managed by the carbonate/bicarbonate equilibrium, and thus the pH of the water. Magnesium is also an important nutritional element. It can also make a significant contribution to the total dietary magnesium intake in water. Magnesium is also a component of important enzyme co-factors (SA Water Quality, 1996).

Ordinary minerals of magnesium are magnesium carbonate and various magnesium silicates. Magnesium interacts with calcium, as well as with several anions and organic acids.

A report by SA Water Quality (1996) has indicated that magnesium salts are important in animal diets. Magnesium is also a constituent of bones and teeth, and an essential element of cellular metabolism and other physiological functions. The absorption of magnesium is enhanced by protein, lactose, vitamin D, growth hormone and the ionophore antibiotics. Ruminants are dependent on a daily supply of magnesium because their homeostatic mechanisms do not regulate blood levels of magnesium sufficiently. Excess intake of magnesium disturbs calcium and potassium metabolism. Symptoms of magnesium toxicosis are lethargy, loss of co-ordination, diarrhoea, decreased feed intake and decreased performance (SA Water Quality, 1996).

2.8.13 Manganese

Manganese is a grey white metal and is found in several oxidation states. It is an important element for humans and animals, however, it is neurotoxic in excessive amounts. At typical concentrations encountered in water, manganese has aesthetic rather than toxic effects (SA Water Quality, 1996).

Manganese uptake occurs by intake from food and water, however more from food. Metabolically, manganese is controlled mainly by excretion through the pancreas, even though direct passage through the gut wall and in the urine also takes place. Manganese is said to be a fairly abundant element, constituting about 0.1% of the earth's crust.

Reasonable excesses of manganese are not toxic to livestock, and manganese is, in fact important for growth and fertility. The SA Water Quality (1996) reported that there is a limited storage of manganese, and little is known about the dietary factors that affect absorption and usage of stored reserves. High concentrations of manganese occur in the bones, liver and kidneys compared to the blood or muscle tissue.

2.8.14 Molybdenum

Molybdenum is a silvery white, very hard metal and is a vital micro-element for all living organisms, however, it is toxic at high concentrations. It plays a role in quite a number of enzymes, particularly the flavoprotein enzyme, xanthine oxidase. Molybdenum is found in association with lead. Molybdenum is often found within the suspended sediments fraction of water. Higher concentrations are generally found in residues and soils and not in solution. Molybdenum is generally used in the manufacturing of hardened alloys and high strength steels, as a lubricant additive, and also as a corrosion inhibitor.

The metabolic interactions of molybdenum with copper and sulphur and its resulting toxicity is strongly influenced by the dietary intake of copper and sulphur (SA Water Quality, 1996).

Molybdenum plays a role as a component of xanthine oxidase, sulphite oxidase and aldhude oxidase, and emerges to improve microbial activity in the rumen. Insoluble complexes are formed between molybdenum and copper and sulphate which decreases the usage of dietary copper. As a result, molybdenum toxicity is directly linked to the concentrations of copper and inorganic sulphate in the diet. The intake of molybdenum at lower levels than those that cause acute toxicity can result in a copper deficiency. Molybdenum appears to have a direct influence on metabolic processes, independent of changes in the metabolism of copper (SA Water Quality, 1996).

Clinical signs of extreme molybdenum intake include persistent diarrhoea, appetite and weight loss, anaemia, lack of co-ordination, infertility, malformation of bone, and loss of pigmentation of hair. Deficiency of molybdenum does occur under natural conditions in South Africa (SA Water Quality, 1996).

2.8.15 Selenium

Selenium is a semi-metallic element with distinct photo-conductivity. Low concentrations of selenium are essential nutritional micro-elements in humans and animals, and the element is an important part of the enzyme glutathione peroxidise. Selenium occurs in association with sulphide ores of heavy metals like copper, iron and zinc, and it is found in different oxidation states (SA Water Quality, 1996).

Selenium forms insoluble metal selenides, which are often incorporated into sediments, especially under anaerobic conditions. It is also bio accumulated by certain plants, subsequently used as indicators in the bio measurement of selenium. Selenium is used in a range of industrial processes. The chemistry of selenium is comparable to that of sulphur. It interacts with sulphur, iron and arsenic, and metals like copper, cadmium and mercury (SA Water Quality, 1996).

Normally, naturally-occurring deficiencies of selenium in livestock are more regularly encountered than selenium poisoning. Selenium is one of the potentially poisonous elements that are supplemented in animal feeds. Concentrations of selenium in plasma, whole blood and red blood cells, and of blood glutathione peroxidise have been used to assess the selenium status (SA Water Quality, 1996). Usually selenium concentration in blood ranges from 30 – 120 ng/dL and is accepted as sufficient to prevent deficiency (Van Ryssen & O'Dell, 2006). A single subcutaneous injection of 50g of selenium in the form of barium selenite/mL was adequate to prevent deficiency in free ranging dairy cattle in KwaZulu-Natal.

Selenium absorption occurs primarily in the duodenum, and the excretion of absorbed selenium takes place primarily through the kidneys. An elevated level in the blood stream concentration of selenium does not occur with concurrent increases in the milk. Selenium guards against the unfavourable effects of arsenic, cadmium and mercury and is also believed to be anti-carcinogenic. It also plays a vital role in the breakdown of toxic polyunsaturated fats (SA Water Quality, 1996).

2.8.16 Sodium

Sodium is an alkali metal which reacts with water to form highly soluble, positively charged sodium. Sodium has been reported to be an essential dietary element and the major cation responsible for extra cellular osmolality. It also plays a vital role in acid-base balance of body fluids and is also involved in a host of other important physiological functions. It metabolically interacts primarily with chloride and potassium. Almost all ingested sodium is absorbed through the intestines and excess sodium is excreted via the kidneys. Muscular activity does increase the requirement for sodium (SA Water Quality, 1996).

Sodium is present in all food in varying degrees. It is ever-present in the environment and normally occurs as sodium chloride, but also appears as sodium sulphate, bicarbonate or nitrate. Sodium is mostly found as solid sodium chloride in areas where geological deposits occur. Levels of sodium in surface water are normally low, due to high rainfall, and high in dry areas with low mean annual precipitation. Sodium is present at high concentrations in domestic waste water. Sodium concentrations are elevated in runoffs from irrigated soils (SA Water Quality, 1996).

The main symptom in livestock of sodium deficiency is lack of appetite, however, decreased palatability and resultant problems tend to occur prior to toxicity effects. Chronic effects range from a decline in water and feed intake, with subsequent production losses, to gastroenteritis and dehydration (SA Water Quality, 1996).

2.8.17 Vanadium

Vanadium is a white, soft, spongy metal, defiant to decay. It occurs in a number of oxidation states, namely, II, III, IV and V. Vanadium salts and compounds are often highly coloured. Compounds of vanadium have a mixture of industrial applications and are used as catalysts in the chemical industry, in glassware and ceramic products, in the textile industry and in the manufacture of dyes. Metabolically, vanadium interacts with chromium and iron and the concentrations of the two elements should be taken into consideration in measuring the effects of vanadium toxicity (SA Water Quality, 1996).

The incidence of poisoning associated with the intake of excessive amounts of vanadium in water used by livestock is unusual, and only limited information exists. It is, however, thought that the iron saturation of transferritin and ferritin takes part in the retention of vanadium. Clinical signs of vanadium toxicity in livestock include diarrhoea, growth reduction and a dull coat (SA Water Quality, 1996).

2.8.18 Zinc

Zinc is a metallic element, with the stable oxidation states being metal (0) and the +II oxidation state, which is the form found in nature. Biologically, zinc is an essential nutritional trace element for plants and animals, but is toxic at high concentrations. The usual form of zinc is the sulphide. It is also found as a carbonate, oxide or silicate and may occur in conjunction with most metal ores like copper and arsenic. Zinc and zinc salts are used in several industrial processes. It is used in electrifying processes and in alloys (SA Water Quality, 1996). Zinc interacts strongly with cadmium, to which it is chemically very alike. It is also an important nutritional micro-element of fairly low toxicity, whereas cadmium, which is not important, is highly toxic to higher organisms. Metabolically, zinc interacts with copper and calcium.

Zinc is vital for the metabolism of proteins, nucleic acids and carbohydrates. Its requirements are affected by dietary and physiological factors. Animals often have a high level of tolerance to a surplus of zinc intake, attributed to the fact that body reserves of zinc are minute and tend to have fast turnover. Less than 10% of metallic zinc, and the carbonate, sulphate and oxide forms, are absorbed from the intestines, for this reason zinc excretion readily occurs. Zinc is more likely to accumulate in bones than in the liver. Symptoms of zinc toxicity are lack of appetite, loss of condition, diarrhoea, haemolysis and icterus. High intakes of zinc have been reported to induce copper deficiency and a high incidence of abortions and stillbirths (SA Water Quality, 1996).

2.9 Wildlife Populations

The exposure of wildlife to persistent toxic substances and adverse health effects are well documented in the literature. Reproductive impairments have been described in avian, fish, and mammalian populations in the Great Lakes in the United States. For example, egg loss due to egg shell thinning has been observed in predatory birds, such as the bald eagle, within the Great Lakes Area (Menzer and Nelson 1980). After feeding on fish from the Great Lakes for two or more years, immigrant birds (eagles) were shown to have a decline in reproductive success (Colburn *et al*., 1993). Effects on the endocrine system and tumour growths have also been detected in fish populations.

2.10 Animal Experiments

Toxicology data in animals which support the association between exposure to persistent toxic substances and adverse health effects are mounting. Animal experiments have demonstrated a wide range of health outcomes from exposure to polychlorinated biphenyls (PCBs), mercury and chlorinated dibenzo-p-dioxins (CDD). Animals exposed orally to PCBs develop effects to the hepatic, immunological, neurological, developmental and reproductive systems. Effects have also been reported in the gastrointestinal and haematological systems (ATSDR, 1998).

When compared with seals given a diet of relatively uncontaminated Atlantic Ocean fish, the seals ingesting contaminated sea herring were found to have impaired natural killer cell activity and T-lymphocyte function. Neurological effects have been seen in monkeys exposed orally from birth to 20 weeks to PCB congener mixture representatives of the PCB mixture found in human breast milk (Rice, 1998). The monkeys were subsequently tested at 2.5 and 5 years of age, and found to have deficits in learning and difficulty in learning complex tasks when compared to controls.

2.11 Health Effects

Developmental, reproductive, neurobehavioral or neurodevelopmental, and immunologic effects have been reported in studies conducted within the Great Lakes basin and outside the basin in the United States. Developmental effects in the form of a decrease in gestational age and low birth weight have been observed in a Lake Michigan cohort exposed prenatally to PCBs (Fein *et al*., 1984). These findings have also been observed in children of women exposed prenatally to PCBs occupationally through the manufacture of capacitors in New York (Taylor *et al*., 1989).

CHAPTER 3

MATERIALS AND METHODS

3.1 METHODOLOGY

3.1.1 Study area

The study was conducted at the Messina Experimental Farm during 2004. The farm is located in the Mopane veld (Accocks, 1988). In the Messina area the woody component of the veldt consists primarily of dense stands of *Colespermum mopane* trees. Annual species of grass such as *Aristida* spp., *Eneapogon* spp. and *Schmidtia pappaphoriodes* are abundant. The experimental site is a relatively pristine and natural environment, free of pollution from mines or agricultural activities. The hypothesis was that this environment would provide an acceptable baseline of mineral content in free roaming ungulates.

3.1.2 Study Animals

Impala were used as sentinel species because they occur all over the Limpopo and Mpumalanga province, they browse and graze and often occur on farms with livestock. Impala are also increasingly used as a source of protein by communal farmers and hunters. The major benefit of impala at the specific study site is that they were not exposed to any licks or supplements used for livestock feeding. 27 impala were culled at the Messina Experimental Farm during 2004. Animals of different sexes, age groups and anatomical location were sampled.

3.1.3 Experimental Procedures

a. Cropping

Animals were shot in the neck or head using a 0.30mm calibre rifle. Both jugular veins were severed as soon as possible after the animal had been shot. After bleeding, all animals were taken to a central processing point for further processing and data collection, and the animals were slaughtered according to the South African Standard Practices (Hoffman, 2000). The animal's body weights (BW), before processing and cold carcass weights (CCW), were recorded 24 hours post mortem. Rams and ewes were sampled and classified into three age groups: young or juvenile (7 to 10 months old), sub-adult (19 to 30 months old) and adult (more than 30 months of age). Classification of age was based on the number of visible permanent incisors, aided by the length and shape of the horns in the case of males. (the experimental design and number of animals sampled are summarised in table below)

Table 3.1: Experimental design illustrating the number of experimental animals included per age category and gender, as well as anatomical locations sampled.

GENDER	AGE		
	JUVENILE	SUB-ADULT	ADULT
RAMS	Liver Thyroid Kidney Kidney fat **(n = 7)**	Liver Thyroid Kidney Kidney fat **(n = 8)**	Liver Thyroid Kidney Kidney fat **(n = 11)**
EWES	Liver Thyroid Kidney Kidney fat **(n = 5)**	Liver Thyroid Kidney Kidney fat **(n = 8)**	Liver Thyroid Kidney Kidney fat **(n = 7)**

b. Body measurements

Body measurements were taken on the right side of the carcass, although not included and reported in the present study, as they were not required and recording of the measurements was part of the general cropping procedures followed at the research station. The following parameters were recorded:

- Body length – the length was taken from the atlas joint along the neck and the curve of the body on the back bone to the tip of the tail.

- Shoulder height – this was measured at the right foreleg of the animal. The measurement was taken from the tip of the hoof to the most protruding part of the scapula. These measurements were used as an indicator of growth and size.
- Horn length – the length of the impala horn was measured along the front curve, from the base to the tip of the horn (Bothma, 1989)
- Horn circumference – this measurement was taken at the base of both horns as close as possible to the head, at right angle to the axis.
- Testicular circumference – the testis were pulled down and the measurement was taken at the widest part of the testis.

c. Processing of the carcass

Only evisceration and removing of the head, feet and intestines took place at the processing facility. Skinning was done approximately 24 hours after cropping. The carcasses were stored in a cold room at 5 degrees Celsius. After 24 hours, the tissue samples of liver, thyroid, kidney and kidney fat, weighing approximately 100g, and male and female reproductive organs, were collected and frozen. After processing, all carcasses were kept in a chiller at 2 to 5 degrees Celsius. Carcasses were hung from the Achilles tendon.

d. Sampling procedure

All tissue samples as mentioned above were sampled for analysis of heavy metal and other elements. The samples were tagged, packed in small plastic bags and immediately frozen until chemical analysis. All the sampled tissues were used for chemical analysis.

Microwave assisted digestion and ICP- MS analysis

A. Apparatus and materials and Instrumentation

1. Programmable milestone 1200 digestion system with Teflon vessels was used to digest all the samples for subsequent mineral analyses.

2. Inductively coupled plasma Mass spectrometer (ICP-MS, Thermo X Series II).

B. Reagents and Gases

Nitric acid 65% AR grade

Hydrogen peroxide 30%-

Argon- 99.99% purity

Hydrogen/Helium mixture

C. Chemical Analytical procedure

Calibration of Microwave equipment

A thermocouple calibrated by the instrument supplier was used to calibrate the equipment.

Sample digestion

The samples were thawed to room temperature before weighing.

A representative sample of about 2g was weighed in the inert polymeric vessels and recorded. 6ml of concentrated nitric acid and 1ml of hydrogen peroxide was added to the sample and left overnight. The inert polymeric microwave vessels were sealed and placed in the microwave system. The temperature profile was specified to permit the reactions and complete dissolution of the elements. The vessels were removed and allowed to cool. The entire contents of the vessels were transferred to 50ml volumetric flasks and topped to 50ml with deionised water. The diluted samples were sent for instrumental analysis by ICP-MS.

Quality control

Blank samples were prepared and digested together with the samples.

Duplicate: The samples were done in duplicate.

Instrumental Analysis

The Instrument was first calibrated with elemental standards for analysis.

All elements were analyzed by normal mode operation. Collision cell technology (CCT) mode was used to analyze Arsenic and Selenium. CCT was used to eliminate the interferences from Argon (Carrier gas) and chlorides.

3.1.4 Statistical analyses

Data was recorded in Microsoft Excel 2003 and statistically analyzed by means of the General Linear Model (GLM) procedure of SPSS version 17.0 in order to compensate for unbalanced experimental design, age, gender and anatomical location were included as factors in the analysis, and the LS Means and Bonferoni method for, multiple comparisons of means, were utilized.

CHAPTER 4

RESULTS AND DISCUSSION

It is important to note that the range of normal trace element values for wildlife are generally unknown, and many trace elements have been shown to differ with age, sex, season and health status. In this instance, direct comparison of tissue concentrations among studies can be difficult. Little data is available on such a wide range of minerals in impala or other wild ungulate species in this area. The purpose of this study was to provide baseline data on mineral status of impala, and to investigate the possibility of using impala as sentinel species for monitoring important elements that may affect livestock and wildlife production in southern Africa.

4.1 Effect of anatomical location on the mineral content of impala

Summary statistics of trace elements sampled from different anatomical locations of impala are summarised in Table 1. Only elements that differed between anatomical locations, or that exhibited interesting trends, will be discussed in detail. Overall, most elements were detected in the liver and kidneys of impala, while smaller concentrations were detected in the kidney, kidney fat and thyroid glands. These specific differences will be discussed in more detail in the forthcoming sections. Minerals that differed significantly ($p<0,05$) between anatomical depots included lithium, titanium, vanadium, chromium, manganese, cobalt, nitrite, copper, zinc, selenium, rubidium, caesium and molybdenum.

Table 4.1: Summary statistics and anova results of trace elements sampled from different tissues in impala, where K = Kidney, KF = Kidney fat, L = Liver, T = Thyroid.

	TISSUE SAMPLES			
TRACE ELEMENT	**K** (x ±SD)	**KF** (x ±SD)	**L** (x ±SD)	**T** (x ±SD)
Li(mg/kg)	$0.193 + 0.112^{a}$	$0.008+0.006^{b}$	$0.154+0.020^{a}$	$0.036+0.052^{c}$
Be(mg/kg)	0.002+0.004	0.000+0.000	0.003+0.016	0.001+0.003
B(mg/kg)	2.318+3.505	1.154+2.120	2.097+3.246	2.019+3.174
Ti(mg/kg)	$22.564+5.030^{a}$	$2.771+1.110^{b}$	$30.876+5.481^{c}$	$13.317+11.624^{d}$
V(mg/kg)	$0.399+0.237^{a}$	$0.066+0.078^{b}$	$0.175+0.131^{c}$	$0.326+0.181^{a}$
Cr(mg/kg)	$0.643+0.999^{a}$	$1.156+0.373^{b}$	$0.527+0.332^{a}$	$0.828+0.341^{a}$
Mn(mg/kg)	$3.729+1.385^{a}$	$0.929+3.458^{b}$	$11.049+3.086^{c}$	$2.709+6.663^{a}$
Co(mg/kg)	$0.168+0.505^{a}$	$0.023+0.017^{b}$	$0.274+0.631^{c}$	$0.061+0.041^{d}$
Ni(mg/kg)	$1.139+1.118^{a}$	$1.063+0.808^{a}$	$2.363+3.084^{b}$	$1.312+0.803^{a}$
Cu(mg/kg)	$9.093+2.342^{a}$	$0.789+0.515^{a}$	$89.587+64.063^{b}$	$2.215+1.416^{a}$
Zn(mg/kg)	$54.204+15.815^{a}$	$15.337+14.437^{b}$	$77.089+24.785^{c}$	$29.975+21.754^{b}$
As(mg/kg)	0.083+0.070	0.044+0.040	0.079+0.097	0.062+0.052
Br(mg/kg)	284.598+146.283	47.727+36.407	160.977+82.547	133.756+115.629
Se(mg/kg)	$4.102+1.444^{a}$	$0.386+0.236^{b}$	$1.612+0.878^{c}$	$0.919+0.572^{b}$
Rb(mg/kg)	$5.870+2.875^{a}$	$0.641+0.505^{b}$	$9.897+4.375^{c}$	$3.362+2.224^{d}$
Sr(mg/kg)	0.495+0.226	0.360+0.299	0.432+0.359	0.536+0.265
Mo(mg/kg)	$0.706+0.408^{a}$	$0.044+0.045^{b}$	$2.580+1.124^{c}$	$0.073+0.523^{b}$
Cd(mg/kg)	0.215+0.337	0.102+0.210	0.173+0.571	0.036+0.056

Sn(mg/kg)	0.060+0.102	0.039+0.103	0.050+0.072	0.075+0.166
Sb(mg/kg)	1.130+7.303	0.067+0.183	0.036+0.051	0.76+0.233
Te(mg/kg)	0.754+2.274	0.340+1.115	0.492+1.281	0.179+0.620
I(mg/kg)	0.222+0.154	0.169+0.215	0.242+0.183	0.269+0.183
Cs(mg/kg)	0.044+0.061[a]	0.011+0.030[b]	0.037+0.035[c]	0.013+0.017[a]
La(mg/kg)	0.046+0.099	0.025+0.042	0.042+0.102	0.034+0.027
W(mg/kg)	0.289+0.649	0.170+0.405	0.243+0.446	0.114+0.260
Pt(mg/kg)	0.731+1.513	0.448+0.990	0.634+1.097	0.310+0.667
Hg(mg/kg)	1.935+3.393	1.205+2.280	1.687+2.505	0.877+1.571
Pb(mg/kg)	123.279+820.036	3.002+8.766	2.938+18.534	1.076+1.038
Bi(mg/kg)	0.245+0.432	0.148+0.292	0.212+0.323	0.125+0.210
U(mg/kg)	0.472+0.664	0.333+0.490	0.456+0.539	0.270+0.377

[a,b,c,] Means in the same row with different superscript letters differ significantly ($p<0.05$)

Lithium

The concentration of lithium tended to be higher in the kidneys compared to kidney fat, liver and the thyroid gland (Figure 4.1.1). The concentration of lithium in the liver was the second highest, followed by the thyroid gland, whereas the concentration of lithium in kidney fat was very low.

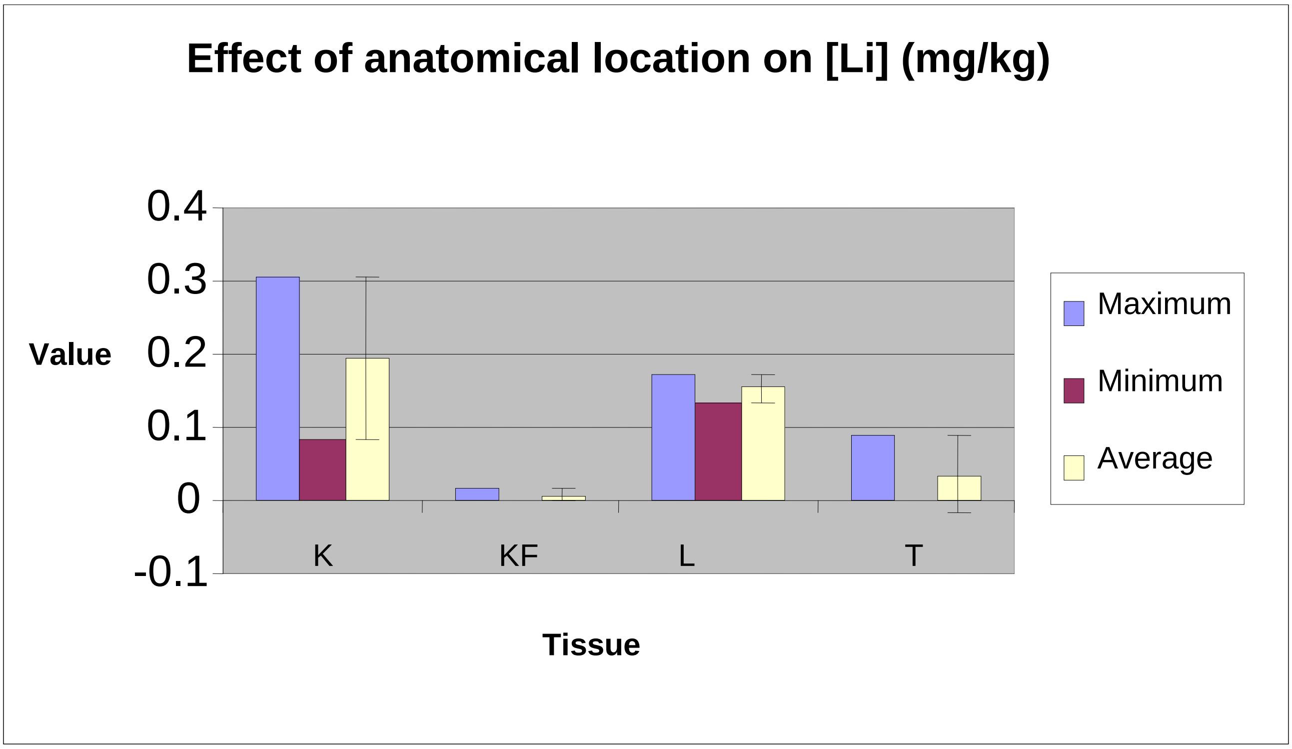

Figure 4.1.1: Effect of anatomical location on lithium content of impala.

The concentration of lithium in kidney tissue is probably a better indicator of lithium status in impala when compared to the other tissues sampled, notably, thyroid and kidney fat, considering that relatively high concentrations of lithium occurred in kidneys ($p<0,05$). The liver also appears to be a good indicator of lithium content in impala because it accumulated in this tissue with relatively small variations between animals sampled.

Lithium is regarded as an essential dietary constituent for some species but deficiencies are unlikely to occur naturally (Underwood and Suttle 1999.). Lithium ranks 27th in abundance among elements in the earth crust and a mean level of 28mg lithium/kg DM has been reported for the soils in Eastern Europe.

Birch (1995) established that interest in lithium stems from findings suggesting that lithium has other beneficial, perhaps fundamental roles, in higher animals, and can be used as a food aversion substance in grazing animals. Reports stated that lithium deprivation depresses fertility, birth weight, lifespan, liver monoamine oxidase activity and several liver and blood enzymes used in the citrate cycle, glycolysis, and nitrogen metabolism in goats (Anke *et al*., 1991).

Titanium

The concentration of titanium in the liver was found to be significantly higher compared to kidney, kidney fat and thyroid. The concentrations of titanium in the kidney was the second highest compared to the other depots. In the kidney fat the concentration of titanium was low.

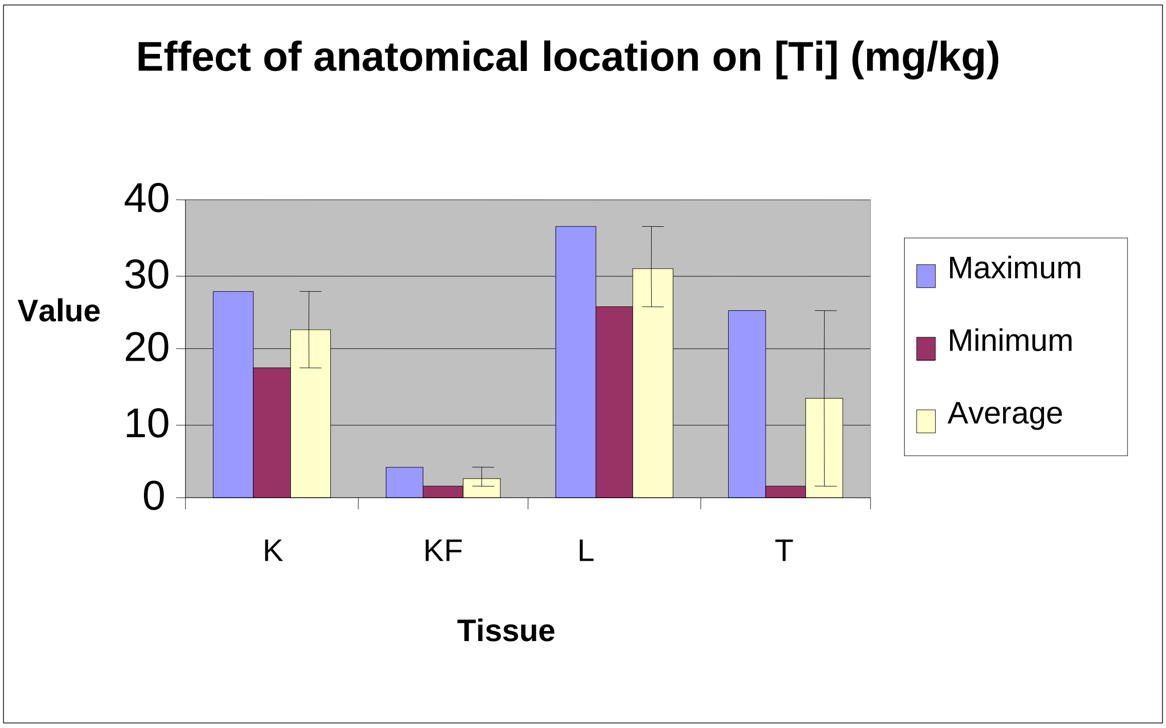

Figure 4.1.2: Effect of anatomical location on titanium content of impala.

Titanium accumulates in liver and kidneys with relatively small variations between animals, which suggest that these tissues could serve as good indicators of titanium content in impala. Variations in titanium content of the thyroid glands were large. Titanium content differed significantly between the different tissues sampled ($p<0.05$), with the highest concentrations occurring in liver (Table 1), followed by the kidney and thyroid gland, and the least accumulation in the kidney fat.

Pais *et al*., (1977) reported some biological interest that was promoted by findings showing physiologically soluble titanium compounds have beneficial actions in animals and plants. There is very little known about the metabolism of titanium. Reports state that it is generally believed that most titanium, especially that from soil contamination, is poorly absorbed. Absorbed titanium is not extensively retained by both plants and animals. Tipton *et al*., (1996) reported findings in human about equal amounts of titanium in faeces and urine of individuals consuming an average of 0.37 and 0.41 mg titanium per day for a month in their diet.

Vanadium

The concentration of vanadium was numerically higher in the kidney compared to the thyroid gland and the liver. The concentration of vanadium in the kidney fat was low compared to the rest of the organs.

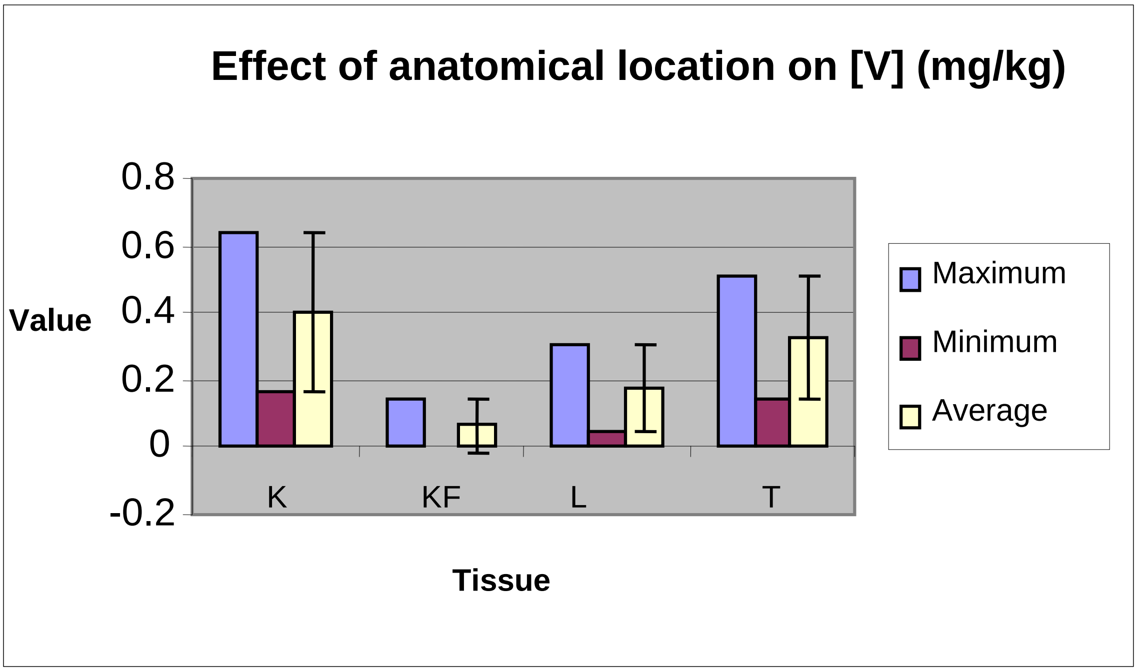

Figure 4.1.3: Effect of anatomical location on vanadium content of impala.

Vanadium accumulated in thyroid and liver and was negligible in kidney fat, suggesting that kidney levels probably provide a better indicator of vanadium status in impala. It is well documented that vanadium tends to accumulate in the liver and kidneys but, in this case, the concentration of vanadium was higher in the kidney compared to the liver (Figure 4.1.3). French and Jones (1993) stated the distribution of vanadium differs throughout the various tissues in the body, notably in the organ tissues, of which the highest concentrations were in the liver, kidney and bone.

Hansard *et al*., (1982) reported that in acute toxicity experiments with three sheep dosed with vanadium at 40 mg/kg BW, vanadium levels in kidney tissue were increased 69 fold at the time of death. There is not much information on health changes of vanadium in ruminants. Vanadium can substitute for phosphate, mimicking the effects of cyclic AMP. As peroxovanadate, it influences free-radical generation and mimics insulin. It is therefore hardly surprising that animals absorb very little vanadium. Sheep absorbed only 1.6% of their dietary supply of vanadium, even when the diet contained 2.6% v/kg (Patterson *et al*., 1986).

Chromium

The concentration of chromium in the kidney and kidney fat were relatively similar, with both being higher compared to the thyroid gland and liver.

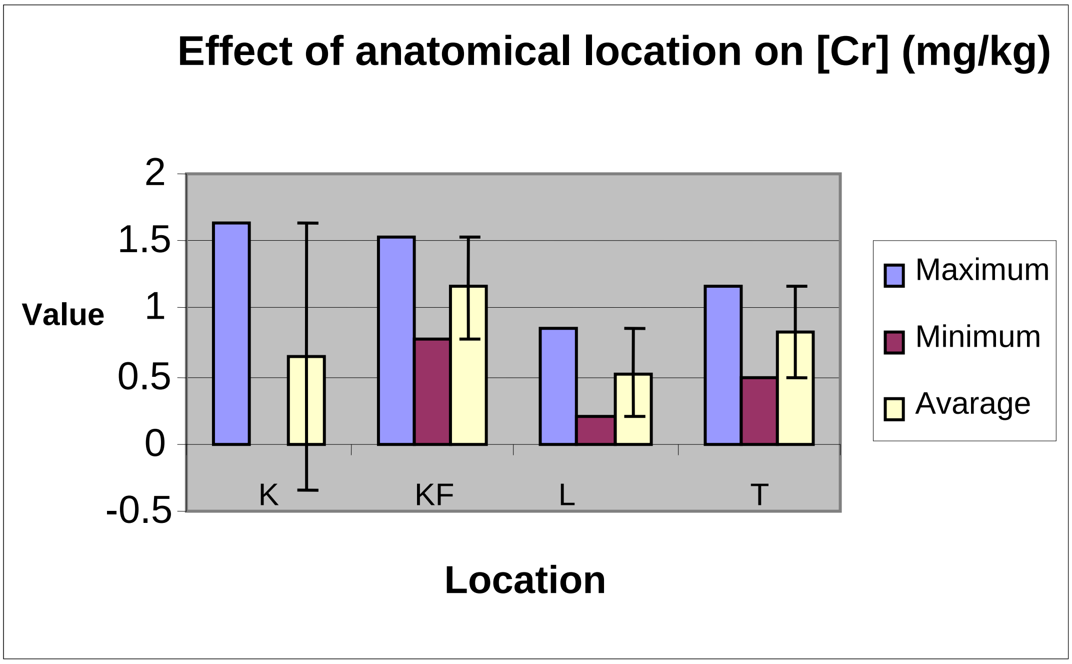

Figure 4.1.4: Effect of anatomical location on the chromium content of impala.

Chromium accumulates more in kidneys and kidney fat, followed by, the thyroid and liver, suggesting that kidney and kidney fat are probably better indicators of chromium status in impala.

In the SA Water Quality report of 1996, it indicates that chromium can also function as a vital element, by being a component of a hormone and a vitamin. It is also found to function as a co-factor with insulin, needed for normal glucose utilisation and growth. The liver is the main storage site, whereas excretion, if absorbed, is generally via the kidneys (SA Water Quality, 1996), however, present results suggest that more chromium accumulates in the kidney, thyroid and kidney fat compared to the liver. Spears (2000) has reported that a number of studies in cattle indicated that chromium supplementation from different sources may increase immune response and reduce incidence of disease, particularly in stressed animals.

Manganese

The concentration of manganese was numerically higher in the liver than in the thyroid gland. The concentrations of manganese in the kidney and kidney fat were similar.

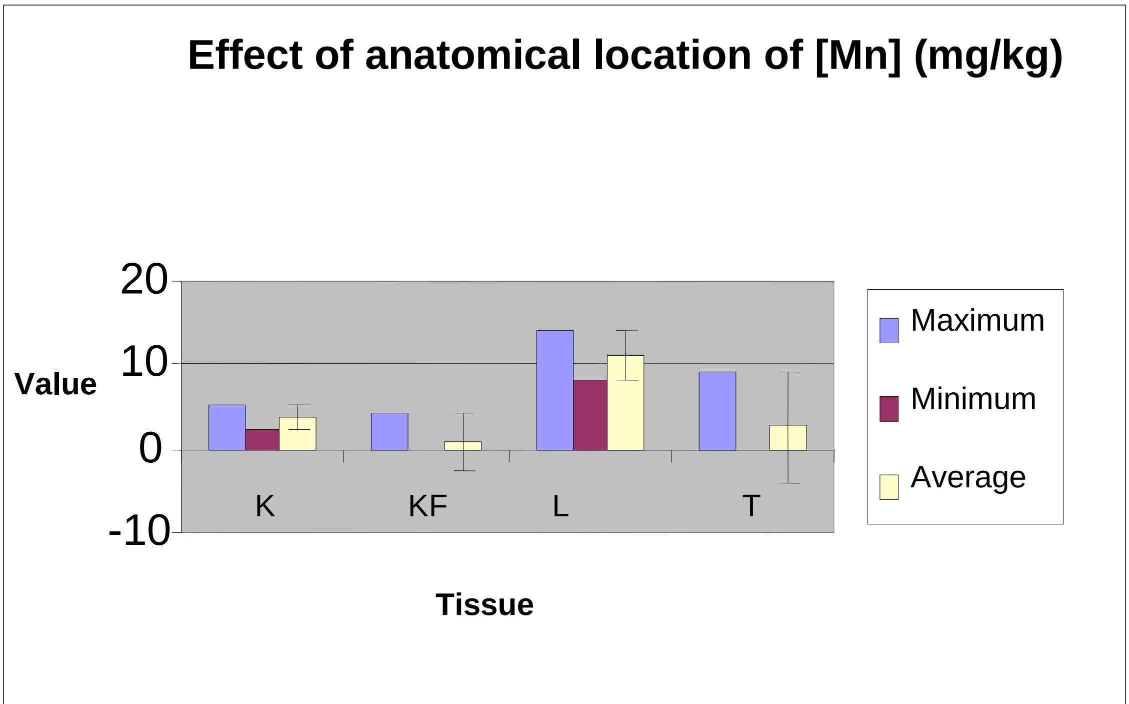

Figure 4.1.5: Effect of anatomical location on the manganese content of impala.

Variation in manganese in the liver was relatively high compared to those in the kidneys, kidney fat and thyroid glands. The concentration of manganese in the liver is probably a better indicator of manganese status in impala. Manganese is poorly absorbed (1% or less) from ruminant diets (Cao *et al*., 2000; Hidiroglow, 1979). Dietary factors that may influence manganese bioavailability have received little attention, probably because manganese deficiency is not considered to be a major problem in ruminants. Limited evidence suggests that high dietary calcium and phosphorus may reduce manganese bioavailability (Cao, *et al*., 2000).

The liver efficiently removes manganese from plasma, whether the manganese is as Mn2+ or bound to 2 macroglobulin, but not Mn3+ bound to the transferrin complex (Gibbons *et al*., 1976**).** The manganese that is taken up by the liver is excreted endogenously via the bile, and accumulations of manganese in the liver often does not reflect dietary intakes of manganese.

Manganese actively participates in redox processes, tissue respiration and bone formation, and affects growth, reproduction, blood formation and the function of endocrine organs. The rapid accumulation of manganese in the mitochondria of liver cells confirms manganese's participation in processes of oxidative phosphorylation.

Cobalt

The concentration of cobalt was highest in the liver (p<0,05), followed by the kidney, while the concentrations were lower in kidney fat and thyroid glands.

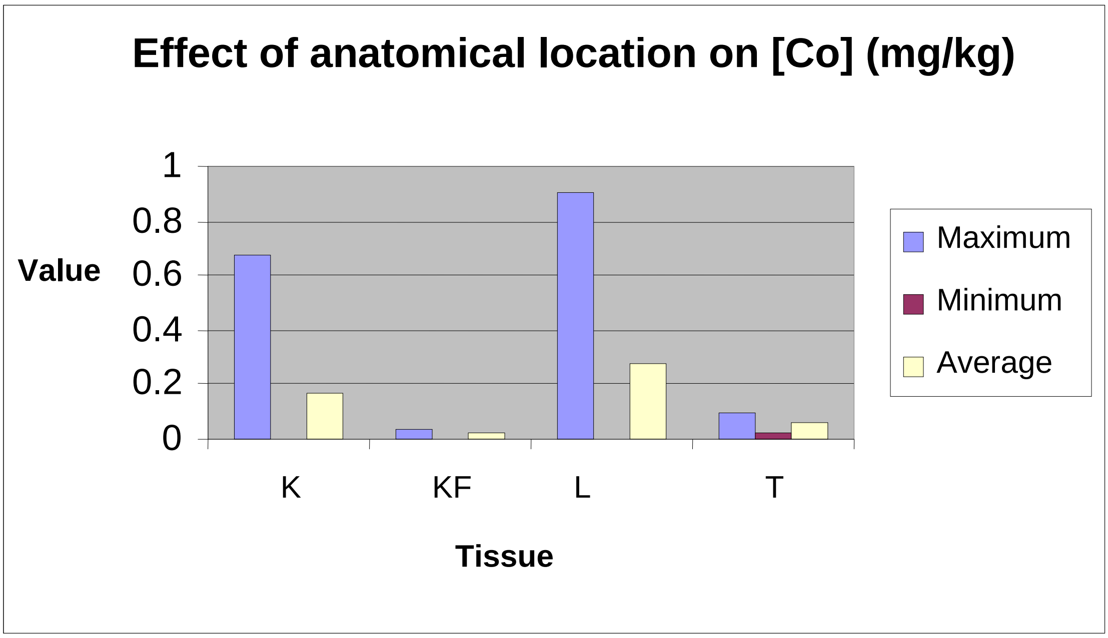

Figure 4.1.6: Effect of anatomical location on the cobalt content of impala.

Variation in cobalt in the liver and kidney were relatively high compared to those in the kidney fat and thyroid glands. The concentration of cobalt in the liver is probably a better indicator of cobalt status in impala. Organs and meats are the best source of vitamin B12, hence the high concentrations of cobalt in the liver and kidney, which concurs with results in Figure 4.1.6. Since the metabolic role of cobalt is as a component of vitamin B12 (Smith, 1997), assessment of cobalt nutrition often centres on measures of B12 status. Concentrations of cobalt in the liver, and performance response of ruminants to cobalt supplementation, can also be used in assessment (McDowell, 1992). As methylcobalamin (MeCbl), cobalt assists a number of methyltransferase enzymes by acting as a donor of methyl groups and is thus involved in carbon metabolism, i.e. the build up of carbon chains.

Methylcobalamin is important for microbes as well as mammals, and is needed for methane, acetate and methionine synthesis by rumen bacteria (Poston and Stadman, 1975). In mammals, MeCbl enables methionine synthatase to supply methyl groups to a wider range of molecules, including formate, noradrenaline, myelin and phosphatidyl ethanolamine (PE). Failure of methylation in vitamin B12 deficient sheep also inhibits folate uptake by the liver (Gawthorne and Smith, 1974). As adenosylcobalamin (AdoCbl), cobalt influences energy metabolism, facilitating the formation of glucose by assisting methylmalonyl-coenzymeA (CoA) mutase in forming succinate from propionate, chiefly in the liver.

Copper

The concentration of copper was negligible in the kidney fat, thyroid and the kidneys. The liver was the only location that contained a higher concentration of copper ($p<0,05$) compared to the other three tissues.

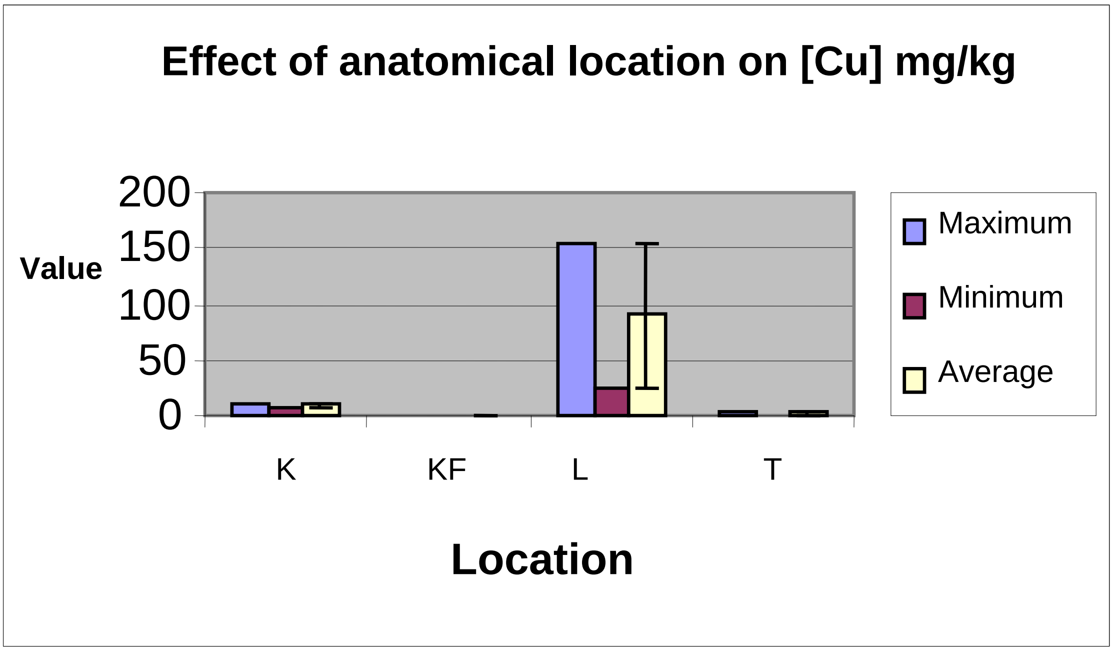

Figure 4.1.8: Effect of anatomical location on the copper content of impala.

Variations in copper in liver were relatively high compared to those in the kidney, kidney fat and thyroid glands. The concentration of copper in the liver is probably a better indicator of copper status in impala.

The concentration of copper in the liver is a direct function of ingestion via the feed. (Georgievskii, 1981). Copper was shown to be essential for growth and haemoglobin formation in experiments done by Hart *et al* (1928). This important discovery was soon followed by experimental evidence that copper is essential for growth, and for prevention of a wide range of clinical and pathological disorders in all types of farm animals.

In sheep, liver contains approximately half of the total copper in the carcass (Langlands *et al*., 1984). In addition to bio-availability of dietary copper, the concentration of copper in the liver is affected by physiological needs, for example, foetal growth. Furthermore, copper is essential for blood formation. It catalyses the incorporation of iron into the structure of heme and assists in the maturation of the erythrocytes. Copper decreases without any change in the haemoglobin concentration. Copper also participates in the process of osteogenesis in the body's protective function in the pigmentation and keratinization of hair and feathers, and in the formation of copper containing proteins with enzymic functions. Higher mean copper levels in liver can be caused by contamination of pasture and by industrial emissions. While pigs are tolerant to an excess of copper in their diet, sheep are extremely sensitive, and cattle are at an intermediate level (Tokarnia *et al*., 2000).

Zinc

The concentration of zinc in the liver was slightly higher compared to that in the kidney and thyroid. Kidney fat contained lower concentrations of zinc ($p < 0,05$), compared to kidney, thyroid and the liver.

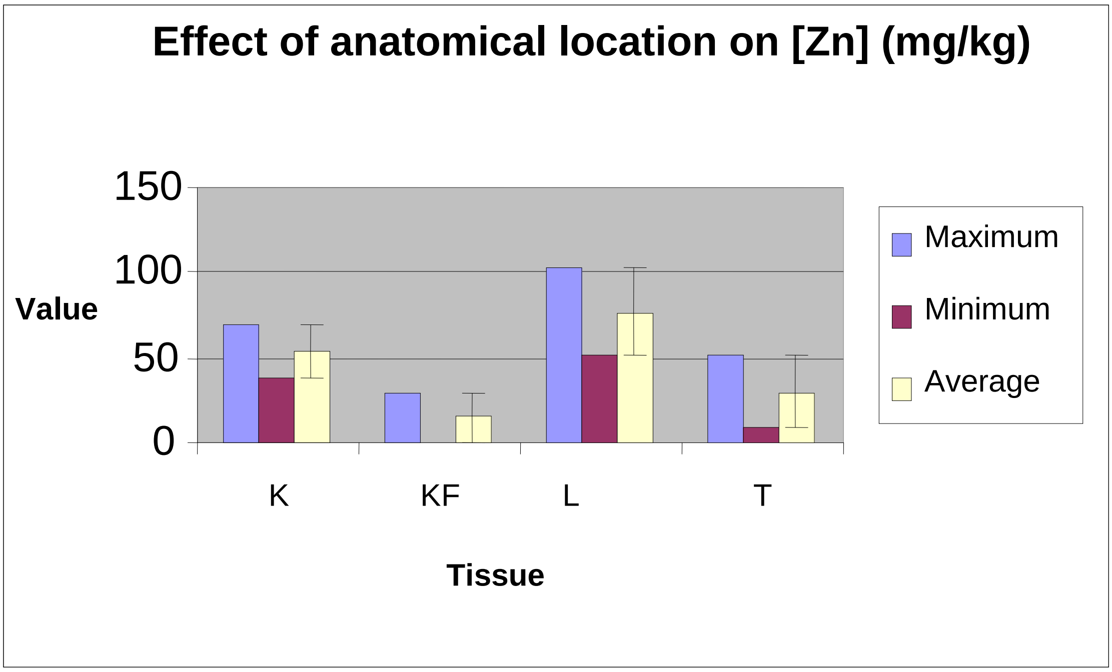

Figure 4.1.9: Effect of anatomical location on the zinc content of impala.

Variations in zinc in liver were relatively high compared to those in the kidney fat, thyroid glands and kidneys. The concentration of zinc in the liver is probably a better indicator of zinc status in impala. There are four isoforms of MT, and increased dietary zinc increases induction of both MT-Ia and MT-II mRNA in liver and kidney tissue, but not in the duodenum, muscle, or skin. Increased concentrations of MT-IA protein account for most of the increased zinc in liver (Lee *et al*., 1994). Concentrations of MT in serum and erythrocytes may be useful as indicators of zinc status that are less affected by infection.

A study demonstrated that zinc concentrations in liver and muscle were higher than those reported for cattle in Sweden (40; 49 mg/kg) and Poland (42; 34 mg/kg) by Johrem *et al.* (1989), and Falandysz (1993). The elements, copper and zinc, accumulate in the liver, which was confirmed in the observed farms in Sweden (Benemariya *et al*., 1993).

Among the various roles of zinc in immunity are gene expression, mitosis, and apoptosis of lymphoid cells, as DNA polymerase, the major enzyme regulating DNA replication, is zinc-dependent (Shankar and Prasad, 1998). Proliferative responses of macrophages, T-cells, or B-cells have been used as early indicators of zinc status in animals.

Rubidium

The concentration of rubidium was highest in the liver ($p<0,05$) followed by the kidneys and the thyroid. Kidney fat concentrations of rubidium were the lowest and insignificant.

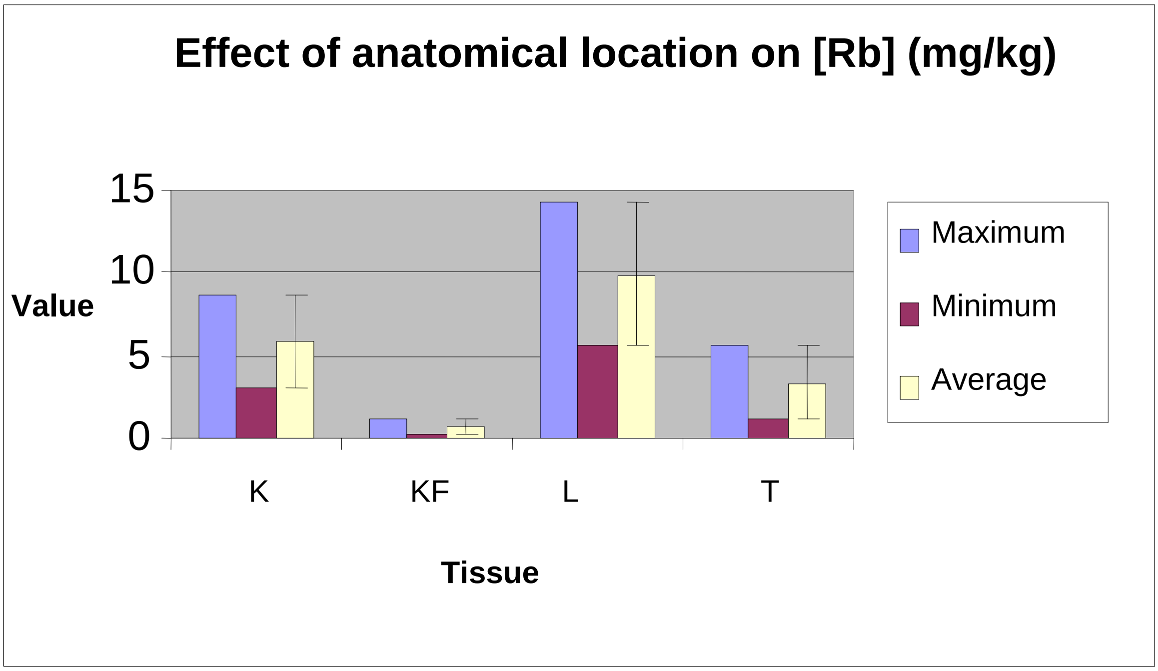

Figure 4.1.10: Effect of anatomical location on the rubidium content of impala.

Variations in rubidium in liver were relatively high ($p<0,05$) compared to those in the kidney, kidney fat and thyroid glands. The concentration of rubidium in the liver is probably a better indicator of rubidium status in impala. Anke and Angelow (1995) found the following mean concentrations of rubidium (ug/g DW) in liver and kidneys for cattle, 36 and 31.8, and sheep, 33.6 and 40.3 respectively. They have also reported that toxic intakes of rubidium markedly increased the rubidium concentration in tissues.

Reports by Glendening *et al*., (1956) found that when dietary intake of rubidium increased from less than 0.02 % (200 mg/kg) to 0.25 % (2,500 mg/kg) through feed supplementation, concentrations in liver, heart, muscle and kidneys of rats were raised from 100-200 mg/kg DW to 8,000-17,000 mg/kg DW.

Molybdenum

The concentration of molybdenum in the liver was higher ($p<0,05$) compared to the kidney, kidney fat, and thyroid. The molybdenum concentration in the kidney fat was the lowest and insignificant, followed by the thyroid gland and kidneys.

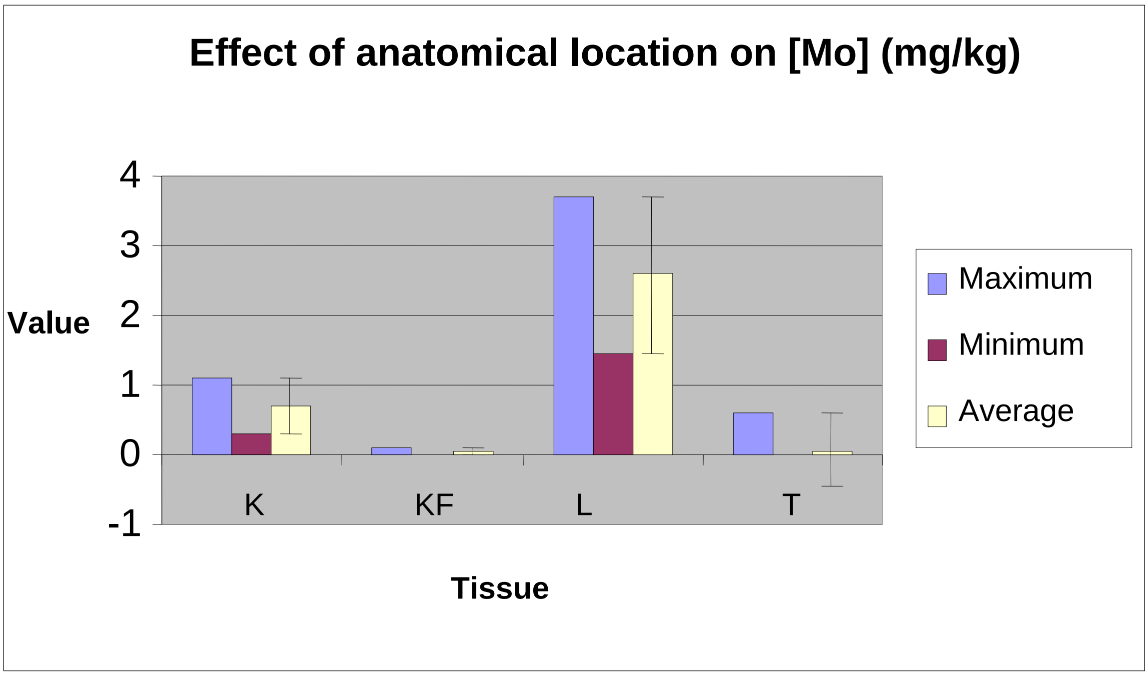

Figure 4.1.11: Effect of anatomical location on the Molybdenum content of impala.

Some kidney fat and thyroid samples did not contain molybdenum. Variations in molybdenum in liver was relatively high compared to those in the kidney, kidney fat and thyroid glands. Molybdenum concentrations in liver probably provide a better indicator of molybdenum status in impala. Since no nutritional deficiencies of molybdenum have been reported in ruminants under practical conditions, no estimates are available on minimum values for molybdenum in blood and tissues. Soluble forms of dietary molybdenum are readily absorbed by animals and cause molybdenum concentrations in serum, whole blood, milk, liver, and kidney to be increased several fold (Kincaid and White, 1988; Wittenberg and Devlin, 1988).

Ward (1994) reported that, with the lowest tolerance to molybdenum toxicity among all species studied, cattle definitely showed overt toxicosis when the dietary molybdenum level was at 100mg/kg DM or higher, regardless of dietary copper or sulphur levels. It was also reported that molybdenum toxicosis may be produced in cattle at levels of 25-50 mg/kg DM. However, reports on the toxicosis caused by <25mg Mo/kg DM is often associated with inadequate available copper. Molybdenum was recently recognized as an essential element (Ward, 1994), it was discovered that it was a component of the enzyme xanthine oxidase which also contains iron. It also plays an important part in the metabolism of purine. Cases of spontaneous molybdenum deficiency in farm animals have never been recorded under practical conditions.

Caesium

The concentration of caesium was high in the kidney, followed by the liver. The concentration level of caesium was lower in the thyroid and the kidney fat.

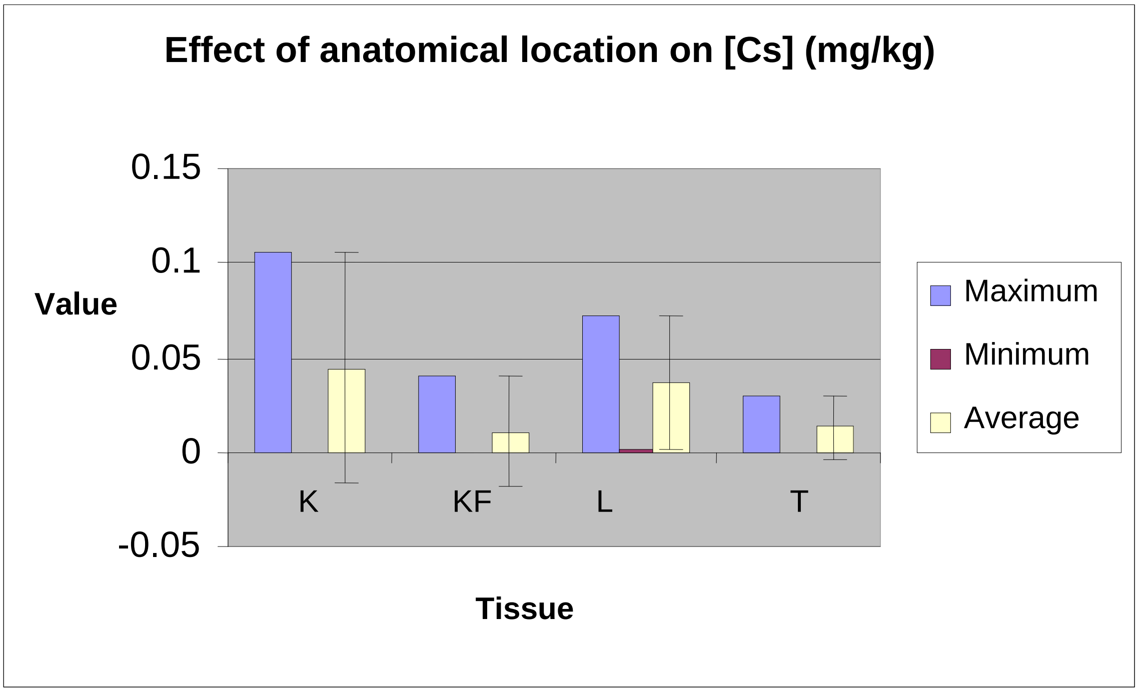

Figure 4.1.12: Effect of anatomical location on the caesium content of impala.

Variations in caesium in the kidneys were relatively high, followed by liver, compared to those in the kidney fat and thyroid glands. The concentrations of caesium in the kidney and liver are probably better indicators of caesium status in impala.

Lead and Bromine

Very high concentrations and high variations in the concentration of lead in kidney, these reflects varying exposure of animals to lead that accumulates in the kidneys. No significant differences in the concentrations of lead were detected between genders, which indicates that males and females are equally exposed to high concentrations of lead. Amongst the commonest source of lead poisoning are: The availability of lead-based paints, discarded oil filters, used crankcase oil, lead storage batteries, or pastures contaminated by industrial lead operations make lead one of the most common causes of accidental poisoning in domestic animals (Demayo et al. 1982). In the case of impala and many other domestic animals, surface water and possibly deep-well or domestic water supplies may contain excessive levels of certain minerals, including lead (ATSDR, 1993).

Bromine

Drinking water is an important route for supplying additional nutrients and pharmaceutical treatments. On the other hand, water may be a channel for potential hazardous chemicals of concern (PHCC). The PHCC become hazardous under circumstances that may be specific to the livestock type and physiological status of the animal, the production environment, ingestion rates of water quality constituents (WQC) and the agonistic or antagonistic effects of other WQC in the drinking water (Meyer *et al*., 1997; Coetzee *et al*., 2000a; b; Meyer & Casey, 2004; Mamabolo *et al*., 2009).

Casey *et al*. (1998; 2001) analyzed various literature and water-source surveys in South Africa and indicated that exposure time, production systems, ingestion rates and species tolerance are important factors that need to be taken into account when formulating water quality guidelines for livestock. Meyer *et al*., (1997) indicated that the verification of the validity of the guidelines in use is required in order to accurately estimate the fitness for use of a water source for livestock production. Evaluation by Casey & Meyer (2001) of a significant number of water samples over a number of years revealed that high concentrations of bromine (Br) occur naturally within groundwater in South Africa, hence a PHCC. The highest quantities of Br present in the water-sources surveyed were

30 - 132 mg Br/L (Meyer *et al*., 1997; Coetzee *et al*., 2000a; b; Meyer & Casey, 2004; Mamabolo *et al*., 2009), a concentration 30 - 130 times higher than the 1 mg Br/L considered by Kempster *et al*. (1980) to be acceptable. Casey & Meyer (2001) recommendation of the relevant safety guideline for Br is 0.01 mg/L. The Safe Drinking Water Committee (1988) reported chronic exposure levels of Br as 2.3 mg/L. This calculation was based on the Suggested No-Adverse-Response-Level (SNARL) where a 70 kg human consuming 2 L of water per day was included. Russian investigators, El'piner *et al. (*1972), recommended a maximum bromide concentration of 0.2 mg/L in drinking water.

Casey *et al*., (1998); NRC, (2005) indicated that the toxicity of bromine as a PHCC depends upon the susceptibility of the species, the amount and time period over which Br is ingested and the solubility of the form of the bromide as found in the water source. The study of iodine (I) x bromide interaction by Baker *et al*. (2003) came to three possible mechanisms by which Br decreases the iodine concentration. The three likely possibilities are that Br: 1) reduces intestinal absorption of I; 2) enhances urinary excretion of iodine or; 3) reduces iodine uptake by the thyroid gland. Iodine concentration did decrease with an increase of bromide inclusion levels, but was insignificant within the thyroid gland. Baker *et al*., (2003); Baker, (2004); NRC, (2005) reported that dietary bromide, on the other hand, was reported to reduce iodine toxicity in chicks The effect of WQCs on the production parameters of broilers was shown by Coetzee *et al*. (2000a; b) and Casey *et al*. (2001). Various manifestations influencing production were reported to occur following the ingestion of bromide over different time periods. An elevated dietary intake of bromide by rat dams in lactation was found to cause a very significant decrease in weight gain in the suckling offspring Pavelka *et al*., (2002). More trials revealed that only half of these sucklings survived and were in a very poor condition. This was complemented by stagnation in the extent of the consumption of diet and water as well as a drop in milk production rate during the nursing period Pavelka, (2003). According to Pavelka *et al*., (2002), this suggests that bromide ingested by the dam was transported via the milk to the sucklings. As indicated by Anke *et al.* (1989) bromide may be an essential element. In three long-term experiments with growing, pregnant and lactating goats, poor bromide nutrition lead to significantly reduced growth, haemoglobin concentration, haematocrit quantities, conception, milk and fat yield, lower longevity of does and kids and an increased abortion rate.

4.2 Effect of gender on the mineral content of impala

Only elements that differed between males and females, or those minerals that exhibited interesting trends, will be discussed in detail. Overall, most elements were detected in samples from male impala, while smaller concentrations were detected in samples from female impala. Minerals that differed significantly between gender include: boron, rubidium, strontium, tin, tellurium, caesium, bromine, antimony and lead. These specific differences will be discussed in more detail in the forthcoming sections.

Table 4.2: Summary statistics (Average ± SD) and ANOVA results of trace elements in samples from male and female Impala (*Aepyceros melampus*)

	SEX	
TRACE ELEMENTS	**MALE(x ± SD)**	**FEMALE (x ± SD)**
Li (mg/kg)	0.020. ± 0.009	0.017 ± 0.010
Be(mg/kg)	0.003 ± 0.004	0.000 ± 0.000
B(mg/kg)	3.023 ± 3.848[a]	0.382 ± 0.677[b]
Ti(mg/kg)	22.280 ± 5.531	23.342 ± 3.364
V(mg/kg)	0.421 ± 0.211	0.339 ± 0.301
Cr(mg/kg)	0.606 ± 0.979	0.747 ± 1.088
Mn(mg/kg)	3.546 ± 1.503	4.235 ± 0.849
Co(mg/kg)	0.170 ± 0.547	0.161 ± 0.037
Ni(mg/kg)	1.171 ± 1.127	1.053 ± 1.137
Cu(mg/kg)	8.918 ± 2.595	9.574 ± 1.413
Zn(mg/kg)	53.654 ± 17.208	55.718 ± 11.647
As(mg/kg)	0.087 ± 0.079	0.070 ± 0.034
Br(mg/kg)	270.465 ± 126.274	323.467 ± 192.253
Se(mg/kg)	3.897 ± 1.573	4.667 ± 0.823
Rb(mg/kg)	6.302 ± 3.205[a]	4.684 ± 1.039[b]
Sr(mg/kg)	0.484 ± 0.256[a]	0.527 ± 0.115[b]
Mo(mg/kg)	0.677 ± 0.468	0.786 ± 0.146
Cd(mg/kg)	0.250 ± 0388	0.118 ± 0.058

Sn(mg/kg)	0.066 ± 0.116[a]	0.045 ± 0.047[b]
Sb(mg/kg)	0.044 ± 0.098	4.115 ± 14.144
Te(mg/kg)	0.866 ± 2.608[a]	0.445 ± 0.873[b]
I(mg/kg)	0.255 ± 0.158	0.129 ± 0.102
Cs(mg/kg)	0.049 ± 0.070[a]	0.030 ± 0.022[b]
La(mg/kg)	0.054 ± 0.114	0.024 ± 0.025
W(mg/kg)	0.304 ± 0.729	0.251 ± 0.367
Pt(mg/kg)	0.742 ± 1.691	0.702 ± 0.912
Hg(mg/kg)	1.945 ± 3.774	1.910 ± 2.150
Pb(mg/kg)	1.040 ± 1.022	459.437 ± 1587.996
Bi(mg/kg)	0.228 ± 0.475	0.292 ± 0.296
U(mg/kg)	0.436 ± 0.727	0.570 ± 0.458

[a,b,c,]Means in the same row with different superscript letters differ significantly ($p<0.05$)

Boron

Illustrations in Figure 4.2.1 show the difference between boron concentrations in males and females. The concentrations of boron were higher ($p<0.05$) in males compared to females.

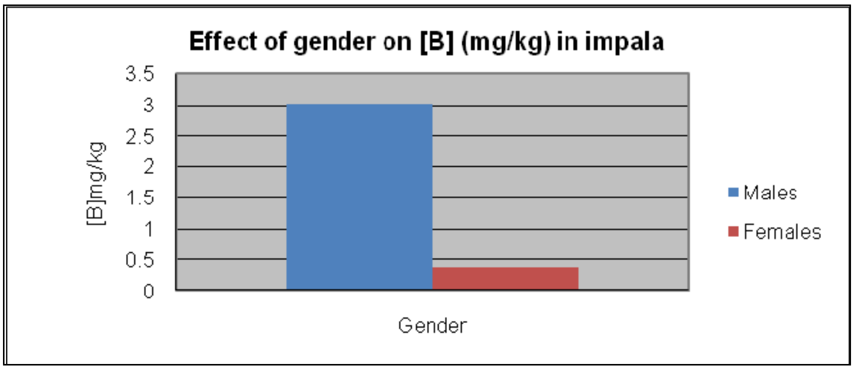

Figure 4.2.1: Effect of gender on the concentrations of boron mg/kg in impala

Variations in boron concentrations in males were relatively high compared to that in females, which were almost negligible. Although results indicate high concentrations of boron in males and negligible concentrations of boron in females, physiological intakes (1-3 mg/kg diet) of boron, based on research findings (Nielsen, 1996, 2002a), suggest that boron is needed for optimal bone health, brain function, and immune function in higher animals and humans. Despite the latter findings, boron is not consistently accepted as an essential nutrient in higher animal as it is in plants.

Rubidium

Figure 4.2.2 demonstrates the different concentrations on rubidium in males and females. There was a slight difference in rubidium concentrations between genders. Males had a slightly higher ($p<0.05$) rubidium concentration compared to females.

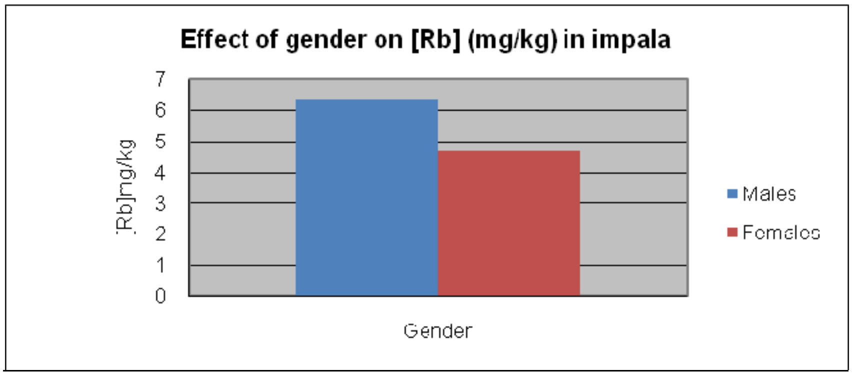

Figure 4.2.2: Effect of gender on rubidium/kg in impala

Variations in the concentration of rubidium in males were relatively high compared to that in females. Biological interest in rubidium has resulted from findings suggesting that it is beneficial, or possibly essential, for higher animals. In comparison to goats fed 1 or 10 mg Rn/kg diet, goats fed less than 0.28 mg Rb/kg diet exhibited decreased feed intake, growth, milk production, and life expectancy, and increased spontaneous abortion (Ankel *et al.*, 1993). Although males exhibited higher rubidium concentrations than females, the results (Figure 4.2.2) suggest that both male and female impala in this study could be used to indicate rubidium status in impala. Schafer and Forth (1983) reported that rubidium is rapidly and highly absorbed by mammals. Human studies have also indicated that rubidium is actively transported from the mother to foetus (Krachler *et al.*, 1999).

Strontium

Strontium was detected in both male and female impala, but the concentrations were higher in females compared to males (Fig 4.2.3).

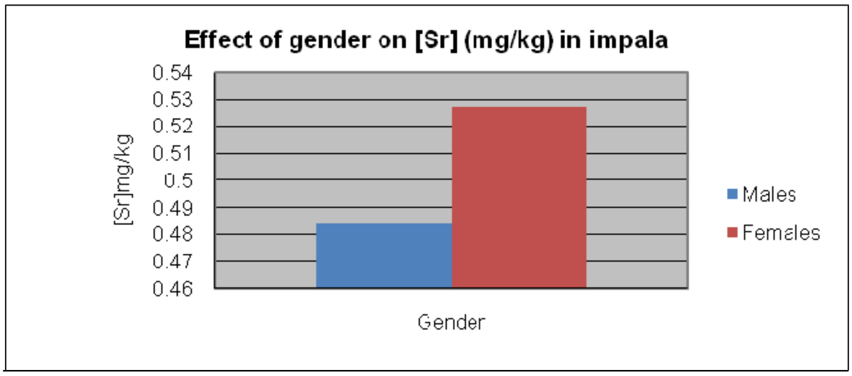

Figure 4.2.3: **Effect of gender on strontium concentrations (mg/kg) in impala**

Variations in the concentrations of strontium in females were relatively high ($p<0,05$) compared to that in males. Strontium is one of the most abundant and potentially hazardous radioactive by-products of nuclear fission, and plants are more efficient than animals in the absorption of strontium. Radioactive strontium is absorbed and deposited in tissues especially the bones, and is also readily transmitted to the foetus and secreted in the milk **(**Hays and Swenson, 1985). The results (Figure 4.2.3) show that females had higher concentrations of strontium. Strontium is preferentially excreted, especially in the urine, after absorption.

Tin

Figure 4.2.4 demonstrates the concentrations of tin which were detected in both male and female impala. The concentration of tin was slightly higher in males compared to males.

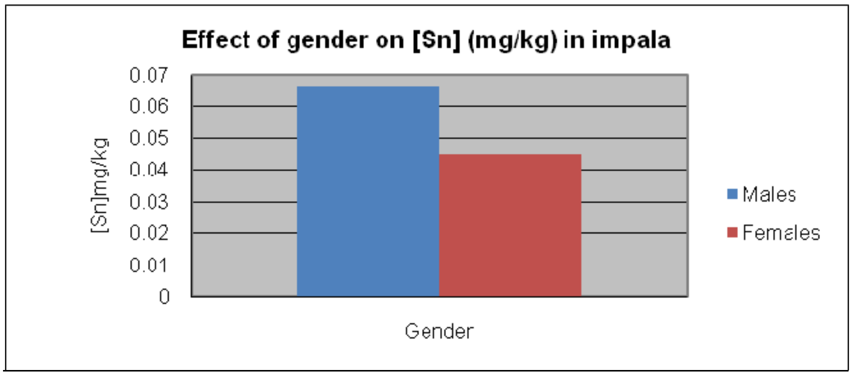

Figure 4.2.4: Effect of gender on tin [Sn] mg/kg in impala

Variations in tin in males were relatively high compared to that in females. The concentrations of tin in females are probably better indicators of tin status in impala. In this study, Figure 4.2.4 illustrates results that both males and females accumulated tin, with the male having the highest accumulation. Findings in NRC (2005) reported that tin is not considered to be an essential element and that exposure of livestock to high levels of inorganic tin is unlikely. If it occurred, the animals which were in marginal nutritional status with regard to zinc or copper, would be most sensitive to chronic high doses of inorganic tin.

Antimony

Small concentrations of antimony were detected in female impala, but the concentrations were negligible from all samples collected from male impala.

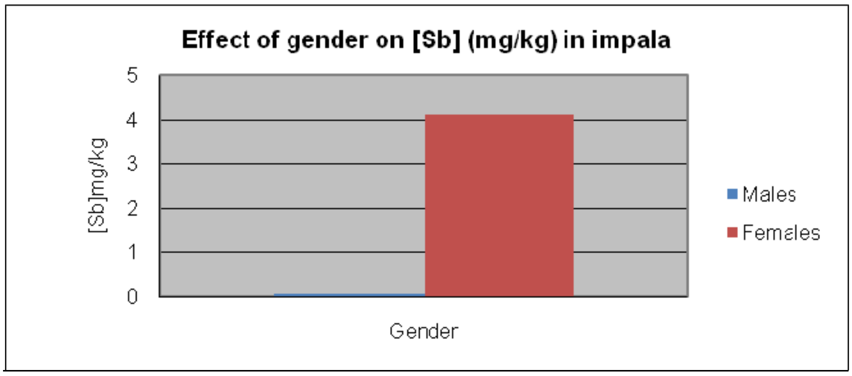

Figure 4.2.5: Effect of gender on antimony [Sb] mg/kg in impala

Variations in antimony in females were higher ($p<0,05$) compared to that in males, which was negligible. The concentrations of antimony in females are probably better indicators of antimony status in impala. Previous studies indicated that lifetime studies with mice fed drinking water supplemented with antimony potassium tartrate, to provide 5mg/L, revealed no demonstrable toxic effects on males, and only a slight decrease in life span and longevity, and some suppression of growth, in females, (Schroeder et al., 1968b**).** This finding concurs with the current results in Figure 4.2.5.

Tellurium

Figure 4.2.6 demonstrates small concentrations of tellurium which were detected in both male and female impala, but the concentrations were slightly higher in males compared to females.

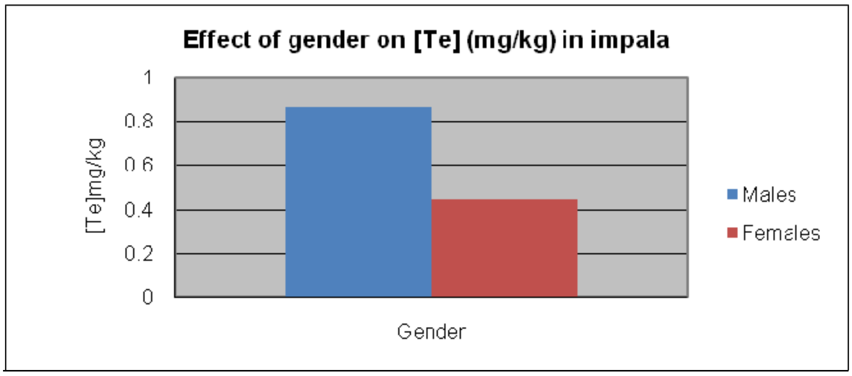

Figure 4.2.6: Effect of gender on tellurium [Te] mg/kg in impala

Variations in tellurium in males were high ($p < 0,05$) compared to that in females. The concentrations of tellurium in males are probably better indicators of tellurium status in impala. There is no information on tellurium to substantiate these current findings.

Caesium

The results in Figure 4.2.7 demonstrate that small concentrations of caesium were detected in both male and female impala, but the concentrations were slightly higher in males compared to females.

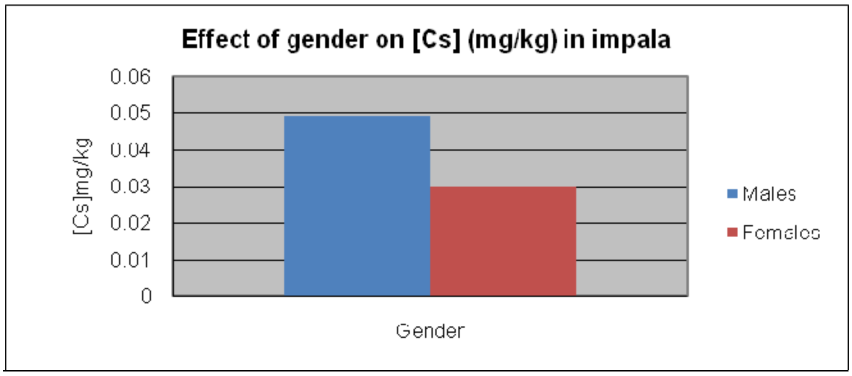

Figure 4.2.7: Effect of gender on caesium [Cs] mg/kg in impala

Variations in caesium in males were relatively high compared to that in females. The concentrations of caesium in males are probably better indicators of caesium status in impala. There is no scientific information to substantiate findings in these current studies.

Table 3: Summary statics of trace elements in impala of different age groups

The summary statistics of trace elements sampled from different age groups of impala are shown in Table 3 below, where 1 = 0-1 year old, 2 = 1-2 year old and 3 = 2-3 years old and older. Only elements that differed between different age groups, or those that exhibited interesting trends, will be discussed in more detail. Overall, most elements were detected in all the three age groups but mostly in juvenile impala (Age 1), which could result from suckling from their mothers.

Table 4.3: Summary statics of trace elements in impala of different age groups

TRACE ELEMENTS	Juvenile (Age 1)	Sub-adult (Age 2)	Adult (Age 3)
Li (mg/kg)	0.014± 0.0108[a]	0.204± 0.009[b]	0.022 ± 0.0124[c]
Be (mg/kg)	0.02± 0.005	0.002± 0.001	0.002 ± 0.004
B (mg/kg)	0.605 ± 1.963[a]	4.584 ± 3.646[b]	2.416 ± 3.699[c]
Ti (mg/kg)	24.673 ± 4.758[a]	20.665 ± 1.843[b]	22.159 ± 5.606[c]
V (mg/kg)	0.424 ± 0.319[a]	0.356 ± 0.133[b]	0.402 ± 0.225b
Cr (mg/kg)	0.736 ± 1.089[a]	1.231 ± 1.936[b]	0.411 ± 0.127[c]
Mn (mg/kg)	4.419 ± 1.254[a]	3.085 ± 0.703[b]	3.604 ± 1.502[b]
Co (mg/kg)	0.201 ± 0.035[a]	0.176 ± 0.600[b]	0.149 ± 0.046[c]
Ni (mg/kg)	1.047 ± 1.142[a]	2.089 ± 1.944[b]	0.880 ± 0.467[c]
Cu (mg/kg)	10.387 ± 1.548[a]	7.638 ± 2.648[b]	8.937 ± 2.311[c]
Zn (mg/kg)	55.056 ± 10.224[a]	45.926 ± 7.414[b]	56.445 ± 19.135[a]
As (mg/kg)	0.130 ± 0.106[a]	0.043 ± 0.025[b]	0.072 ± 0.043[b]
Br (mg/kg)	387.867 ±183.905[a]	213.950 ± 71.557[b]	257.637 ± 120.345[c]
Se (mg/kg)	5.233 ± 1.136[a]	3.696 ± 1.118[b]	3.689 ± 1.414[b]
Rb (mg/kg)	5.355 ± 1.603[a]	6.077± 3.053[b]	6.052 ± 3.328[b]
Sr (mg/kg)	0.434 ± 0.088[a]	0.517 ± 0.321[b]	0.518 ± 0.240[b]
Mo (mg/kg)	0.950 ± 0.245[a]	0.479 ± 0.330[b]	0.662 ± 0.445[c]
Cd (mg/kg)	0.275 ± 0.294[a]	0.161 ± 0.116[b]	0.203 ± 0.403[c]
Sn (mg/kg)	0.117 ± 0.176[a]	0.040 ± 0.045[b]	0.039 ± 0.048[b]
Sb (mg/kg)	4.184 ± 14.124[a]	0.019 ± 0.030[b]	0.020 ± 0.024[b]

Te (mg/kg)	2.329 ± 4.014[a]	0.019 ± 0.024[b]	0.233 ± 0.630[c]
I (mg/kg)	0.213 ± 0.213	0.210 ± 0.065	0.229 ± 0.147
Cs (mg/kg)	0.085 ± 0.106[a]	0.030 ± 0.023[b]	0.029 ± 0.022[b]
La (mg/kg)	0.075 ± 0.111[a]	0.017± 0.019[b]	0.040 ± 0.107[c]
W (mg/kg)	0.768 ± 1.079[a]	0.028 ± 0.034[b]	0.144 ± 0.280[c]
Pt (mg/kg)	1.880 ± 2.445[a]	0.072 ± 0.111[b]	0.391 ± 0.726[c]
Hg (mg/kg)	4.471 ± 5.409[a]	0.410 ± 0.532 [b]	1.206 ± 1.755[c]
Pb (mg/kg)	460.042±1587.805[a]	0.923 ± 0.853[b]	0.787 ± 0.639[c]
Bi (mg/kg)	0.609 ± 0.655[a]	0.033 ± 0.046[b]	0.138 ± 0.228[c]
U (mg/kg)	1.020 ± 0.938[a]	0.103 ± 1.142[b]	0.327 ± 0.426[c]

[a,b,c,]Means in the same row with different superscript letters differ significantly (P<0.05)

Lithium

Figure 4.3.1 illustrates the small concentrations of lithium that were detected in sub adult impala (Age 2). By contrast the concentrations of lithium in juvenile (Age 1) and adult (Age 3) impala were negligible.

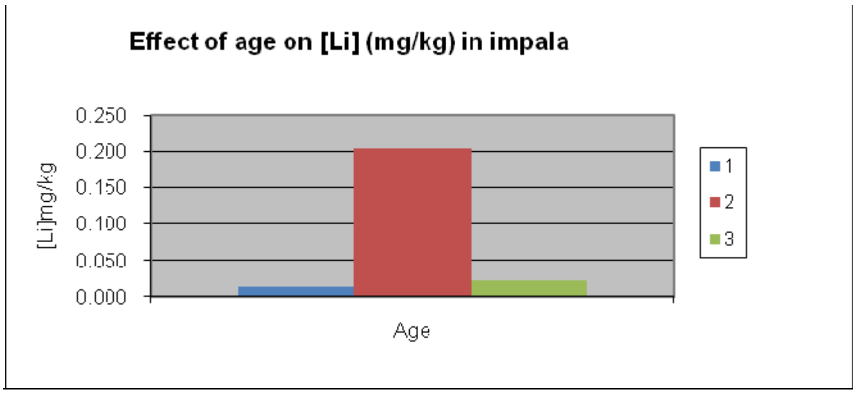

Figure 4.3.1: Effect of age on lithium [Li] (mg/kg) in impala

Variations in lithium in sub adults were relatively high ($p<0,05$), compared to those in juveniles and adults. The concentrations of lithium in sub adults are probably better indicators of lithium status in impala. Lithium concentrations in tissue samples are directly dependant on lithium intake NRC (2005). Tissue concentrations of lithium in animals reported in literature are generally lower than 0,5 mg/kg NRC (2005), which concurs with the findings of the present study with values that were less than 0, 2 mg/kg. These results suggest that impala in this specific environment did not consume feed that contained high concentrations of lithium. In cattle, it was found that intakes of 100mg/kg of lithium reduced feed intake (Anke, 1991). Since the concentrations of lithium detected in tissues of impala were far below these values, no adverse effects are expected in terms of feed intake or production losses in impala.

Beryllium

Figure 4.3.2 illustrates the small concentrations of beryllium detected in juvenile impala (Age 1), the concentrations in sub adult (Aged 2) and adult impala were negligible.

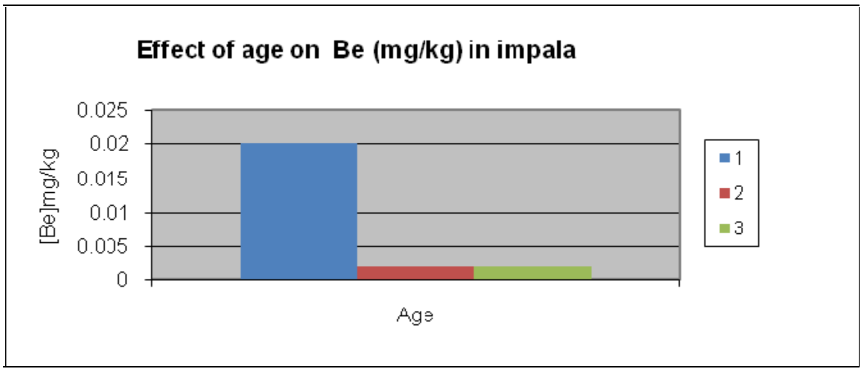

Figure 4.3.2: Effect of age on beryllium [Be](mg/kg) in impala

Variations in beryllium in sub adults and adults were small compared to high beryllium concentrations in juveniles, which could have resulted from suckling milk from their mothers. The concentrations of beryllium in juveniles are probably better indicators of beryllium status in impala. No literature could be found that described beryllium as an element found in ungulates.

Boron

Figure 4.3.3 illustrates that concentrations of boron was detected in sub-adult (Age 2) and adult impala, but the concentrations in juvenile impala were very low. The concentrations in 2 year old impala were higher (p<0,05) compared to adult impala.

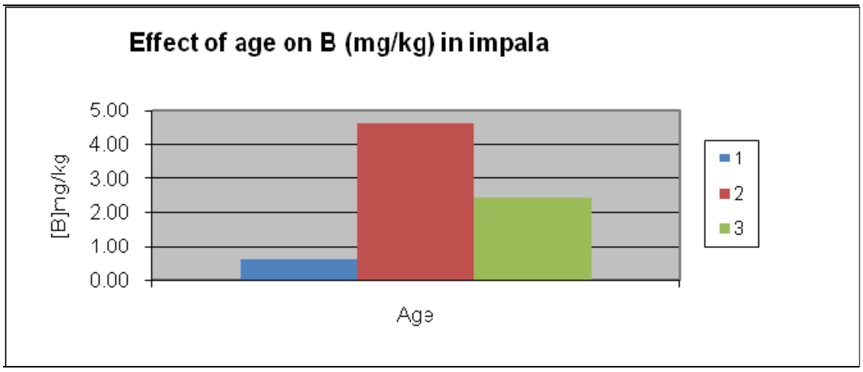

Figure 4.3.3: Effect of age on boron [B] (mg/kg) in impala

Variations in boron in sub-adults were relatively high (p<0,05) compared to those in juveniles and adults. The concentrations of boron in sub adults are probably better indicators of boron status in impala. Substantial evidence form experiments comparing very low intakes (<100ug/kg diet for animals, 0.25ug/d for humans) with physiological intakes (1-3 mg/kg diet) of boron suggested that boron is required for optimal bone health, brain function and immune function in higher animals and humans (Nielsen, 1996, 2002a). The NRC (2005), however, found out that, although the evidence is similar, boron is not consistently accepted as an important nutrient for higher animals as it is for plants, due to lack of clearly defined specific biochemical functions.

IPCS (1998) reported that the most sensitive indicators of boron toxicity in animals have been decreased weight and rib abnormalities in the developing rat foetus, which occurs with boron intakes of about 13mg/kg body mass per day. It was furthermore suggested that 10 mg /kg body mass per day may be a reasonable MTL for animals. Findings in this current study indicate the highest boron tissue concentrations in sub-adult impala (Figure 4.3.3). As reported by NRC (2005), it is unlikely that boron toxicity under normal environmental conditions is a concern for animals.

Titanium

Figure 4.3.4 illustrates that titanium was detected in all three age groups of impala, but higher concentrations were detected in juvenile impala compared to the concentrations in sub adult (Age 2) and adult impala which were low, but slightly higher in adults than in medium aged animals.

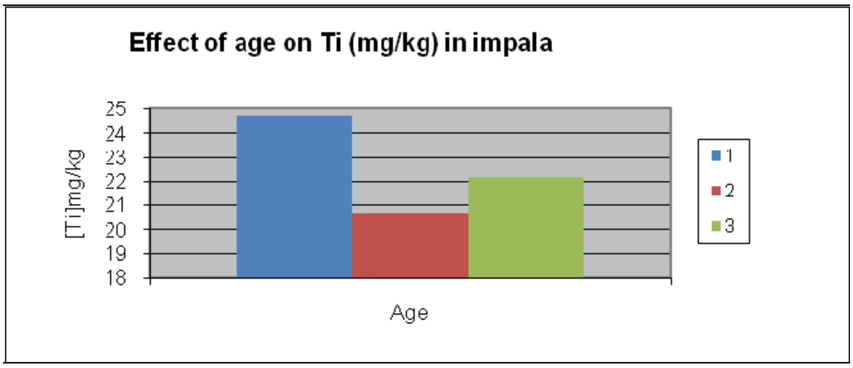

Figure 4.3.4: Effect of age on titanium [Ti] (mg/kg) in impala

Variations in titanium in juveniles were high ($p<0,05$) compared to titanium concentrations in sub-adults and adults. The concentrations of titanium in juveniles are probably better indicators of titanium status in impala

Vanadium

Figure 4.3.5 illustrates that small concentrations of vanadium was detected in all age groups in impala. Higher concentrations were detected in juvenile impala compared to the concentrations in sub adult (Age 2) and adult impala which were low, but higher in adults than in sub-adults.

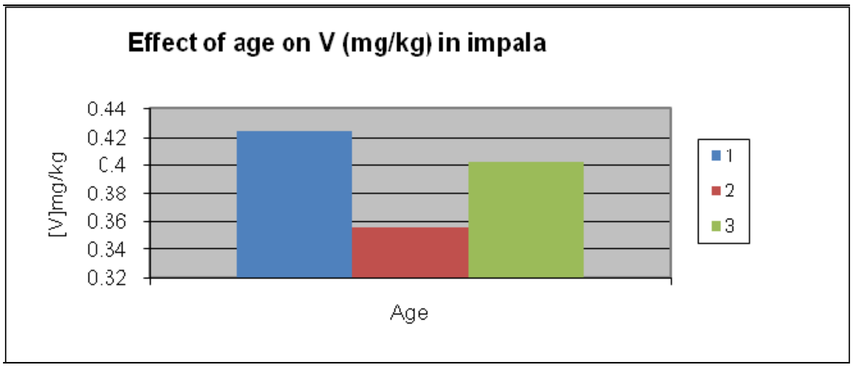

Figure 4.3.5: Effect of age on vanadium [V] (mg/kg) in impala

Variations in vanadium in juveniles were high ($p<0,05$) compared to vanadium concentrations in sub-adults and adults. The concentrations of vanadium in juveniles are probably better indicators of vanadium status in impala. Although the results in the present study indicate higher concentrations in juveniles, a report by the National Research Council (NRC) (1980) concluded that, in the species tested, vanadium is essential for normal growth and proper physiological roles.

Chromium

Figure 4.3.6 illustrates that small concentrations of chromium was detected in sub-adult impala (Age 2), followed by minute concentrations in juvenile impala (Age 1) and adult impala (Age 3) were also minute. Concentrations in impala (Age 2) was higher compared to impala (Age 1) which was in turn higher than in impala (Age 3).

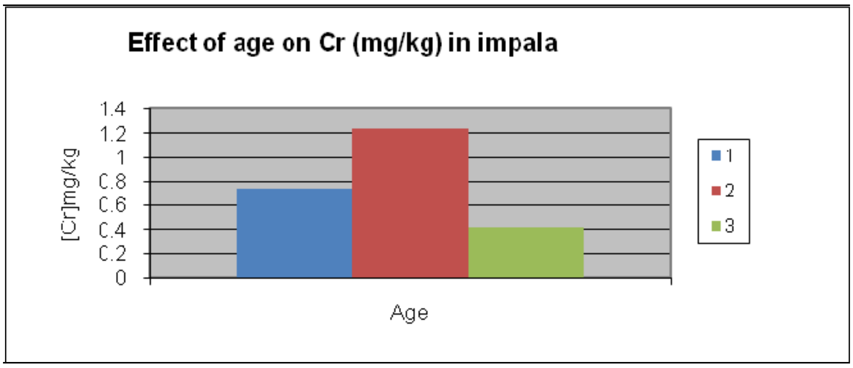

Figure 4.3.6: Effect of age on chromium [Cr] (mg/kg) in impala

Variations in chromium in juveniles were minimal compared to high chromium concentrations in sub-adults ($p < 0,05$). The concentrations of chromium in sub-adults are probably better indicators of chromium status in impala.

Manganese

Manganese was detected in three age groups and there was a small difference in manganese concentrations between Ages 1,2and 3, i.e. Juvenile, sub adult and adult impala

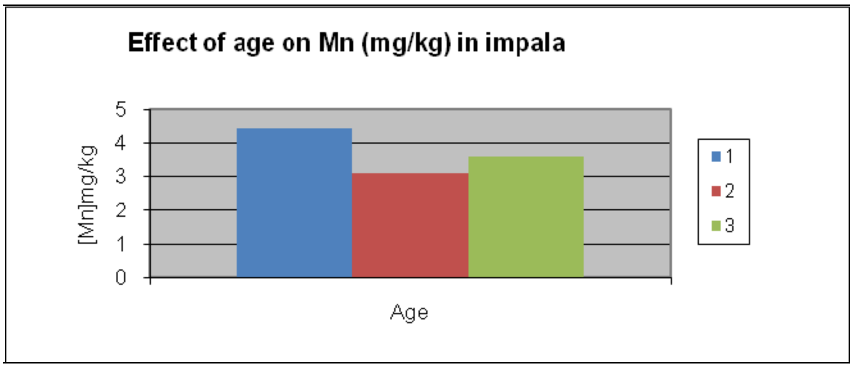

Figure 4.3.7: Effect of age on manganese [Mn] (mg/kg) in impala

Variations in manganese in juveniles were higher compared to manganese concentrations in sub-adults and adults. In this case, concentrations of manganese in impala (Age 1) would probably be better indicators of manganese status in impala. Manganese is also instrumental in skeletal development and growth. Georgievskii (1982), reported that age related variations in the manganese content of individual tissues are not explicit, and that the concentration in liver decrease on passing from a purely milk diet to mixed diet.

Cobalt

Concentrations of cobalt were detected in all three age groups and only a small difference in cobalt concentrations between juvenile (Age 1), sub adult (Age 2) and adult (Age 3) was detected.

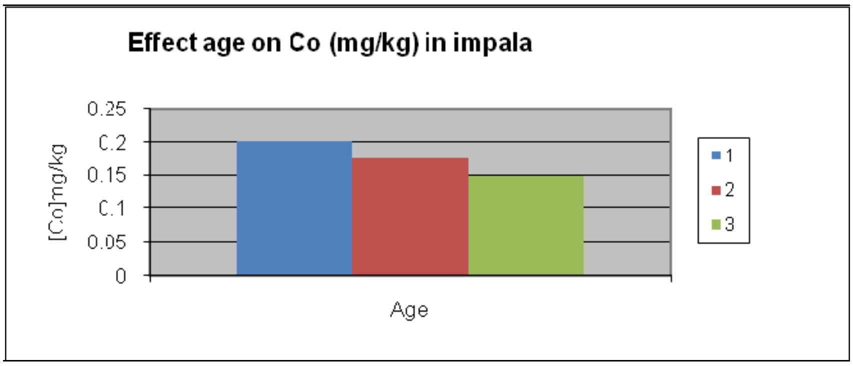

Figure 4.3.8: Effect of age on cobalt [Co] (mg/kg) in impala

Variations in cobalt in juveniles were high compared to cobalt concentrations in sub-adults and adults. Although slight differences of cobalt concentrations were detected in the three age groups. Although there were slight differences in cobalt concentrations, the juvenile impala would probably be better indicators of cobalt compared to sub adults and adult animals. Kirchgessner *et al.*(1994) reported that, in weanling rats fed physiological concentrations of cobalt ($CoCl_2$), apparent and true absorption of cobalt averaged 28 and 29.8 percent respectively, whereas, Toskes *et al*. (1973) reported that mice absorbed 26 percent of an oral dose of labelled cobalt.

Cobalt is required for the synthesis of vitamin B12. In domestic ruminants, cobalt deficiency results in inappetence and loss of body weight, emaciation, weakness, decreased growth, unthrifty appearance, diarrhoea, and anaemia (Smith, 1990; Radostits *et al*., 2000).

Nickel

Figure 4.3.9 illustrates the small concentrations of nickel detected in sub adult impala (Age 2), small concentrations in juvenile impala (Age 1) and adult impala (Age 3) were also minute. Concentrations in Age 2 was higher compared to Age 1, followed by Age 3.

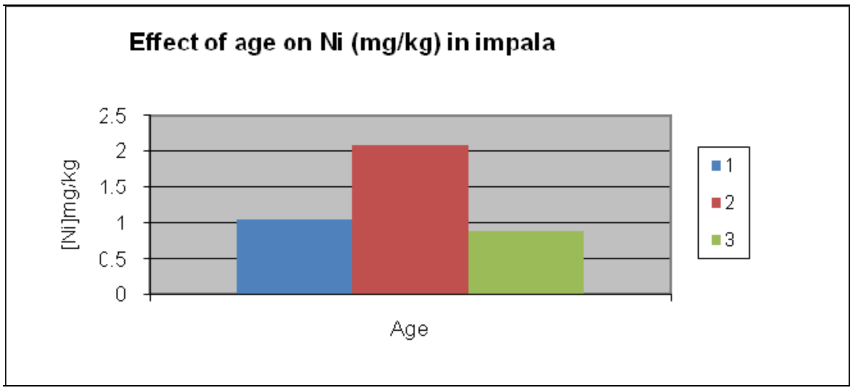

Figure 4.3.9: Effect of age on nickel [Ni] (mg/kg) in impala

Variations in nickel in juveniles and adults were low compared to high nickel concentrations in sub-adults ($p<0,05$). Concentrations of nickel in sub-adults probably better indicates nickel status in impala. There is no research information on nickel to substantiate findings in Figure 4.3.9.

Copper

Concentrations of copper was detected in all impala aged 1, 2 and 3. Concentrations were slightly higher in juvenile impala (Age 1) compared to sub adult impala (Age 2) and adult impala (Age 3) with their concentration being almost the same.

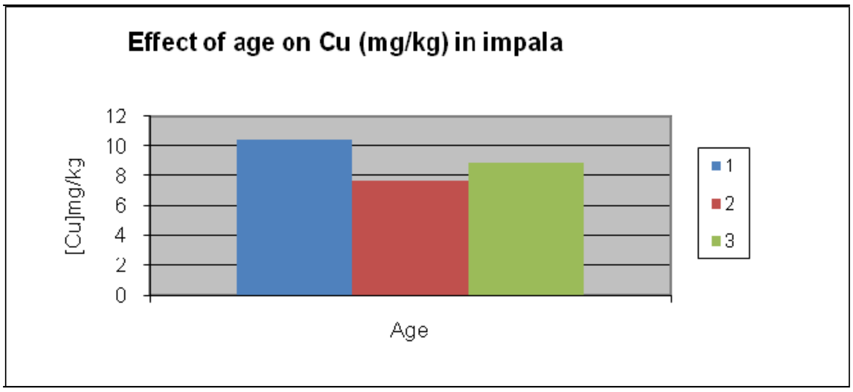

Figure 4.3.10: Effect of age on copper [Cu] (mg/kg) in impala

Variations of copper concentrations in juveniles were high, although there were no significant differences compared to copper concentrations in sub-adults and adults.

Before development of a functional rumen, copper absorption is high (70–85%) in milk-fed lambs but decreases to <10% after weaning (Underwood, and Suttle, 1999). It is well documented that copper requirements vary greatly in ruminants, depending on concentrations of other dietary components, especially sulphur and molybdenum.

The highest liver copper values, of 143 ppm, have been found in neonatal gazelles. Liver copper levels in foetuses and neonates are usually much higher than in adults, and a healthy foal has a level of copper 7 times higher than that of adults (Radostits *at al*., 1994). Liver of newborn ruminants normally contains high concentrations of copper (> 200 mg of Cu/kg liver DM; Hidiroglou and Williams, 1982; Branum et al., 1998) that are affected by maternal copper status. For example, the concentration of copper in liver of foetuses from cows with > 25mg of Cu/kg of liver DM was higher than that in liver of foetuses from dams with < 25 mg of Cu/kg of liver DM (Gooneratne and Christensen, 1989). Lambs with swayback had 17 mg Cu/kg liver, compared to normal lambs with 109 mg Cu/kg liver (McC. Howell and Davison, 1959).

Zinc

High concentrations of zinc were detected in all impala Aged 1, 2 and 3. Concentrations were slightly higher in juvenile impala (Age 1)and higher in adult impala (Age 3) compared to sub adult impala (Age 2).

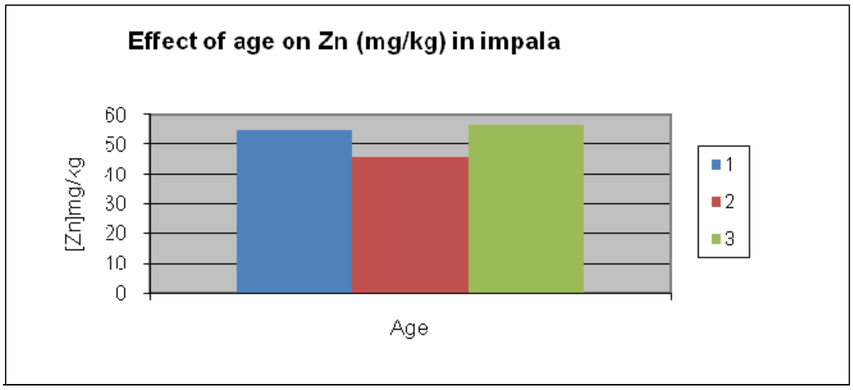

Figure 4.3.11: Effect of age on zinc [Zn] (mg/kg) in impala

Variations in zinc in juveniles and adults were high compared to zinc concentrations in sub-adults. Concentrations of zinc in plasma fluctuate with age, stress, infections, and feed restriction. Plasma zinc is very high (2.3 g/mL) in newborn calves and drops to 1.2 g/mL by 12 wk of age (Kincaid and Hodgson, 1989). Plasma zinc, as part of an acute phase response, is initially reduced by infection (Wellinghausen and Rink, 1998), only to become elevated within a few days. Serum zinc is also decreased by hyperthermal stress and ketosis in cows and is increased in cows with mastitis and in older cows (Wegner *et al*., 1973).

Calves readily absorb and bind large amounts of zinc as metallothionein (MT) in liver, in response to elevated zinc intakes (Kincaid *et al*., 1976). For example, diets supplemented with 600 ppm zinc fed to young calves caused zinc in liver to increase by 600% but did not affect zinc in liver of mature cows (Kincaid *et al*., 1976). Once the added zinc is removed from the diets of calves, concentrations of zinc in liver return to normal within a few weeks (Kincaid and Cronrath, 1979).

Arsenic

Figure 4.3.12 illustrates that small concentrations of arsenic were detected in all three groups of ages in impala, but higher concentrations were detected in juvenile impala, (Age 1) compared to the concentrations in sub adult impala (Age 2) and adult impala (Age 3), which were low but higher in adults than in medium aged.

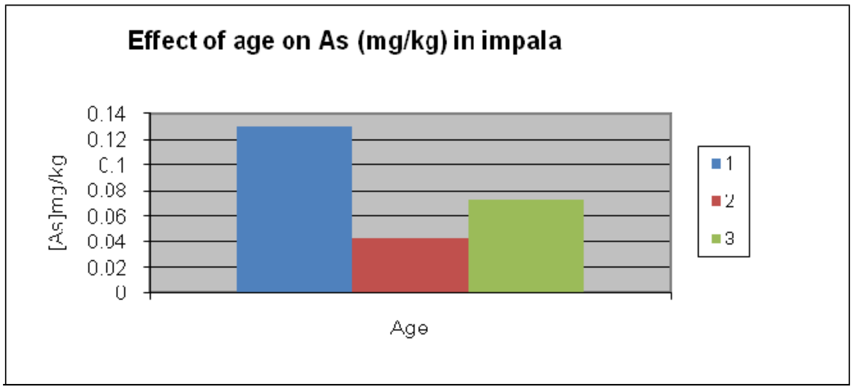

Figure 4.3.12: Effect of age on arsenic [As] (mg/kg) in impala

Variations in arsenic in juveniles were high (p< 0,05) compared to arsenic concentrations in sub-adults and adults. Age, sex and pregnancy apparently affect arsenic toxicity. Findings reported by Vahter *et al*. (2000) indicated that children are more susceptible to arsenic toxicity.

Bromine

Figure 4.3.13 illustrates that high concentrations of bromine was detected in all three groups of Ages (1, 2 and 3) in impala, but higher concentrations were detected in juvenile impala (Age 1) compared to the concentrations in sub adult (Age 2) and adult impala (Age 3) which were low and slightly higher in adults than in medium aged.

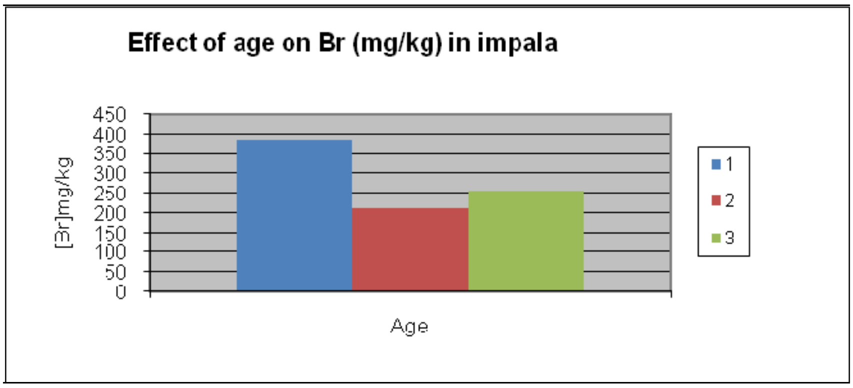

Figure 4.3.13: Effect of age on bromine [Br] (mg/kg) in impala

Variations in bromine in juveniles were high ($p<0,05$) compared to bromine concentrations in sub-adults and adults. There are no known essential biochemical functions of bromine and it is not usually considered as an essential nutrient (NRC, 1980). Natural bromine can have adverse effects on thyroid function and thus thyroxin synthesis because it competes with iodine absorption in the thyroid gland.

Selenium

Figure 4.3.14 illustrates small concentrations of selenium were detected in all three groups of ages (1, 2 and 3) in impala, but slightly high concentrations were detected in juvenile impala, (Age 1) compared to the concentrations in sub adult impala (Age 2) and adult impala (Age 3), of which their selenium concentrations were the same.

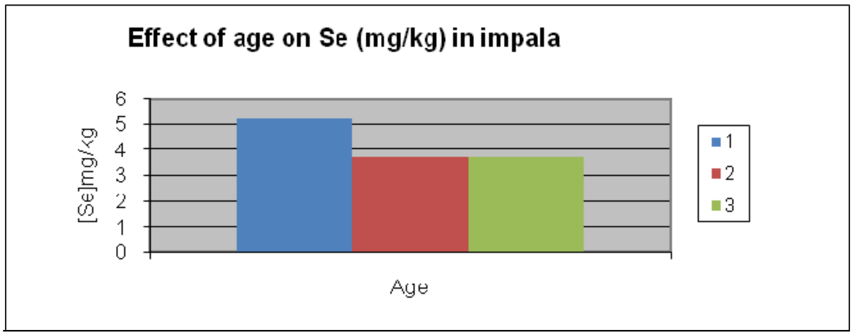

Figure 4.3.14: Effect of age on selenium [Se] (mg/kg) in impala

Differences of selenium concentrations in juveniles were high ($p<0,05$) compared to selenium concentrations in sub-adults and adults. Selenium is an essential nutrient and the role of selenium in animal health is complex. Diseases due to selenium deficiency are recognized in livestock worldwide, generally in association with selenium-deficient soil (Radostits *et al*., 2000).

Concentrations of selenium in whole blood of newborn calves and their dams are highly correlated ($r = 0.74$, $P <0 .05$; Kincaid and Hodgson, 1989). Cows in late pregnancy need 3 to 5 mg of Se/d to ensure adequate selenium reserves in tissues of newborns (Abdelrahman and Kincaid, 1995). Relatively large amounts of selenium are transferred from the dam to the foetus during the last trimester of pregnancy therefore, selenium levels in maternal blood are reduced unless selenium intakes of cows exceed 3 mg/d. The efficiency of maternal transfer of selenium to the foetus is affected by the chemical form of the selenium in the diet. Compared to selenite, more selenium from selenomethionine is transferred from the dam to the foetus and into milk (Kincaid and Rock, 1999; Knowles *et al*., 1999).

Rubidium

Figure 4.3.15 illustrates that rubidium was detected in all three groups of ages (1, 2 and 3) in impala, but slightly higher concentrations were detected in both sub adult, (Age 2) and adult impala (Age 3) compared to juvenile impala (Age 1).

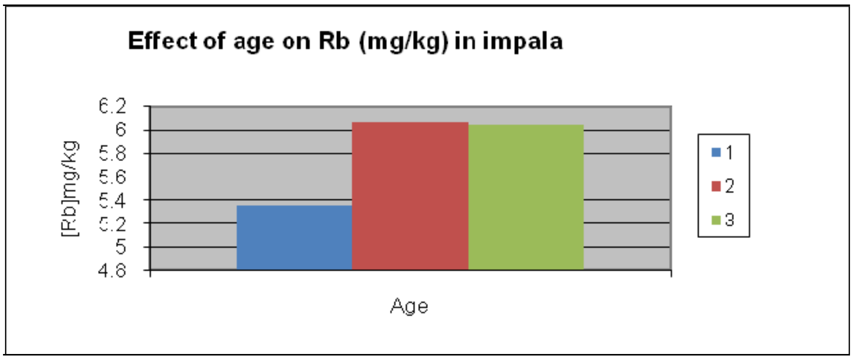

Figure 4.3.15: Effect of age on rubidium [Rb] (mg/kg) in impala

Variations in the concentration of rubidium indicate that sub adult and adult impala are better indicators of rubidium status in impala.

Strontium

Figure 4.3.16 illustrates that small concentrations of strontium were detected in all three groups of ages (1, 2 and 3) in impala. Slightly higher concentrations of strontium were detected in both sub adult, (Age 2) and adult impala (Age 3) compared to juvenile impala (Age 1).

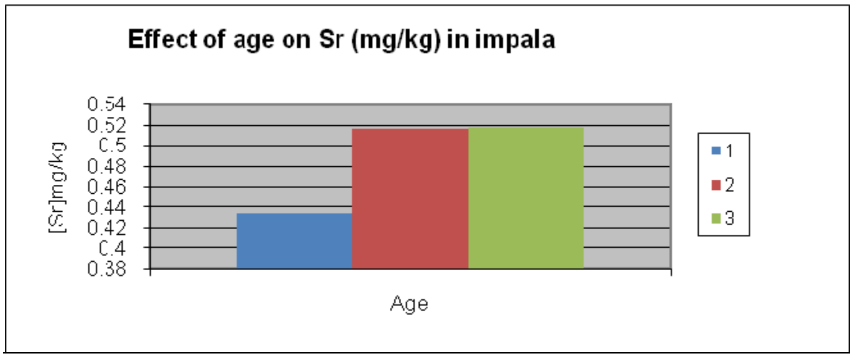

Figure 4.3.16: Effect of age on strontium [Sr] (mg/kg) in impala

Variations in the concentration of strontium as indicated in Figure 4.3.16 indicate that sub adults and adult impala are more exposed to strontium than juvenile impala. The higher levels of this element in sub-adults and adults probably suggest that these two age groups are more likely to ingest strontium than juveniles. A report by the NRC (2005) indicated that mature animals can tolerate higher levels than young. The findings above indicate that cattle and swine can tolerate 2,000 mg Sr/kg diet (0.2 %), chicks can tolerate 3,000 mg Sr/kg diet (0.3 %), and hens can tolerate 30,000 mg Sr/kg diet (3.0 %) when dietary calcium is adequate. The report also states that these amounts are 100 to 1,000 times greater than those normally found in animal feeds. Thus strontium is not a toxicological concern for animals.

Molybdenum

Figure 4.3.17 illustrates small concentrations of molybdenum were detected in all age groups (1, 2 and 3) in impala. Lower concentrations of molybdenum were detected in both medium (Age 2) and adult impala (Age 3) compared to juvenile impala (Age 1), which was slightly higher.

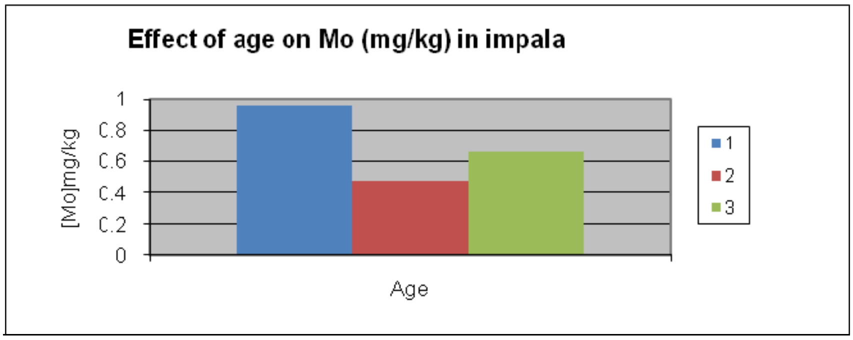

Figure 4.3.17: Effect of age on molybdenum [Mo] (mg/kg) in impala

Variations of molybdenum concentrations were higher ($p<0,05$) in juvenile impala compared to sub adults and adult impala. This finding indicates that juvenile impala are better indicators of molybdenum status. Kincaid (1980) reported that, based on the responses of growth, liver copper concentration, and plasma copper distribution, it was suggested that the minimal toxic concentration of molybdenum in drinking water for calves was between 10 and 50 mg/L, and the critical copper:molybdewnum ratio is <0.5 when animals were given diets containing 13 mg Cu/kg and 0.29 % sulphur.

Gipp *et al*. (1967) and Kline *et al*. (1973) reported that two independent studies indicated that growing swine showed no apparent adverse reaction to 27 or 50 mg of Mo/kg of feed.

Cadmium

Figure 4.3.18 illustrates small concentrations of cadmium were detected in all age groups (1, 2 and 3) in impala. Lower concentrations were detected in both medium (Age 2) and adult impala (Age 3) compared to juvenile impala (Age 1), which was slightly higher.

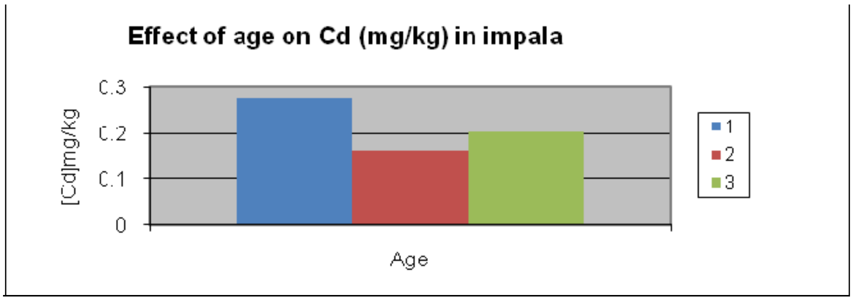

Figure 4.3.18: Effect of age on cadmium [Cd] (mg/kg) in impala

Bokori and Fekete (1995) reported that cadmium is not considered an essential nutrient for animals, however, a number of studies with rodents, chickens and livestock, have reported increased weight gain when low levels of cadmium were added to the diets.

Matsuno *et al*. (1991b) reported that, in beagles, the biological half-life of cadmium fed at either 1, 2, 10, or 50 mg/kg diet is between 1 and 2 years. Due to the reported low elimination rate, cadmium accumulates with age, and older animals have significant levels of cadmium in their kidneys, even if the levels in their diets and water were consistently low. The present study (Figure 4.3.18) indicates high tissue concentrations in juvenile impala (Age 1). This could be due to suckling milk from the mothers. Since cadmium accumulates over time and has a long biological half-life Dietz *et al*. (1998), positive correlations between age of the animal and cadmium concentration in kidney and/or liver have been fairly consistent in many studies in deer, moose and other free-ranging ungulates (Froslie *et al*., 1986; Scanlon *et al*., 1986; Crête *et al*, 1987; Glooschenko *et al*., 1988; Brazil and Ferguson 1989; Gamberg and Scheuhammer, 1994; Paré *et al*., 1999; Gustafson *et al*., 2000; O'Hara et al., 2001; Custer *et al*., 2004).

Tin

Very small concentrations of tin were detected in all age groups (1, 2 and 3) in impala (Figure 4.3.19), but very low concentrations which were almost negligible, were detected in both sub adult (Aged 2) and adult impala (Age 3) compared to juvenile impala (Age 1), which was slightly higher.

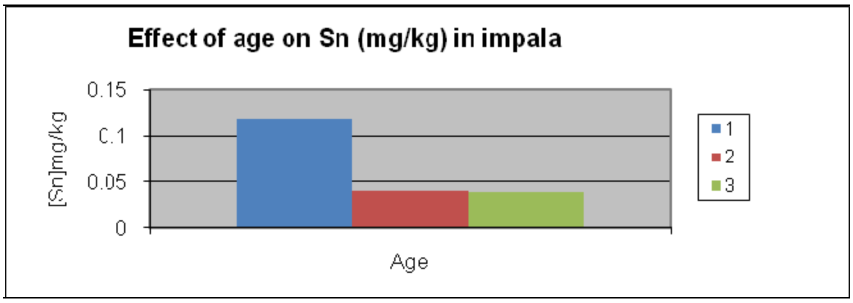

Figure 4.3.19: Effect of age on tin [Sn] (mg/kg) in impala.

Variations in concentrations of tin in impala of different age groups indicates that the juvenile impala had accumulated more tin ($p<0,05$) than the sub adult and adult impala (figure 4.3.19). Sullivan *et al*. (1984) reported findings that neonatal rats absorbed tin more than adult rats when both were gavaged with solutions containing tin (0.9 % versus 0.01 % of dose, respectively). These findings concur with the results in Figure 4.3.19.

Antimony

A small concentration of antimony was detected in juvenile impala (Age 1), ($p<0,05$), but the concentrations were negligible in all samples collected from sub adult impala (Age 2) and adult impala (Age 3).

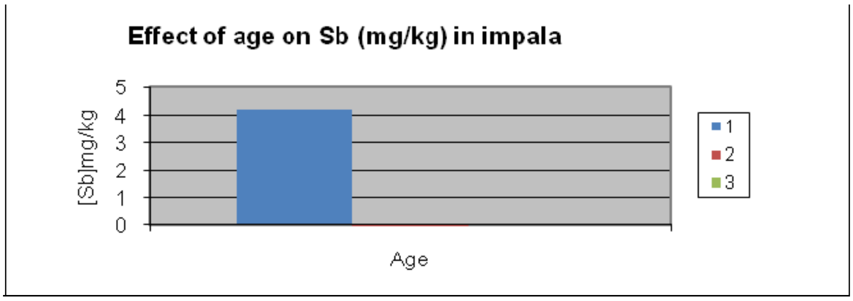

Figure 4.3.20: Effect of age on antimony [Sb] (mg/kg) in impala

Variations in the concentration of antimony indicate that juvenile impala are better indicators of antimony status in impala (Figure 4.3.20).

Tellurium

A very small concentration of tellurium was detected in juvenile impala (Age 1), ($p<0,05$) but the concentrations were negligible from all samples collected from sub adult impala (Age 2) and almost negligible in adult impala (Age 3).

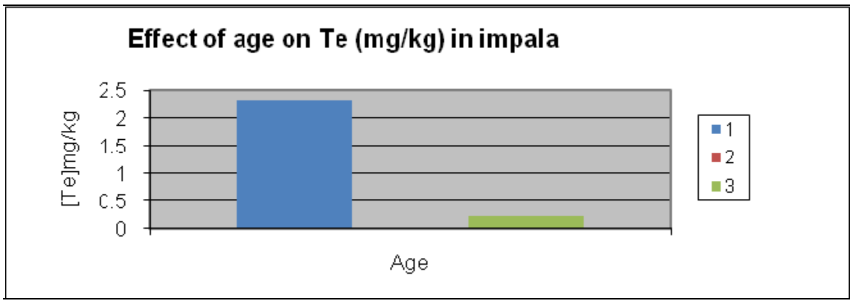

Figure 4.3.21: Effect of age on tellurium [Te] (mg/kg) in impala

Variations in the concentration of tellurium indicate that juvenile impala are better indicators of tellurium status in impala.

Iodine

Small concentrations of iodine were detected in all three age groups (1, 2 and 3) in impala, but lower concentrations were detected in both juvenile (Age 1) and sub adult (Age 2) impala compared to adult impala (Age 3), which was slightly higher ($p<0,05$).

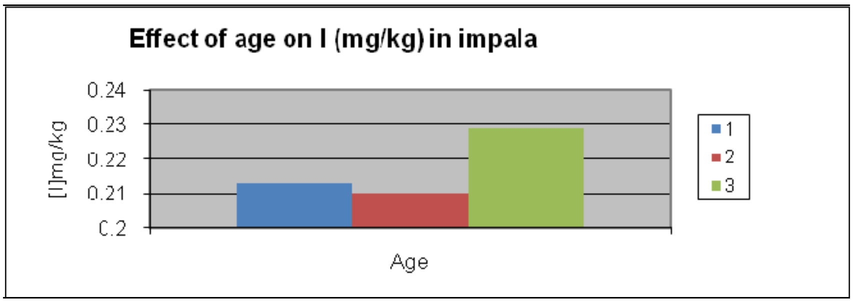

Figure 4.3.22: Effect of age on iodine [I] (mg/kg) in impala

The NRC (2005) report states that iodine is an essential element for animal species, including humans, mainly because it is an important component of the thyroid hormones

(they regulate cell activity and growth in virtually all tissues and therefore are essential in intermediary metabolism, reproduction, growth and development, hematopoiesis, circulation, neuromuscular functioning, and thermoregulation.)

The colostrum of dairy cows is higher in iodine than milk, and in late lactation there is a decrease in concentration NRC, (2005). Although, in the present study, it is indicated that iodine concentrations are higher in adult impala and low in sub-adults and juveniles (Figure 4.3.22). This finding is not in contrast with other reports and it could be because iodine concentration is directly influenced by intake from feed and water.

NRC publications on recent estimates of iodine requirements (mg/kg diet) where the diet does not contain goitrogens are as follows: chicken and cats, 0.35; turkey, 0.40; beef cattle, 0.50; dairy cattle, 0.25 (growing), rats and mice, 0.15. Non human primates, 2 (reviewed by McDowell, 2003.) Miller *et al*, (1975) reported that lactating animals require more iodine as a result of approximately 10 % or more of the iodine intake possibly being excreted in milk, depending upon the rate of milk production.

Iodine requirements may also be influenced by genetic differences, climate, and environment. Reports state that thyroid hormone secretion in certain animals have been shown to be inversely associated with environmental temperature NRC (2005)**.** ARC (1980) reported that cattle, sheep and goats show a significant decrease in thyroid hormone production during the summer.

Caesium

Small concentrations of caesium were detected in all three age groups (1, 2 and 3) in impala, but lower concentrations were detected in both middle (Age 2) impala and adult impala (Age 3), compared to juvenile impala (Age 1), which were slightly high ($p<0,05$).

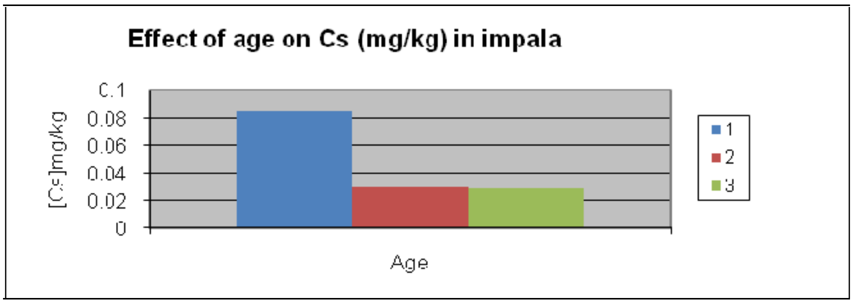

Figure 4.3.23: Effect of age on caesium [Cs] (mg/kg) in impala

Variations in the concentration of caesium indicate that juvenile impala are better indicators of caesium status in impala.

Lanthanum

Concentrations of lanthanum were detected in all three age groups (1, 2 and 3) in impala, but lower concentrations were detected in both middle (Age 2) impala and adult impala (Age 3), compared to juvenile impala (Age 1), which was higher.

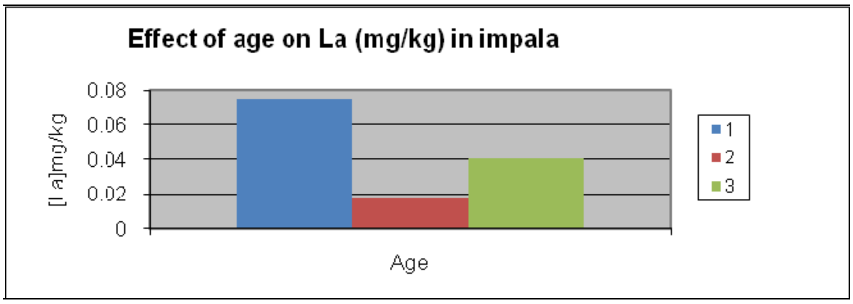

Figure 4.3.24: Effect of age on lanthanum [La] (mg/kg) in impala

Variations in the concentration of lanthanum indicates that juvenile impala are better indicators of lanthanum status in impala

Tungsten

Small concentrations of tungsten were detected in impala (Age 1) ($p<0,05$), but the concentrations were negligible from samples collected in impala Age 2 and 3.

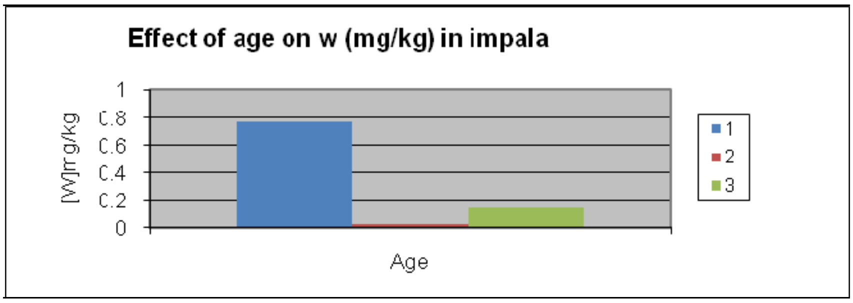

Figure 4.3.25: Effect of age on tungsten [W] (mg/kg) in impala

Variations in the concentration of tungsten indicate that juvenile impala are better indicators of tungsten status in impala.

Platinum

Small concentrations of platinum were detected in impala (Age 1) ($p<0,05$), but the concentrations were negligible from samples collected in impala Age 2 and 3.

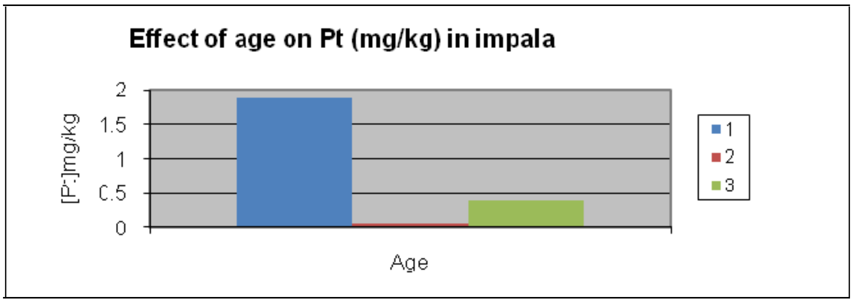

Figure 4.3.26: Effect of age on [Pt] (mg/kg) in impala

Variations in the concentration of platinum indicate that juvenile impala are better indicators of platinum status in impala.

Mercury

High concentrations of mercury were detected in impala (Age 1) ($p<0,05$), but the concentrations were almost negligible from samples collected in impala Age 2 and 3, of which the concentrations of mercury were minute.

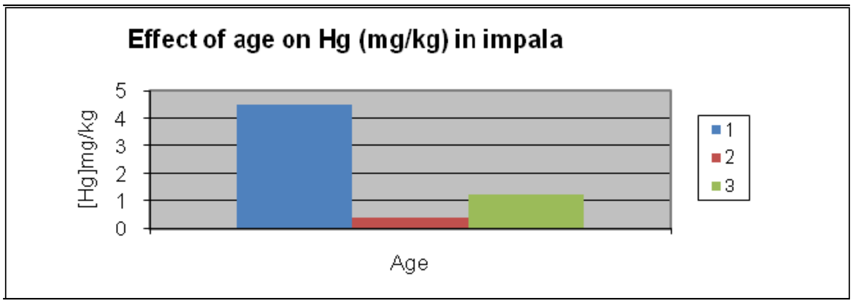

Figure 4.3.27: Effect of age on mercury [Hg](mg/kg) in impala

Johnston and Savage (1991) reported that mercury is not known to be an essential element for animals, although, in several experiments in rodents, pigs, and chicks, low levels of inorganic mercury increased growth rate, however, this effect was not seen in all experiments.

Reports stated that age is a primary factor that influences the absorption of inorganic mercury, i.e. 1 week old suckling mice absorbed 38 % mercuric chloride, whereas adult mice absorbed only 1 percent of dose in standard diets and 7% in milk based diet (Kostial *et al*., 1979). These findings are in contrast with the current study (Figure 4.3.27).

NRC (2005). Also reported that the accumulation of mercury in tissues takes many months to plateau, and older animals usually have higher levels than young animals.

Titanium

Higher concentrations of titanium were detected in impala (Age 1) ($p<0,05$), but the concentrations were almost negligible from samples collected in impala Age 2 and 3, of which the concentrations of titanium were minute.

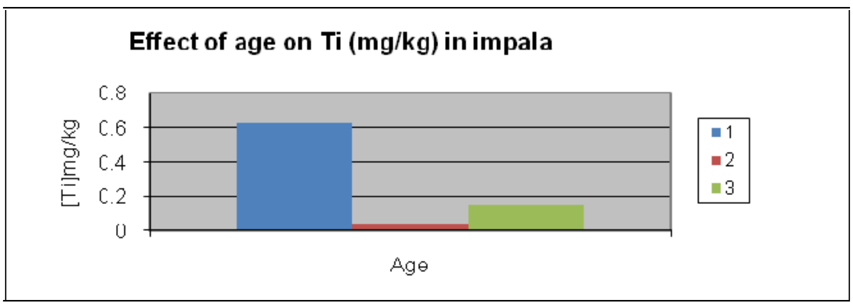

Figure 4.3.28: Effect of age on titanium [Ti] (mg/kg) in impala

There is limited or no information on titanium literature to substantiate current findings in Figure 4.3.28.

Lead

A higher concentration of lead was detected in impala (Age 1) ($p<0,05$) , but the concentrations were negligible from samples collected in impala Age 2 and 3.

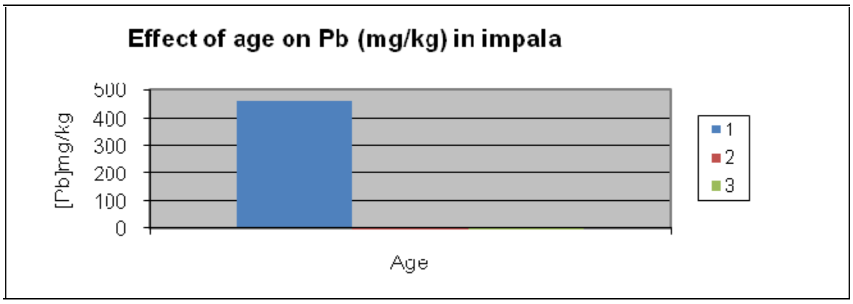

Figure 4.3.29: Effect of age on lead [Pb] (mg/kg) in impala

Reichlmayr-Lais and Kirchgessner (1981); Kirchgessner *et al*. (1991) and Manser, (1991) reported in several studies that the addition of lead to the diet of rats and pigs improved growth rate and lipid metabolism. Improved egg production in chickens was reported by Mazliah *et al*., (1989). However, lead is not known to be an essential nutrient for animals and does not take part in any known beneficial biochemical functions (NRC, 2005).

NRC (2005) reported that the efficiency of lead absorption is hugely influenced by the chemical form of the lead, the level of other dietary constituents, and the age and physiological state of the animal. Young animals absorb lead considerably more efficiently than older animals. This finding concurs with the current results (Figure

4.3.29). Pearl *et al*. (1983) reported that the apparent absorption of lead in adult sheep is 15 percent when included in the diet at 1,000 mg/kg.

ASTDR (1999) reported that physiological states (e.g. pregnancy, parturition, osteoporosis, infection, or prolonged immobilisation) that are associated with increased bone resorption promote the release of lead, and entry into blood and milk. It was also reported that a mother with a high body lead burden can transfer lead transplacentally to her developing foetus. Goyer (1990) also reported that maternal and foetal blood lead is nearly identical, so lead passes through the placenta unencumbered.

Bismuth

High concentrations of bismuth were detected in impala (Age 1) ($p<0,05$), but the concentrations were almost negligible from samples collected in impala Age 2 and 3.

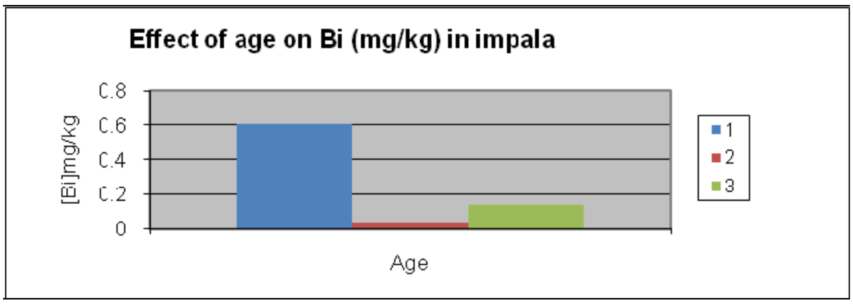

Figure 4.3.30: Effect of age on [Bi](mg/kg) in impala

Reports stated that bismuth is not considered an essential nutrient for plants or animals and has no known essential biochemical functions in normal metabolism NRC, (2005).

Uranium

High concentrations of uranium were detected in impala (Age 1) ($p<0,05$), but concentrations of uranium in impala aged 1 and 2 were almost negligible.

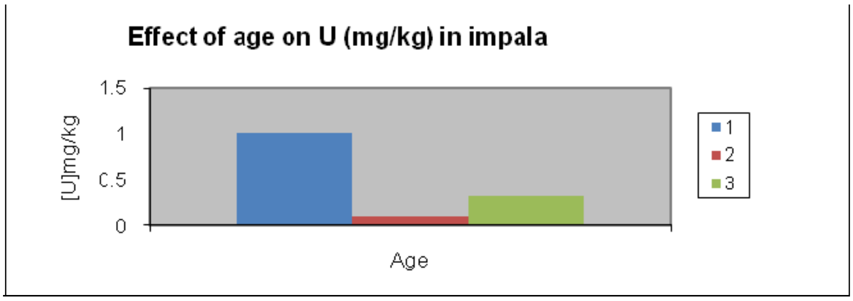

Figure 4.3.31: Effect of age on [U] (mg/kg) in impala.

(There is no literature on uranium in wild and domestic animals).

CHAPTER 5

CONCLUSIONS

The purpose of this study was to provide baseline data on the mineral status of impala and to investigate the possibility of using impala as sentinel species for monitoring important elements that may affect livestock and wildlife production in southern Africa. Impala can be used as sentinel species because they are wild ungulates that are grazers and browsers. This puts impala in a favourable position to give an indication of the status of browsers and grazers. From a livestock point of view, impala could serve as sentinel species, although they also browse, which complicates the interpretation of data in livestock because cattle and sheep are predominantly grazers. However, the grazing behaviour of impala and goats are similar, so the present finding would be valuable for goat production in this region.

As stated in the results and discussions on the effect of anatomical location on the mineral content of impala, liver has been shown to be the one location where trace elements are detected, in particular, titanium, manganese, cobalt, nitrate, copper, zinc, rubidium, molybdenum and caesium. Kidneys were the second most important anatomical location in this regard, where lithium, vanadium and chromium were predominantly detected. Minerals did not accumulate in large concentrations in kidney fat or the thyroid gland.

In terms of the effect of gender on the mineral content of impala, most elements were detected in samples from male impala, while smaller concentrations were detected in samples from female impala. This finding suggests that male impala give a better indication of mineral status than female impala. Minerals that differed significantly between genders include boron, rubidium, strontium, tin, tellurium and caesium.

Results on the effect of age on the mineral profile of impala suggest that juvenile impala exhibited most elements. This finding could be attributed to the fact that they suckle milk from their mothers, as well as through the feed they ingest. Overall, most elements were detected in all three age groups.

The study was of value regarding the use of impala as sentinel species, however there is more potential for further investigation. The brain, skeletal muscle and heart muscle may be used in the future for sampling processes, and it would be useful to sample impala in different geofigureical locations to study the interactions and accumulation of minerals with age, and between anatomical locations and genders. It would be interesting in future studies to compare the mineral status of impala and commercial or indigenous goats since they exhibit similar feeding behaviour.

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