ECOTOXICOLOGICAL ASSESSMENT OF RIFT VALLEY LAKES IN KENYA AND THE POTENTIAL HEALTH IMPACT ON THE LESSER FLAMINGO POPULATION

By

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DECLARATION

This thesis is my original work and has not been presented for a degree at any other University

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DEDICATION

This work is dedicated to my husband Elijah Tenai and our children Jovita and Hansel Tenai; to my parents John and Esther Chemuren and to my grandmother Martha Longu.

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LIST OF ABBREVIATIONS

AAS	Atomic absorption spectrophotometer
ATSDR	Agency of Toxic Substances and Disease Registry
GDP	Gross Domestic Product
Pb	Lead
As	Arsenic
Cd	Cadmium
Cr	Chromium
NRC	National Research Council
TISAB	Total ionic adjustment buffer
GFAAS	Graphite furnace atomic absorption spectrophotometer
US EPA	United States Environmental Protection Agency
LNK	Lake Nakuru
LO	Lake Oloidien
CL	Crater Lake
LE	Lake Elementaita
IUCN	International Union for Conservation of Nature
ND	Not detected
EIA	Expandable Ion Analyzer
Rmv	Relative millivolt

Abstract

The Kenyan Rift Valley lakes which include; lakes Elementaita, Nakuru, Oloidien and Crater are located in Nakuru County. The lakes host a large number of the Greater Flamingo (*Phoeniconaias roseus*), Lesser Flamingo (*Phoeniconaias minor*), Great White Pelican (*Pelecanusono crotalus*) and other water birds. The Lesser Flamingo congregate in vast numbers at certain times in these lakes creating major tourist attraction sites which contributes significantly to the Gross Domestic Product (GDP) of Kenya. Lesser flamingos have been classified as near-threatened due to their declining population. In recent years, there have been massive die-offs, particularly within the Lesser Flamingo population; the most recent occurred towards the end of 2013. These mass deaths have been attributed to diverse causes that either relate to the availability of food for flamingos as influenced by the salinity or toxicology of the lake waters or direct impacts upon the birds' population. The exact cause(s) of the deaths have not been conclusively established. Environmental pollution may be a contributing factor to the die offs. This study aimed at assessing the levels of fluoride and metals which included lead, cadmium, chromium and arsenic with the aim of establishing whether they have detrimental health impact on the birds' population directly or indirectly. This was an eco-toxicological assessment of the above lakes. The methodology involved collection of water samples (n=40), sediments samples (n=51) for ecotoxicological analysis. Live and dead Lesser Flamingos (n= 6; n=2, respectively) opportunistically collected from the lakes were also sampled. Live birds were clinically examined in the field and then euthanized for post-mortem examination. Analysis of samples for fluoride was done by use of Orion fluoride meter model EIA 940. Lead, arsenic, cadmium and chromium were analysed using Graphite Furnace Atomic Absorption Spectrophotometer (GFAAS) model-Specter AA-10 Varian. The data was entered in excel work sheet and analysed using “Instat +” computer statistical package. The results of the toxicological study showed that the mean concentration (±SD) of fluoride in water samples were 55 ± 28.87, 30.75 ± 2.99, 116.9 ± 70.25, 247.9 ± 170.4 ppm for lakes Crater, Oloidien, Elementaita and Nakuru, respectively. The high fluoride levels in Lake Nakuru could be attributed to the relatively high contribution from rivers with a mean of 19.25 ± 16.26 ppm and Nakuru town sewerage (6.5 ± 0.58 ppm) in addition to the natural leachate from volcanic soil, sediment and other lakes. However this fluoride concentration in water and soil sediments differed significantly ($P < 0.05$) between lakes. Mean fluoride concentration in the tissues of birds varied significantly ($p < 0.05$) from 122.5 ± 40.93 ppm in the bones, 3.12 ± 2.45 ppm in the skin and 0.40 ± 0.21 ppm in the muscle. These concentrations were positively correlated with soil fluoride levels. Lead and arsenic were found to be in high concentration in soil sediments in all four lakes while chromium and cadmium were in low concentration. The concentration (±SD) of lead in the soil sediments were 212.5 ± 44.25, 567.3 ± 46.12, 430.1 ± 122.1, 273.7 ± 67.12 ppb while that of arsenic were 409.3 ± 243.9, 512 ± 66.96, 354.4 ± 294.9, 265.5 ± 290.8 ppb for lakes Crater, Elementaita, Nakuru and Oloidien, respectively. Soil sediments analysed from the inflow of the Nakuru sewerage drain (1754 ± 22.81 ppb) and rivers to Lake Nakuru (1129 ± 107 ppb) had the highest concentration of lead. The concentration (±SD) of lead in the flamingo tissues were 11.09 ± 22.69, 141.6 ± 37.45, 1.89 ± 1.89, 17.36 ± 21.67, 24.59 ± 30.79, 16.68 ± 8.83, 13.9 ± 8.54 ppb while that of arsenic were 10.2 ± 2.72, 6.82 ± 2.57, 5.24 ± 2.27, 10.43 ± 3.25, 19.12 ± 4.68, 16.92 ± 6.1, 12.45 ± 3.87 ppb for muscle, bone, brain, heart, kidney, liver and lungs, respectively. Arsenic, cadmium, chromium and lead were also observed in bird tissues. Metals in the Lesser Flamingo tissues were below the toxicological levels that are reported in literature to be harmful, except lead which was above the level recommended by the US Environmental Protection Agency. It was concluded from the results that the toxicants detected were not the main cause of the die-offs and other factors may be involved. It is recommended that more data should be collected in order to conclusively determine the cause of the die-offs and population decline in the Lesser Flamingo.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

The Kenyan eastern Rift Valley lakes which include Lakes Elementaita, Nakuru, Oloidien and Crater in Nakuru County, host a significant number of water birds. The birds include the Greater Flamingo (*Phoeniconaias roseus*), Lesser Flamingo (*Phoeniconaias minor*), Great White Pelican (*Pelecanusono crotalus*), among others. These birds, particularly the Lesser Flamingo, congregate in vast numbers in these lakes, providing key tourist attraction and this, in turn, has contributed significantly to the GDP of Kenya. In recent years, however, there have been massive die-offs, particularly within the Lesser Flamingo population; the most recent occurred towards the end of 2013. These mass deaths have been attributed to a number of diverse causes that either relate to the availability of food for flamingos as influenced by the salinity or toxicology of the lake waters or direct impacts upon the birds' population. As these lakes catchments fall directly within a combination of agricultural and industrial regions, the run-offs and the resulting effluents will make the waters highly prone to chemical contamination. However, the potential impacts of these agricultural and industrial pollutants on the health of birds in the lakes are not well documented.

Furthermore, the use of agrochemicals is documented in agricultural production in the study region. Developments in both agriculture and industrial sector have contributed to a better quality of life, but they have also increased the risk of exposure to hazardous chemicals and industrial waste products. Lack of human and financial resources causes constrain to the government initiatives to control and regulate the trade in agrochemicals, industrial chemicals and waste disposal. In these circumstances, the threat of environmental damage from agricultural run-offs as well as industrial pollution is a strong reality, where water discharge from urban run-off, faulty sewage systems, rain-water sheet run-off, river and stream contribute significantly to the contamination of the water shed that feeds these Rift Valley lakes.

The four lakes investigated in this study lie within the Kenyan segment of the Great Rift Valley and are an essential part of the flamingos' flyway. Furthermore, the lakes serve as a stopover and wintering grounds for a number of species of migratory water birds during their annual migration.

1.2 Justification

The Lesser Flamingo contributes significantly to ecotourism which, in turn, contributes substantially to the country's GDP and brings in a large amount of revenue annually to Kenya. Indeed, tourism is regarded as the second largest sector of Kenya's economy and is estimated at contributing about 10% of the GDP and is Kenya's leading foreign exchange earner, generating around Kshs. 65.4 billion in 2007 (Udoto, 2012). Birds are the key attractions to Kenya which boasts having 1,137 species. The Lesser Flamingo is one of these attractions as they have been known to congregate in millions in these alkaline Rift Valley lakes causing a spectacular tourist attraction. However, mass die-offs have had a severe negative impact on numbers and as been felt as a ripple-effect in the tourism industry. Furthermore, the Lesser Flamingo has been classified as near-threatened (IUCN, 2006) due to declining population numbers and a reduced number of suitable breeding sites, many of which are threatened by human activities. This situation is now compounded by the high mortalities observed of this species on many of these lakes in Kenya during their migrant winter foraging over the past decade, where, in previous years, they covered the lake surface in their multitudes. Environmental contaminants may be responsible for either having a negative impact directly on the flamingos or have an impact on the food availability of the species. Although some work has been undertaken on the toxicants in the Rift Valley lakes (Koeman *et al.*, 1972; Gikunju *et al.*, 1992; Kairu, 1996; Nelson *et al.*, 1998; Ndetei and Muhandiki, 2005) no recent studies have been undertaken on fluoride and metal concentration within these lakes system. It was therefore considered important to assess the levels of these contaminants as possible contributing factors that could potentially have a detrimental health impact on the population directly or indirectly. This study focused on investigating the presence of particular environmental contaminants within the water bodies and sediment and the possible implications to the health of the birds themselves.

1.3 Objectives

1.3.1 General objective

The overall objective of the study was to assess the ecotoxicology of the Rift Valley lakes in Kenya and the potential health impact on the Lesser Flamingo population.

1.3.2 Specific objectives

1. Establish the clinical signs and post mortem lesions on the Lesser Flamingos during a die off.
2. Identify and quantify fluoride in water, soil sediments and the Lesser Flamingo biological samples.
3. Determine the presence and level of heavy metals (Pb, As, Cd, Cr) in water, soil sediments and the Lesser Flamingo biological samples.

1.3.3 Hypothesis

There are no fluoride and heavy metals in the lake water, soil sediment and biological tissues of the Lesser Flamingo in the four lakes under study.

CHAPTER TWO

LITERATURE REVIEW

2.1 Background information

Ecotoxicology is the study of the effects of toxic chemicals on biological organisms, especially at the population, community and ecosystem level. It predicts the effects of pollution so as effective and efficient method of control can be carried out before detrimental health effects are reached. It integrates the effects of stressors at all levels of biological organisation from molecular to whole communities and ecosystem. It is important to look at the ecotoxicology of the Rift Valley lakes in order to control detrimental effects that can affect the life of the Lesser Flamingos and other water birds.

The largest population of the Lesser Flamingos estimated to be 1.5 – 2.5 million occur in the alkaline-saline lakes of the Great Rift Valley in East Africa (Delany and Scott, 2006). It is an obligate filter feeder and feeds during the night and early morning when the surface of water is calm, primarily by swimming and filtering the algae near the surface with a specialised bill that contains upto 10 000 microscopic lamellae (Del Hoyo *et al*., 1992). These birds can take in toxicants as they feed on the lakes. Some of the toxicants that were investigated in this study includes fluorides and heavy metals, that is, lead, arsenic, cadmium and chromium.

Fluorine is the most electronegative of all chemical elements and is therefore never encountered in nature in the elemental form. It represents about 0.06 to 0.09% of the earth’s crust (Wedepohl, 1974). The bulk of fluoride is found in the constituents of silicate rocks, where the complex fluorophosphates like apatite (Ca_{10} $(PO_4)_6F_2$), seems to be one of the major fluoride minerals; (Rutherford *et al*., 1995).

Although a clear definition of what makes up a “heavy metal” is not available, it is the elements density that is the defining factor. Generally, heavy metals are defined as having a specific density of more than 5 g/cm^{3}and the most severe heavy metals to humans are associated with those of lead, cadmium, mercury and arsenic (Järup, 2003). It is therefore presumed that the impact of these heavy metals will also have a severe negative effect on the Lesser Flamingo population within the Rift Valley lakes.

The term heavy metal also refers to any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentrations. These metallic chemical elements have a relatively high density and are toxic or poisonous at low concentrations (Irwandi and Farida, 2009) with some, such as Copper, Selenium, and Zinc being essential in the bodies of living organisms for maintaining metabolism but can exert toxic effects if the homeostatic mechanism maintaining their physiologic limit is unbalanced (Hammond and Beliles, 1980). Generally, they do not break down further into less harmful constituents and they can bio accumulate within organisms, particularly within aquatic environments (Furness and Rainbow, 1990; Akan *et al.*, 2009). Sediments are the natural collectors of pollutants where persistent contaminants are stored and partially buried, but can be potentially re- suspended, thus re-entering the pelagic food web (Pusceddu *et al.*, 2005). Heavy metals are regarded as severe pollutants due to their toxicity, persistent and bioaccumulation problem (Schafer *et al*., 1999; Tam and Wong, 2000).

2.2 Lesser Flamingo population distribution, behavior, habitat and threats

The Lesser Flamingo breeds mainly in the Rift Valley lakes of East Africa in Ethiopia, Kenya and Tanzania. Some smaller breeding congregations occur in West Africa, South Africa, India and Pakistan. When they are not breeding, they occur in every sub-Saharan country and from Arabian Peninsula to Pakistan. The largest population of the Lesser Flamingos estimated to be 1.5 – 2.5 million occur in the alkaline-saline lakes of the Great Rift Valley in East Africa (Delany and Scott, 2006). Smaller populations occur in Rann of Kachchh in North Western India, estimated to be approximately 390,000 birds, in South

Africa, it is estimatedto be 55,000 – 65,000 birds, and in West Africa estimated to be 15,000 – 25,000 birds (Delany and Scott, 2006). The overall population trend is decreasing owing to habitat degradatioon and also disturbance (Delany and Scott, 2006). In most of Africa, the birds population is on a decline as suggested by others (Rose and Scott, 1997; Simmons, 1996). The bird makes extensive movement in response to adverse environmental conditions (Del Hoyo *et al*., 1992; McCulloch *et al*., 2003; Childress *et al*., 2007). These birds breed in huge colonies of many thousands of pairs usually in company of Greater Flamingo, *Phoeniceous roseus* (Del Hoyo *et al*., 1992). The Lesser Flamingo breeds on large undisturbed alkaline and saline lakes, salt pans or coastal lagoons, far out from the shore after seasonal rains floods and isolate remote breeding sites from terestial predators and soft muddy material for nest building (Del Hoyo, *et al*., 1992; McCulloch and Irvine, 2004). This bird feeds on highly specialised diet which almost consist entirely of microscopic blue - green algae and benthic diatoms found only in alkaline lakes, salt pans and saline lagoons and eustaries (Del Hoyo, *et al*., 1992). There are only three main breeding sites existing in Africa and all facing threats and requiring protection (Simmons, 1996). The proposed soda ash mining and hydroelectric power schemes affects the main breeding site in Lake Natron, Tanzania which was put on hold but had not been withdrawn. Other threats include; land claim water pollution and disturbance and also fears that Lake Bogoria is suffering from malnutrition (Simmons, 1996).

2.3 Fluorides

2.3.1 Sources of fluorides

Fluoride in the ground/surface water is derived from the weathering and subsequent leaching of fluoride-bearing minerals in rocks and soils like fluorspar, cryolite and fluorapatite. A substantial amount of this fluoride is retained in subsoil horizons, where it complexes with aluminium that is most likely associated with phyllosilicates (Huang and Jackson, 1965).

Larsen and Widdowson (1971) observed that the solubility of fluoride in soils is highly variable and has the tendency to be higher at pH <5 and >6 values. It appears that the predominant retention mechanism is that of fluoride exchange with the OH group of amorphous materials, such as Al-hydroxides (Flühler *et al*., 1982; Barrow and Ellis, 1986). In this case, fluoride resulting in a simultaneous release of Al and Fe replaces the crystal lattice OH- of clay minerals. Other fluoride retention mechanisms include the binding of fluoride to soil cations (e.g., Ca_2, $A1_3$), or fluoride precipitation as CaF_2, as in calcareous soils (Gupta *et al*., 1982). Fluoride found in the air is released to the atmosphere by natural sources such as volcanoes and various anthropogenic sources (NRC, 2006). According to ATSDR (2003), atmospheric releases of inorganic fluoride to the atmosphere can come from power plants burning coal, aluminium production plants, phosphate fertilizer plants, chemical production facilities, steel mills, magnesium plants, and manufacturers of brick and structural clay.

2.3.2 Studies undertaken on fluorides in the Kenyan Rift Valley lakes

High fluoride content in the Kenya waters has been reported by several researchers (Bakish, 1974; Jones *et al*., 1977; Clarke *et al*., 1990; Harper *et al*., 1990; Gikunju *et al*., 1995). The major source of fluoride entering into the hydrological system in Kenya can be traced to volcanic activity associated with Rift Valley formation and chemical weathering of volcanic rocks (Yuretich, 1982; Nanyaro *et al*., 1984; NRC, 2006). The volcanic rocks of the Rift Valley system are predominantly alkaline rocks rich in sodium and fluoride (Harper *et al*., 1990) whereby the rocks are richer in fluoride here than the analogous rocks in other regions of the world (Gachiri and Davies, 1993). Concentration of fluoride through evaporation has also been reported to be responsible for the extremely high fluoride concentrations found in Kenyan lakes (Jones *et al*., 1977; Nanyaro *et al*., 1984; Clarke *et al*., 1990). And this process is reported to be so effective that the fluoride concentration is several orders of magnitude higher than occurs in the normal groundwater and river water (Gaciri and Davies, 1993).

Considerable amounts of fluoride are also discharged directly into hydrological system in the form of waste waters and other wastes resulting from mining and ore-processing operations at the Kenyan Fluorspar Mine in the Kerio Valley (Gaciri and Davies, 1993). This is indicated by the widespread occurrence of fluorosis among inhabitants and cattle in the surrounding region (Nyaora *et al*., 2002).

Fluoride has a dual significance in that it helps in the normal mineralization and formation of bones but when consumed in inadequate quantities (less than 0.5 ppm) may lead to health problems such as the deficiency of mineralization of bones, particularly among the young. On the contrary, when fluoride is consumed in excess of 1.0 ppm, it leads to several health complications observed in all ages (Vaish and Vaish, 2002). Being a cumulative bone seeking mineral, the resultant skeletal changes are progressive where higher fluoride concentration exerts a negative effect on the course of metabolic processes and an individual may suffer from skeletal fluorosis, non-skeletal manifestation or a combination of the above (Wagner *et al*., 1993). Birds may be affected too by the same phenomenon.

2.4 Heavy metals

2.4.1 Lead poisoning

2.4.1.1 Background information

Lead (Pb) is biologically a non-essential toxic metal that exist in several stable oxidation states that are absorbed and accumulated by aquatic and terrestrial organisms (Schafer *et al*., 1999; Tam and Wong, 2000; Ahmed and Bibi, 2010). Lead is released into the aquatic environment from sources such as chemicals, fertilizers and gasoline containing lead (Schafer *et al.,* 1999; Rashed, 2001)**.**

2.4.1.2 Common sources of exposure

Environmental contamination may occur due to industrial use of lead in factories that process lead-acid batteries or produce lead wire or pipes, metal recycling, foundries, colour pigments, protection screens, lead fishing weights and ballast weights (Schafer *et al*.,1999; Mañay *et al*., 2008). Lead also enters in the aquatic environment through a number of ways including erosion and leaching from the soil, domestic and industrial waste discharges, Pb-dust fallout from the atmosphere and combustion of petroleum products. Lead is mainly soluble in soft and slightly acidic water (Moore and Rainbow, (1987) and the content in soil may be contributed by broken-down lead paint, residues from lead-containing gasoline or pesticides used in the past (Woolf *et al*., 2007). Most of these sources exist around the study sites.

2.4.1.3 Toxicosis

Acute toxicity can occur due to exposure from industries such as battery manufacturers, lead smelters and spray painting; and also due to processes that can cause lead mobilisation from bone (Schafer *et al*., 1999). Chronic low grade exposure of lead comes from the soil, plants and water while high grade exposure is from ingestion of lead (Vicky, 2013). Lead poisoning and elevated concentrations in tissues have been reported in birds which they may consume as grit (USGS National Wildlife Health Center, 2009). Lead toxicity in birds may present with neurological signs with severely affected birds dying suddenly (Phil, 2010). Lead can kill a bird if consumed at a rate of 25µg/Kg body weight per day (Kendall and Scanlon, 1982).

2.4.2 Arsenic poisoning

2.4.2.1 Background

Arsenic has no known importance within biological systems or organisms and inorganic arsenicals can be methylated in the body of mammals.

2.4.2.2 Occurrence and sources of exposure

Elemental arsenic is rarely found in nature whereas inorganic arsenic is released to the environment as a by-product during mining, roasting and melting of zinc or lead containing ores, (Schafer *et al*., 1999). Arsenicals are used in the glass industry and also as pesticides and herbicides in agriculture and forestry (Schafer *et al*., 1999) which are the main activities that takes place in Nakuru County. All these can be released to an aquatic environment during the rainy season. Natural weathering of rocks and soil adds around 40,000 tons of arsenic to oceans yearly, accounting for <0.01 mg/l input to water on a global basis (NRCC, 1978)

2.4.2.3 Toxicosis

Lethal effects of inorganic arsenic occur due to inhibition of gluconeogenesis and glycogenolysis (Schafer *et al*., 1999). Kubota *et al*. (2003) and Kunito *et al*. (2008) concluded in their studies that higher trophic marine animals includinng birds may have a unique metabolism of arsenobetaine which plays an important role in accumulation of arsenic.

2.4.3 Cadmium poisoning

2.4.3.1 Background

Cadmium has an extra-ordinary long biological half life which makes it accumulate in the body of animals (Tam and Wong, 2000). The epidemic intoxication which occurred in Japan in 1950's brought public attention on cadmium's relevance as a dangerous environmental contaminant (Schafer *et al*., 1999).

2.4.3.2 Occurrence and sources of exposure

Cadmium has a wide distribution and is associated with lead, zinc and phosphates. Environmental contamination is associated with industrial effluent. More than half is used in making alloys and galvanizing. It is also used as a stabilizer in plastics, in radiation screens and batteries as well as in pigments (Schafer *et al*., 1999). Lake Nakuru receives sewage effluent containing industrial effluent which come from the increased number of industries.

2.4.3.3 Toxicosis

Cadmium is a cumulative poison and the kidney is the primary target organ for cadmium toxicity (Schafer *et al*., 1999). Higher cadmium and lead concentration are the co-factors in the development of histopathological lessions including circulatory disturbance, degenerative changes, inflammation and leukocytic infiltration of the liver and kidney in birds (Binkowski *et al*., 2013).

2.4.4 Chromium poisoning

2.4.4.1 Background

Chromium exists in various oxidation states. Hexavalent form is a highly toxic non-essential metal to micro-organisms, plants and higher organisms including birds. The hexavalent form Cr (VI) is more toxic than the mobile Cr (III) form (Kotaś and Stasicka, 2000; Jabari *et al*., 2009).

2.4.4.2 Occurrence and sources of exposure

Environmental contamination is associated with industrial effluent since it has widespread industrial use (Reemste and Jekel, 1997; Mwinyihija *et al*., 2005, 2006; Jabari, *et al*., 2009).

2.4.5 Studies undertaken on heavy metals in birds associated with Rift Valley lakes

Heavy metals studies in the Kenyan saline lakes have been undertaken by a number of researchers; (Kairu, 1996; Nelson *et al.,* 1998; Ndetei and Muhandiki, 2005) among others. Heavy metal resedues in birds were assessed by Koeman *et al*. (1972) and Kairu (1996). In his findings Kairu (1996) noted that the concentration of cadmium was highest in the Great White Pelican's (*Pelecanusono crotalus*) kidney tissues and the concentration of mercury was highest in the Great White Pelican's liver where as the concentration of arsenic was highest in the Great Cormorant (*Phalacrocorax carbo*) kidney tissues. Analysed bone tissue samples had no concentration of cadmium, mercury and arsenic above the detection limit of 0.5µg/kg. Kairu (1996) also observed that the kidney had relatively higher mean concentration of arsenic, lead and cadmium than the liver. Findings on the concentration of mercury in the liver and the kidney was consistent with that previously reported by Koeman *et al*. (1972). However, arsenic concentration was noted to have been significantly lower in Kairu's (1996) study than Koeman's *et al*. (1972) study. The highest mean cadmium concentration (1.3µg/kg) by Kairu (1996) was observed in flamingos and was nearly identical to the value (1.35µg/kg) obtained by Koeman *et al*. (1972). In his study, Kairu (1996) concluded that the temporal variability of metal concentration in Lake Nakuru at the time, appeared to suggest that the concentrations of cadmium, mercury and arsenic in the primary and secondary consumers had remained rather stable for the two decades.

Nelson *et al.* (1998) stated that toxic trace metals had been implicated as the potential cause for the then flamingo kills at Lake Nakuru between 1993 and 1995. Nelson *et al*. (1998) predicted that chromium and lead exposure occurred largely through ingestion of suspended solids whereas copper and zinc exposure was through ingestion of both suspended solids and their primary food source a cyanobacterium *Spirulina platensis*.

Nelson *et al.* (1998) noted that higher exposure doses were predicted when metal concentrations were determined from sediment concentrations and lower when using suspended solids concentrations. They also noted that decrease in *S. platenisis* population increased the clearing rate of the flamingos and further increased the predicted metal exposure through ingestion of suspended solids. They concluded that in addition to their findings, it would be informative to determine the phase distribution of trace metals under the more acidic conditions found in the flamingo's digestive tract.

2.4.6 Threats on the Lesser Flamingo's health within the Rift Valley lakes

Climatic change can also affect the environment and in turn the organisms that depend on it. Lesser Flamingos are faced with a number of threats apart from heavy metals which may include insufficient/lack of food, change in the depth of the lake which may be due to flooding either from the heavy rains or a lot of water from the in flow rivers. Studies undertaken by (Svengren, 2002) first acknowledged that Lake Nakuru was popular for flamingo feeding due to the abundance of their food and shallowness of the lake which allows for grazing. In some seasons, changes in the climate and water chemistry causes changes in salinity and nutrient concentration which in turn reduces the growth of cyanobacteria. When this phenomenon occurs simultaneously within the neighbouring lakes, the Lesser Flamingos fly in vain in search for the food. This was believed to have initiated the mass deaths of the birds between 1993 and 2000. Among other reasons that were implicated was the growth of other algae including the toxic ones which exposed the birds to their toxins. It was also deduced that stress exposed to the birds due to lack of food led to the breakdown of adipose tissue to provide energy. This caused the stored pesticides and heavy metals in the adipose tissue to be released inside the body. The increase concentration affected the central nervous system in birds (Svengren, 2002) .

Cyanobacteria communities in the lake shore of Lake Bogoria contained cyanobacteria toxins (Krienitz *et al*., 2003). Stomach and faecal content contained cyanobacteria cells, fragments and cyanobacteria toxins hence the possibility that the toxins contributed to the mass deaths (Krienitz *et al*., 2003). Krienitz *et al*. (2003) also concluded that the observed ophistotonus behaviour of the flamingos, especially the convulsed position of extremities and neck when dying was due to neurological effects.

In relation to the above theories, among other factors concerning the Lesser Flamingo mortality especially in the Kenyan Rift Valley saline lakes, further investigations are required. The levels of pesticides and heavy metals need to be monitored closely in order to control pollution in the lakes.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The study was conducted in Kenya which extends between latitude 4.5^o N and 4.5^o S and between longitudes 34^o E and 42^o E. The study was undertaken in four Rift Valley lakes in Nakuru County (Figure 1) during the month of December, 2013. The four lakes include lakes Oloidien, Nakuru, Elementaita and Crater all located to the northwest of the capital city Nairobi. Lake Nakuru is supplied by a number of rivers and Nakuru town sewerage but it has no outflow, Lake Elementaita is supplied by a number of rivers and hot springs and Lakes Oloidien and Crater are closed lakes with neither inflow nor outflow and the source of water is underground water springs. The four lakes under study host a significant number of the Lesser Flamingos during their stop over. Deaths have been witnessed recently in the four lakes hence the study.

3.2. Sample size determination and collection

3.2.1 Sample size determination

Sample size determination was done purposefully. Water and sediment sampling sites were chosen purposefully depending on the discharge points into the lake and six further sites were done randomly at least 100 metres from discharge points which were considered less impacted. Birds were opportunistically sampled.

3.2.2 Collection of water samples

A Sampling permit was obtained from the Kenya Wildlife Service (Appendix 7) for the purpose of the birds sampling. Sampling was undertaken during the month of December, 2013 for a period of one week. Sampling was done in the morning between 7.00 a. m and

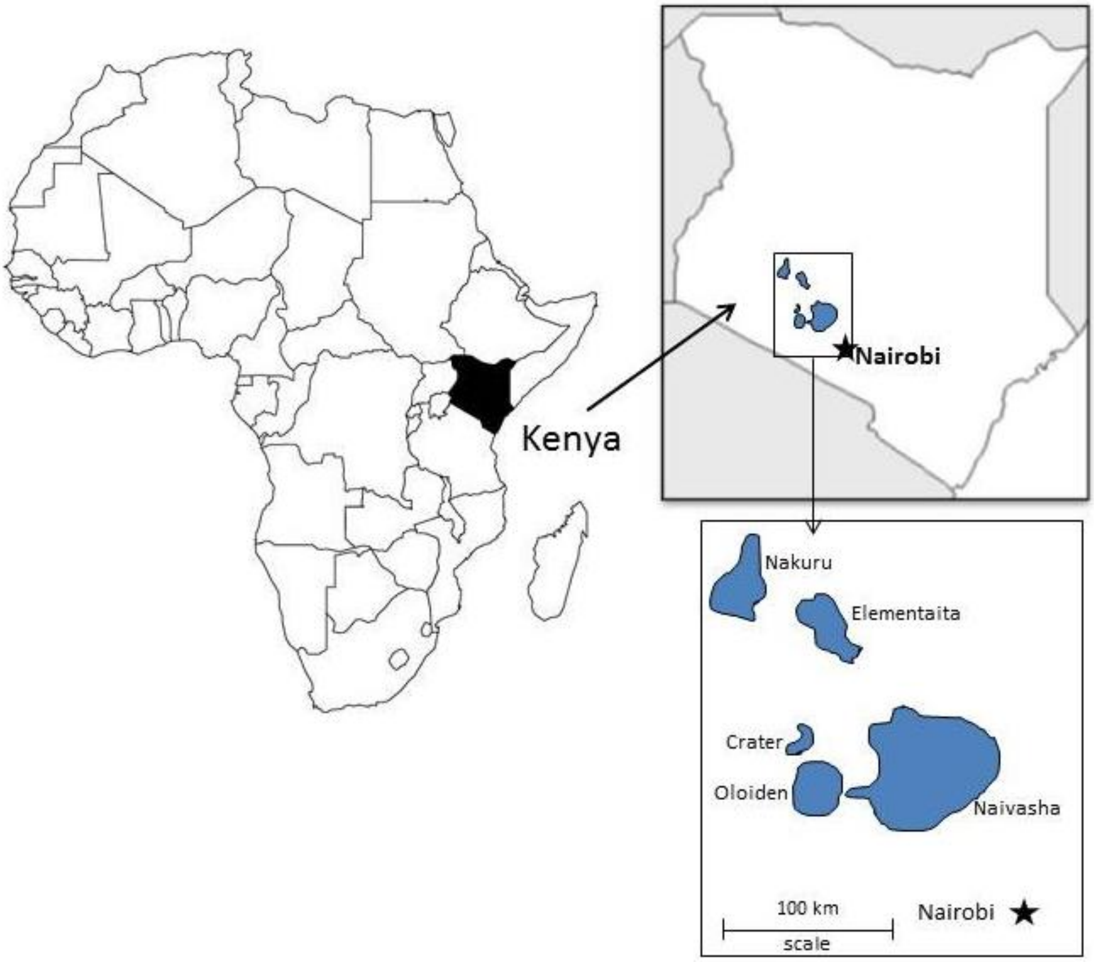

Figure 1: Location of Kenya in Africa and lakes Nakuru, Elementaita, Crater and Oloidien in Nakuru County, Kenya

11.00 a.m. Water samples were collected from the surface of streams, rivers and lakes in sterile polyethylene plastic containers. About 300 mg of sediments were scooped from the lake and river bed and packed in sterile polyethylene plastic containers. Polyurethane hand gloves were worn when the samples were being collected. Each sample was collected and packed separately at the sampling sites. Labelling of each sample was done for ease of identification and packed in a cool box. Water sampling sites in Lake Nakuru were chosen purposefully depending on the discharge points into the lake and six further sites were done randomly at least 100 metres from discharge points. Lake water and sediment samples were collected in replicates of two to three from discharge points of the following rivers Makalia, Nderit, Naishi and sewerage drain to Lake Nakuru. Other sites located at least 100m away from inflows of major discharge points were selected to represent sites that are considered less influenced by rivers and sewerage flowing into the lake. Replicates of two to three, lake water and soil sediment samples in two sites were each collected in Lakes Oloidien and Crater while replicates of two to three samples of both water and sediments were collected in four sites from Lake Elementaita. Birds sampling was done opportunistically. A total of 40 water samples, 51 sediment samples and 8 birds were sampled (Table 1).

Figure 2 and 3 show sample collections from Lake Nakuru sewerage drain and the discussion between the supervisor and the student, respectively during sample collection from Lake Nakuru.

Table 1: Samples collected from each of the lakes under study

Site	Type of sample	Number of samples
Lake Nakuru	Water	12
Rivers(L. Nakuru)	Water	8
Sewage drain(L. Nakuru)	Water	4
Lake Nakuru	Sediment	15
Rivers(L. Nakuru)	Sediment	9
Sewage drain(L. Nakuru)	Sediment	3
Lake Elementaita	Water	8
Lake Elementaita	Sediment	12
Lake Oloidien	Water	4
Lake Oloidien	Sediment	6
Crater Lake	Water	4
Crater Lake	Sediment	6
Total birds	Bird’s tissues	8

Figure 2: Collection of water sample in a sewerage drain in Lake Nakuru, Kenya

Figure 3: Discussion between student and supervisor during sample collection in Lake Nakuru, Kenya

3.2.3 Collection of biological tissues

Lesser flamingos were opportunistically collected from the lakes. The birds were trapped at Lakes Oloidien, Crater and Elementaita by making use of a noose carpet or those that were too weak were caught by hand. The noose carpet is a 1m x 1m grid covered in wire mesh onto which nylon nooses are fixed. The noose carpet was submerged in shallow water in the region the birds were feeding. The birds were released from the nooses and placed inside a cloth bag with suitable aeration. Birds that were found to be in very poor physical condition were euthanized by use of sodium thiopental, by injection directly into the heart. The birds were carried away from the lake shore where they were dissected on a disinfected plastic table and the tissues removed aseptically (Figure 4).

The following morphometric measurements were taken: body mass, cumin width and length, tarsus length, body length (tail tip to beak tip), wing length (end of humerus to tip of 10th primary feather), foot width (webbed foot splayed open) and a body score condition (1-good, 2-poor, 3-extremely poor) based on the sharpness of the sternal keel (degeneration of breast muscle) and lack of body fat deposits. The bird was carefully dissected and each of the internal organs was removed and packed individually in a zip lock bag, labelled and placed in a cool box. Organs of interest included the brain, heart, lungs, kidney, liver, bone, breast muscle and feathers. The dissection table and instruments were washed and disinfected before dissecting the next bird. New pair of latex gloves was worn during each dissection. The bird was carefully examined for ectoparasites (Figure 5). Any external parasites from the individual birds were collected and placed in a small sealed vial with 70 % alcohol. Post mortem findings were recorded accordingly. The organs were then transported to the University of Nairobi, Department of Public Health Pharmacology and Toxicology for storage and further analysis.

Figure 4: Dissection of Lesser Flamingo in Lake Oloidien, Nakuru County, Kenya

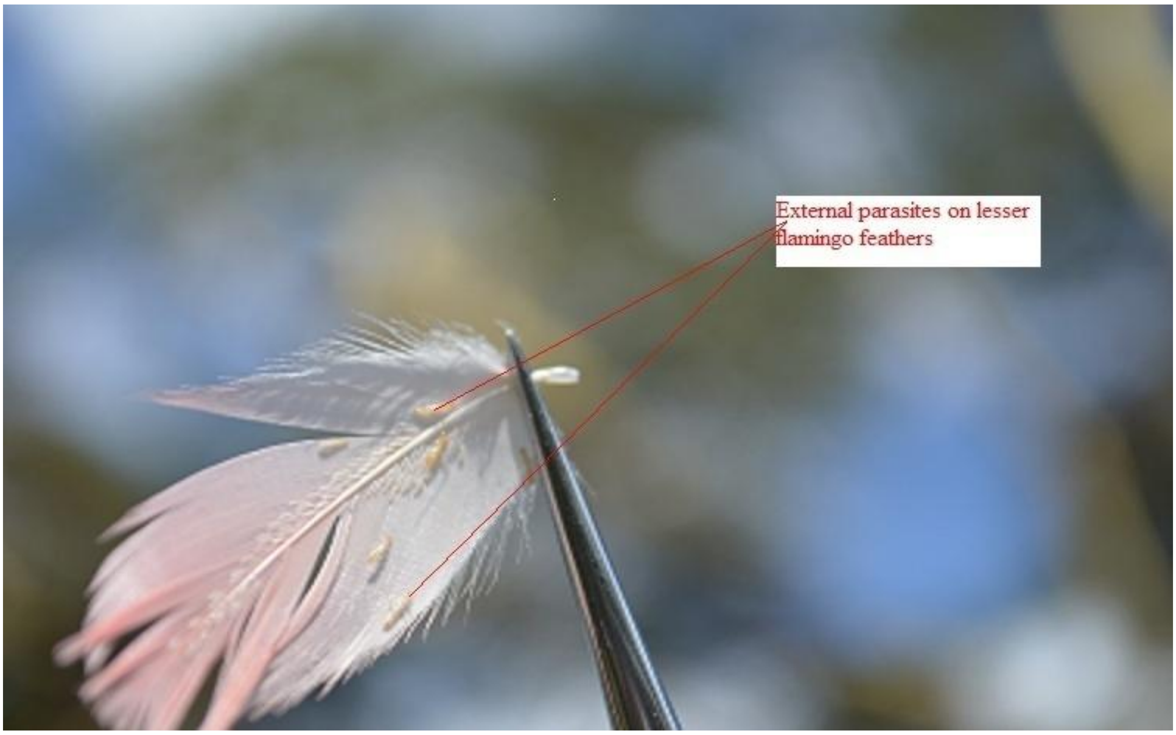

Figure 5: External parasites on Lesser Flamingo feathers

3.3 Laboratory analysis

3.3.1 Identification of lice

Lice specimens were identified using Leica EZ4D stereo microscope at the Kenya Wildlife Services, Veterinary department Laboratory. The identification of lice was carried out according to relevant literature (Price *et al*., 2003).

3.3.2 Fluoride analysis

3.3.2.1 Instruments and equipment

Fluoride combination electrode (96-09, Orion Research Incorporated, Cambridge Mass, USA)

Automatic voltage regulator (model CVR 500 AXSamlex®, England)

Magnetic stirrer and a glass coated metal bar, 5mm x 11mm

Polyethylene tubes (15 ml)

Twenty millilitre plastic cups (NDD/TL, Norsk Dental Depot, Oslo, Norway)

Pipette tips (1000 microlitres, 9604, Treff, Degershein, Switzerland)

Half litre plastic bottles for holding distilled water

Twenty millilitre plastic disposable straight pipettes

Fifty millilitre graduated glass pipettes, forceps, outer polypropylene tubes (15 ml), and inner polypropylene tubes

3.3.2.2 Reagents

All reagents used were of analytical grade

Electrode filling solution (Orion, 90-00-01)

Fluoride standard solution (100 ppm) 94-09-07 Orion®

TISAB III (Total ionic adjustment buffer), 940911, Orion®

10 M perchloric acid

14.3 M nitric acid

Sodium hydroxide pellets (May and Baker Limited, Dagenham, England)

Tri-sodium citrate (BDH Limited Poole England)

Acid mixture: equal parts of 10 M perchloric acid and 14.3 M nitric acid

Base mixture: 7.8 M sodium hydroxide and 1.0 M tri-sodium citrate in the ratio of 3:10

Blank II solution: A mixture of 150 ml of 7.8 M sodium hydroxide, 500 ml of 1.0 M tri-sodium citrate and 100 ml of acid mixture

3.3.2.3 Cleaning procedure

(a) Plastic tubes

The tubes were soaked in soapy solution for 2 to 3 hours then cleaned with tap water, soap and a test tube brush. The tubes were then rinsed in distilled water and allowed to dry.

The tubes were thereafter soaked in 7.8M sodium hydroxide for 24 hours after which they were soaked in 35% nitric acid for 24 hours.

The tubes were finally rinsed with distilled water and then dried in the oven at 60°C.

(b) Glass ware

Glass was cleaned with tap water, soap and a test tube brush, and then rinsed with 0.5 M perchloric acid 3 times before rinsing with distilled water 3 times and then kept in the oven to dry at 60°C.

3.3.2.4 Sediment and flamingo tissue sample preparation

3.3.2.4.1 Sediment preparation

The soil sediment was removed from the freezer and allowed to thaw on the laboratory bench.

3.3.2.4.2 Flamingo tissue sample preparation

The bench was cleaned with soap and distilled water and rinsed with acetone. Foil paper was spread on the bench and the freezer samples placed on the foil and allowed to thaw. The sample code and organ type was noted and recorded.

3.3.2.4.3 Extraction of the soil sediment and biological tissue samples in a closed chamber

Thirty grams of the wet sediment sample was weighed using a Mettler electric weighing balance model PM-4600 and dried overnight at 105^{o}C in glass petri dishes. The sample was then crushed using a pestle and a mortar and the dry weight taken. The samples were placed in plastic tubes and 45 mg was extracted for analysis. A volume of 0.4ml of concentrated perchloric/nitric acid mixture (1:1) was added to the sample in a fume chamber and 2.6 ml of base mixture (7.8M in NaOH +1M tri-sodium citrate) at a ratio of 3:10 was placed in a digestion tube. The preparation was incubated at 60^{o}C in the oven for 1 hour then cooled in the fridge for 10 minutes and left to stand in the bench for a further 5 minutes then shaken vigorously. Finally the sample was poured into the analysis tube ready for analysis. The pH of the samples was within the recommended range during the analysis (Appendix 9). Blank II solution was used as the background solution in sediment and the flamingo tissue samples.

3.3.2.5 Water sample preparation

The procedure was run in duplicate. Three millilitres of water sample was placed into a 15 ml graduated plastic tube and 0.3 ml of Tisab III was added to prepare for analysis. The pH of the water sample was between 5 and 7 after adding Tisab III (Appendix 8). Distilled water was used as the background solution.

3.3.2.6 Preparation of standard solution

Fluoride standard solution of 0.05, 0.1, 1.0, and 10.0ppm were prepared by diluting the 100ppm standard solution with distilled water. Three hundred microlitres (0.3 ml) of Tisab III was added to 3 ml of each of the fluoride standard solution in a 15 ml tube.

3.3.2.7 Development of a standard curve

The lowest fluoride concentration which had been mixed with Tisab III was used. A magnetic stirrer was inserted into the graduated tube and run for at least 4 hours. Other standards of 0.01, 0.1 and 10ppm were run in duplicates in ascending order, each for at least 10 minutes or until it stabilized. Their RMV (relative millivolt) was taken and an average calculated and a calibration curve plotted. The lowest standard was run and the electrode was washed with distilled water and wiped dry with velvex soft tissue ready for sample analysis. The difference between a tenfold increases in fluoride concentration was between 54 and 60 RMV.

3.3.2.8 Sample analysis using Orion fluoride meter

Processed samples were analysed using the Orion fluoride meter model EA 940. A clean glass covered stirrer was inserted into the tube with the sample to be analysed. The sample was allowed to run for at least 10 minutes or until it stabilized. The reading was taken and compared with that of fluoride standard curve.

3.3.3 Heavy metal analysis

3.3.3.1 Tissue sample extraction

Five grams (5 g) of wet tissue from the flamingo organs/ tissue (liver, heart, kidney, lungs, bone and muscle) was individually weighed and placed in a beaker and digested with 20ml of concentrated nitric acid in a fume chamber. Five millilitres of the acid was added at a time until the reaction fumes in the beaker were clear. It was then allowed to stand overnight for digestion to take place. Two millilitres of hydrogen peroxide was added to complete the digestion. The mixture was then filtered into a 50 ml volumetric flask using Whatsman no 1 filter paper. The residue in the filter paper was washed with distilled water and the filtrate added up to the 50ml mark. The filtrate was then placed in 100ml polyethylene bottles ready for analysis.

3.3.3.2 Sediment sample extraction

Thirty grams of the wet sample was weighed using a Mettler electric weighing balance (model PM-4600) and dried overnight at 105°C in glass petri dishes. The sample was crushed using a pestle and a mortar and the dry weight taken. Two and a half grams of dry soil sediment was weighed and placed in a beaker. Twenty millilitres concentrated nitric acid was added. This was then digested in a hot plate until the volume was10 ml. The mixture was filtered in a 50ml volumetric flask using Whatsman No 1 filter paper. The residue was washed with hot distilled water, then filtered and added to the 50ml mark of the volumetric flask. This was then poured to 100ml polyethylene bottles ready for analysis.

3.3.3.3 Water sample extraction

Fifty millilitres of the water sample was placed in a beaker and 5 ml of concentrated nitric acid was added. The sample was then boiled and allowed to evaporate to half of the volume and then allowed to cool down and filtered with Whatsman No 1 filter paper into a 50ml volumetric flask and topped up with distilled water to the 50ml mark. The digested sample was then placed in 100ml polyethylene bottles ready for analysis.

3.3.3.4 Preparation of the standard solution

Stock solutions for each metal (Pb, As, Cd, Cr) was prepared by dissolving 1mg in 1:1 nitric acid and diluted to 1 litre to give a stock solution of 1000 ppb. From each of the stock solutions working standards of 0.05, 5, 10 and 100 ppb was made and calibration curves plotted.

3.3.2.5 Graphite Furnace Atomic Absorption Spectrophotometer (GFAAS) analysis

Aliquots of the filtrates were used to estimate the concentration of the metal by GFAAS. The samples were analysed for lead, arsenic, cadmium and chromium using AAS Model-Specter AA-10 Varian at the Ministry of Mining, Department of Mines and Geology. The setting of the instrument for metal analysis is presented in table 2.

3.4 Data analysis

Data collected was stored in excel worksheet. The data was analysed using freeware computer package “Instat +”. Descriptive statistic such as the mean was used to analyse the data on toxicant levels within the different lakes and biological samples. ANOVA was used to determine the significant difference ($p<0.05$) of statistical means of the metals and fluoride in water and sediment within the different lakes and also the different sampling sites.

Table 2: Operating conditions of Graphite Furnace Atomic Absorption Spectrophotometer

Element	**Cadmium**	**Chrome**	**Lead**	**Arsenic**
Sample	W+S+T	W+S+T	W+S+T	W+S+T
Wavelength (nm)	228.2	357.9	283.3	193.7
Drying temperature^{o}C	110-130	110-130	110-130	395
Pylorisis temperature^{o}C	600	1500	850	
Atomization temperature^{o}C	1700	2250	1650	2100
Clean out temperature^{o}C	2450	2450	2450	2300
Matrix modifier	$NH_4H_2PO_4$	$Mg(NO_3)_2$	$NH_4H_2PO_4+Mg(NO_3)_2$	

Key: W-water, S- soil sediment, T-lesser flamingo tissue

CHAPTER FOUR

RESULTS

4.1 Clinical and post mortem results for flamingos

Flamingos were found dead on the lake shore in Lake Oloidien (Figure 6). Some were very weak, could not fly away, and had difficulty in walking and could be caught very easily by hand. Few weak birds were found in Crater Lake while in Lake Elementaita, three dead birds were found. Birds feeding in the lakes were generally of poor body condition, ,massive emaciation with empty crops, lack of fat tissue, had distended gall bladder and adrenal gland (Table 3) and showed signs of lethargy and depression on capture. The morphometric measurements ranged from 1000 – 1300 g, 755 – 870 mm, 565 – 665 mm, 180 – 235 mm, and 90.8 – 107.3 mm for body mass, body length, wing length, tarsus length and culmin width, respectively. From the morphometric measurements, the males were generally larger than the female. There were insignificant variations within and between the lakes (Table 3). Most of the birds sampled were infested with lots of external parasites of the genus *Anatoecus spp* from the family of *phthiraptera*, sub order *ischnocera* (Figure 7). These are chewing lice which infest the Lesser Flamingos.

Figure 6: Dead Lesser Flamingo on the lake shore in Lake Oloidien, Nakuru County, Kenya

Figure 7: *Anatoecus spp*

Table 3: Clinical and post mortem findings of Lesser Flamingos collected from lakes Oloidien, Crater and Elementaita

Sample	Location	Sex	Mass (g)	Body length (mm)	Wing length (mm)	Tarsus length (mm)	Culmen length (mm)	Body score [1]	Gall bladder	Adrenal gland	Crop[3]	Fat score [2]	Parasites
1	Oloidien	Male	1000	820	600	225	102.75	3	Distended	Distended	3	3	None
2	Oloidien	Male	1090	855	635	225	102.7	3	Distended	Normal	3	3	Ectoparasites
3	Oloidien	Male	1122	870	655	235	107.3	2	Normal	Distended	2	3	None
4	Oloidien	Male	1118	825	665	235	96.9	2	Distended	Distended	3	3	Ectoparasites
5	Oloidien	Male	1103	825	650	220	99.6	2	Distended	Distended	3	3	None
6	Crater	Male	1300	865	640	205	100	2	Distended	Normal	3	2	Ectoparasites
7	Crater	Male	1100	833	615	225	97.4	2	Distended	Normal	3	3	Ectoparasites
8	Elementaita	Female	1150	755	565	180	90.8	1	Normal	Distended	1	2	None

[1] Body score; 1=good, 2=poor, 3=extremely poor
[2] Fat score: 1=high, 2=little, 3=none
[3] Crop: 1=full, 2=medium, 3=empty

4.3 Results of fluoride analysis

The fluoride concentrations of the four lakes sampled are presented in Table 4. The results indicate that all the study sites had fluoride in the water and sediment. Lake Nakuru indicated significantly higher concentrations of fluoride than the other three lakes (247.9± 170.4ppm; ANOVA; $P<0.05$, $F =7.1$), followed by Lake Elementaita (116± 70.25ppm). The other two lakes and rivers into Nakuru had relatively low fluoride levels. However, Crater Lake had significantly higher (ANOVA, $F = 42.1$, $P<0.05$) concentrations of fluorides in the soil sediments (8.58 ± 0.67ppm) followed by Lake Elementaita and Lake Nakuru that had almost equal mean concentration of fluoride in the soil sediments (7.04 ± 0.96ppm and 6.67± 2.52ppm, respectively). Lake Oloidien, the rivers discharging into Lake Nakuru and the sewerage drain had low levels of fluoride. The largest fluoride concentration in flamingo tissues were significantly higher in the bone material (122.5 ± 40.93ppm, ANOVA, $F = 99.6$, $P<0.05$)) with low levels on the skin and muscle. The mean concentration of fluorides in the muscle was negligible compared to bone and skin concentration.

Table 4: Mean fluoride concentration ± SD (ppm) in water, sediment and tissue samples from the four Rift Valley lakes in Nakuru County, Kenya

Locality	**n**	**Fluoride Water**	**n**	**Fluoride Sediment**	**n**	**Tissue**		
						Bone	**Muscle**	**Skin**
Crater Lake	4	55 ± 28.87	6	8.58 ± 0.67	1	156.7 ± 49.33	0.53±0.06	7.33±0.58
Lake Oloidien	4	30.75 ± 2.99	6	1.75 ± 0.52	3	111.1 ± 33.33	0.36±0.22	4.11±1.83
Lake Elementaita	8	116.9 ± 70.25	12	7.04 ± 0.96	none	none	none	none
Lake Nakuru	12	247.9 ± 170.4	15	6.67 ± 2.52	none	none	none	none
Discharge points to Lake Nakuru(Rivers)	8	19.25 ± 16.26	9	0.32 ± 0.29	none	none	none	none
Sewage drain to Lake Nakuru	4	6.5 ± 0.58	3	0.72 ± 0.25	none	none	none	none

4.4 Results of heavy metals analysis

The mean concentration of lead, chromium, cadmium and arsenic collected from water of the four lakes in Nakuru County in the month of December are shown in Table 5. Lake Elementaita had the highest mean concentration of lead (14.24 ± 8.86 ppb) followed by Lake Nakuru (12 ± 14.24 ppb). There were no detectable levels of lead from Lake Oloidien and the Crater Lake. Rivers discharging to Lake Nakuru and sewerage drain had the least lead concentrations.

Chromium mean concentration from the highest to the lowest was; Lake Nakuru (5.25 ± 5.67 ppb) followed by Lake Elementaita (3.42 ± 4.16 ppb) and Crater Lake (0.21 ± 0.26 ppb). There were no detectable levels of chromium in Lake Oloidien. Concentration of chromium was highest from the rivers discharging to Lake Nakuru with a mean of 18.55 ± 19.92 ppb with River Nderit discharging the highest concentration of 40.2 ppb (Appendix 4).

The concentration of arsenic was highest in Lake Oloidien with a mean of 11.37 ± 11.21 ppb and it was the only metal detected in Lake Oloidien water. Lake Nakuru had the lowest mean concentration of arsenic (2.34 ± 3.1 ppb). The rivers discharging to Lake Nakuru and the sewerage drain had the least arsenic concentration. Cadmium was not detected above the detection limit of 0.05µg/kg in the waters of all the four lakes under study. In general, it was observed that Lake Nakuru and Lake Elementaita have high concentrations of the four heavy metals.

The mean concentration of lead, chromium, cadmium and arsenic in soil sediments are shown in Table 5. All the metals under study were found in soil sediments of the four lakes except cadmium which was not detectable in sewerage drain soil sediment in Lake Nakuru. Lead and arsenic were found in high concentration in soil sediments of all the

Table 5: Mean metal concentrations ±SD (ppb) in water and sediment from the four Rift Valley lakes in Nakuru County, Kenya

Locality			Metals in water					Metals in sediment		
	n	Pb	Cd	Cr	As	n	Pb	Cd	Cr	As
Crater	4	ND	ND	0.21 ± 0.26	4.35 ± 3.81	6	212.5 ± 44.29	36.2 ± 39.7	136.4 ± 48.4	409.3 ± 243.9
Elementaita	8	14.24± 8.86	ND	3.42 ± 4.16	9.68 ± 3.36	12	567.3 ± 46.12	64.8 ± 40.47	64.85 ± 18.73	512 ± 66.96
Nakuru	12	12 ± 14.24	ND	5.25 ± 5.67	2.34 ± 3.1	15	430.1 ± 122.1	76.69 ± 48.31	57.85 ± 17.29	354.4 ± 294.9
Oloidien	4	ND	ND	ND	11.37 ± 11.21	6	273.7 ± 67.12	0.4673 ± 0.52	50.27 ± 3.98	265.5 ± 290.8
River Nakuru	8	1.2 ± 2.23	ND	18.55 ± 19.92	0.77 ± 1.36	9	1129 ± 107	82.88 ± 17.83	99.72 ± 24.38	198.4 ± 49.87
Sewage Nakuru	4	1.52 ± 1.76	ND	4.47 ± 0.96	0.50 ± 0.44	3	1754 ± 22.81	ND	91.3 ± 6.26	ND
Benchmark levels*		8.1	9.3	50	36		21000	1000	8100	6000

*Benchmark levels for water concentrations (µg/L) for Pb from US EPA (1999) and for Cd, Cr and As from US EPA (1987). For sediment benchmark levels (µg/kg): Pb and Cd from MacDonald (1993), Cr from Long *et al*., (1995), As from Persaud *et al*., (1993)

Key: Pb – lead, Cd – cadmium, Cr – chromium, As – arsenic, n – number of samples, ND – not detected.

lakes. Chromium and cadmium were generally found in low concentration in soil sediment of all the four lakes. Sewerage drain in Lake Nakuru had the highest level of lead mean concentration of 1754 ± 22.81 ppb while the rivers draining to Lake Nakuru had lead mean concentration of 1129 ± 107 ppb with River Nderit discharging the highest lead concentration of 1291 ppb (Appendix 5) among the rivers. The highest concentration of lead from the soil sediment was detected from the sewerage drain, 1774 ppb (Appendix 5). The metal concentration of both water and soil sediment samples were significantly varied ($p<0.05$).

Table 6 shows the mean distribution of metals in various tissues of the Lesser Flamingo. Arsenic, chromium and lead was observed in all the tissues analysed. Cadmium was detected only in the liver and kidney. Chromium was more concentrated in the kidney followed by cadmium while lead is more concentrated in bone. Cadmium is more concentrated in the liver. Chromium is least concentrated in the muscle while lead is least concentrated in the brain. The mean concentrations of all the other metals except cadmium are fairly distributed among the tissues. Lead concentration was above the level recommended based on the daily intake amount. Mean metal concentration in the tissue of birds varied significantly ($p< 0.05$).

Table 6: Mean metal concentrations ± SD (ppb) in birds' tissue samples collected from the four Rift Valley lakes in Nakuru County, Kenya

	Muscle	**Bone**	**Brain**	**Heart**	**Kidney**	**Liver**	**Lungs**
Lead * 25							
Crater Lake (n=2)	32±45.25	111.5±17.68	0.21±0.29	47±18.38	70.5±14.85	21.5±4.95	20.5±4.95
Oloidien (n=5)	4.94±9.61	162±31.14	2.94±1.6	7.17±12.28	7.74±13.13	17.1±9.15	13.6±8.08
Elementaita (n=1)	ND	100	ND	9	17	4.9	2.2
Nakuru	None	None	None	None	None	None	None
Cumulative mean	**11.09±22.69**	**141.6±37.45**	**1.89±1.89**	**17.36±21.67**	**24.59±30.79**	**16.68±8.83**	**13.9±8.54**
Cadmium *1450							
Crater (n=2)	ND	ND	ND	ND	62.7±1.27	27.3±15.98	ND
Oloidien (n=5)	ND	ND	ND	ND	37.46±47.32	64.96±28.72	ND
Elementaita (n=1)	ND	ND	ND	ND	71.8	78.9	ND
Nakuru	None	None	None	None	None	None	None
Cumulative mean	**ND**	**ND**	**ND**	**ND**	**48.06±38.76**	**57.29±29.56**	**ND**
Chromium*1000							
Crater (n=2)	0.82±0.97	10.65±8.98	10±8.49	10.16±8.29	27.4±4.38	8.08±4	7.3±3.11
Oloidien (n=5)	1.29 ±1.82	10.94±6.38	10.52±6.55	8.26±3.52	255.2±329	21.96±19.35	7.76±3.53
Elementaita (n=1)	1.1	11.9	12.6	9.3	23.7	21	5.94
Nakuru	None	None	None	None	None	None	None
Cumulative mean	**1.15±1.44**	**10.99±5.91**	**10.65±5.96**	**8.87±4.2**	**169.3±276.1**	**18.37±16.02**	**7.42±2.98**
Arsenic*2460							
Crater (n=2)	11.37±4.15	7.65±4.88	5.35±0.92	13.3±5.24	19.45±8.27	11.18±3.29	8.14±1.29
Oloidien (n=5)	9.65±2.75	7.23±1.23	5.17±2.97	10.02±1.99	17.67±2.78	19.18±6.29	14.7±2.90
Elementaita (n=1)	10.6	3.07	5.4	6.77	25.7	17.1	9.84
Nakuru	None	None	None	None	None	None	None
Cumulative mean	**10.2±2.72**	**6.82±2.57**	**5.24±2.27**	**10.43±3.25**	**19.12±4.68**	**16.92±6.1**	**12.45±3.87**

Key:

ND – not detected; none - no sample collected; Pb – lead; Cd – cadmium; Cr – chromium; As - arsenic

*****Benchmarks levels for birds' concentration µg/kg BW- day for Pb from Kendall and Scanlon (1982), Cd from White and Finley (1978), Cr from Haseltine *et al*. (1985) and As from US Fish and Wildlife Service (1969).

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

Birds feeding in the lakes were generally of poor condition, indicated signs of starvation, as witness from empty crops, lack of fat reserves, an extended gall bladder and adrenal glands (Table 3). They also showed signs of lethargy and depression on capture. While the cause of these clinical signs remained unknown, it was speculated that this could either be due to starvation or a toxin that prevents adequate food intake. The implications were that either (a) the birds were affected directly (toxin poisoning the system) or (b) indirectly (the status of the water was influenced affecting the availability of the flamingos food). Morphometric measurements of birds showed that the males were bigger than the female which agrees with earlier studies (Zimmerman *et al*., 1999). There were insignificant variations on morphometric features of the birds within and between the lakes. The morphological components related to feeding (culmin length and body mass) and the components associated to movement (wing and tarsus length) showed insignificant variation among birds. The flamingos legs and necks are longer in relation to body size and are well known for their unusual bill shape (Del Hayoyo *et al*.,1992). The mean body weight of the Lesser Flamingos (1122.88 g) was below that reported by other researchers 1760 g (Brown, 1973); 1800 g (Bartholomew and Pennycuick, 1973) and 1525 g (Tewodros and Afework, 2014). This weight could be attributed to poor nutritional state of the birds. In comparison to the studies done by Tewodros and Afework (2014) in Lakes Abijata and Shalla in Ethiopia, wing and tarsus length were longer, the culmin length was in the same range, while the body weight was lower. The birds were also infested with lots of parasites of the genus *Anatoecus ssp*. (*Phithiraptera: Ischnocera*). This parasite has been reported to infest the Lesser Flamingos in Africa; Clay (1973). *Anatoecus ssp* are chewing lice, they cause disturbance to the birds as they feed. There is the possibility that these lice can interfere with the feeding habit of the birds leading to starvation or not fully getting the required nutrients. This can in turn lower the immunity and the birds become susceptible to other infectious and non infectious agents.

The Rift Valley Lakes are faced with a number of pollution problems. There are a number of agricultural farms along the rivers which discharges to these Lakes. Some pesticides and fertilizers may contain fluorides and metals which can be washed off to these rivers which finally drained to the lakes. They can accumulate in the lakes and become a source of toxicants to the aquatic life causing chronic toxicosis.

In this study, the results obtained showed that all the study sites had fluoride concentration in water and soil sediments. Too much fluoride when consumed by the aquatic life affects their health. The waters of Lake Nakuru showed the highest mean concentrations of fluoride (Table 4). The variation among the sites within Lake Nakuru was witnessed and this could be attributed to evaporation and dilution from the inflow water which has caused the water levels in Lake Nakuru to rise. This phenomenon may cause differences in concentration of the fluoride within the different sites. This also concurs with previous studies done on the Kenyan lakes by Jones *et al*. (1977); Nanyaro *et al*. (1984); Clake *et al*. (1990) and Kilham (1990) which showed high fluoride variation in concentration which was attributed to evaporative concentration. Lake Oloidien had the least fluoride concentration in lake water which could be due to the dilution from the overflow from the main Lake Naivasha which is a fresh water lake. The results on fluoride concentrations are in agreement with the studies done earlier by a number of researchers (Bakish, 1974 ; Jones *et al*., 1977; Clake *et al*, 1990 ; Harper *et al.*, 1990 ; Gikunju *et al.*, 1995) which indicate high presence of fluoride in the Rift Valley lakes.

There was considerable amounts of fluoride being discharged from the rivers to lake Nakuru with River Nderit contributing the highest concentration (40ppm) among the rivers. The level of fluoride in the sewage discharge was very low (6.5± 0.58ppm). Lake Nakuru is a closed lake without an exit. These fluoride being discharged into the lakes could build up to increase the fluoride concentration in the Lake Nakuru. During the rainy seasons agricultural chemicals that contain fluoride are washed off to the rivers discharging to the lake. This also supports the studies done by Gaciri and Davies (1993) in Western Kenya which showed that considerable amounts of fluoride is also discharged directly into hydrological system in the form of waste waters and other wastes. The concentration levels of fluoride were generally low in the soil sediments compared to the fluoride concentration levels in the water of respective lakes.

Fluoride was detected in the biological tissues of the Lesser Flamingos. The bone tissue contained the highest concentration of fluoride which concurs with the studies done by Gikunju *et al.* (1992) showing that calcified tissues accumulate fluoride. Excess fluoride causes bones to be brittle and make them to break easily and this could be the cause of inco-ordination seen on the Lesser Flamingos while they were walking. In WHO (2002) report, they observed that there were few data from which to estimate total exposure to and the bioavailability of fluoride, and there were inconsistencies in reports on the characterization of its adverse effects in humans which could be the same case with birds. Intake of fluoride in water and foodstuffs is the primary causative factor for endemic skeletal fluorosis in humans (Liteplo *et al.*, 2002). There is also the possibility that the Lesser Flamingos consumes fluorides in the river water (Brown, 1973) and from the lake sediments as they feed since they are filter feeders. This could also expose them to skeletal fluorosis. There were considerable amount of fluoride levels in the river water and lake sediments. This was the first documentation on the levels of fluoride on the Lesser Flamingo tissues.

The results on heavy metal analysis showed that waters of Lake Nakuru and Lake Elementaita had lead, chromium and arsenic with lead being found in high levels. The Crater Lake had high levels of arsenic, little levels of chromium and no lead was detected. Lake Oloidien had the highest levels of arsenic which could be attributed to the run offs from the flower farms surrounding the lake and the spill of the Lake Naivasha waters which is highly polluted (Harper *et al.*, 1990). It is also possible that the arsenic leaches from the volcanic soils to the water hence the high concentration. Chromium and lead were not detected in Lake Oloidien. In general, it was observed that Lake Nakuru and Lake Elementaita had the highest levels of the four heavy metals. This may suggest that the rivers flowing in and the sewerage drain to Lake Nakuru contribute to the levels of these metals apart from the ones leaching naturally from the ground. Analysis of variance for metal in water revealed that the mean concentration of all the metals was significantly varied ($p<0.05$) with Lake Nakuru levels being significantly high. There is a lot of inflow from the rivers which has flooded the lake and this could be the possible cause of the high variation. Flooding of Lake Nakuru could be also due to the increase intensive farming and urbanization around the area which can reduce the soil ability to absorb water due to the addition of water to the soil during irrigation. It is also possible that the conservation of the Mau forest allows the seasonal rivers to pour a lot of water to Lake Nakuru. The levels of cadmium, chromium and arsenic in the waters of the four lakes under study were below the benchmark levels (Table 5) except for lead levels in Lake Elementaita (14.24 ppb) and Lake Nakuru (12 ppb) which were above the recommended levels (8.1 ppb) by the US Environmental Protection Agency.

Lead, cadmium, chromium and arsenic were found in soil sediments of the four lakes except cadmium which was not detected in sewerage drain to Lake Nakuru. The order of concentration from the highest to the lowest was; Lead > arsenic > chrome > cadmium. Presence of lead in Lakes Elementaita, Oloidien and Crater could be attributed to leakage of petrol from the boats and also from the soil since generation of geothermal power is within Naivasha Sub County which can cause leaching of lead from the soils. Sewerage drain and the rivers draining to Lake Nakuru had a high mean concentration of lead (1754 ±22.81 ppb) and (1129 ± 107 ppb), respectively and they are the possible causes of lead detected in Lake Nakuru. Comparing the present average levels of chromium (57.85 ppb) and lead (430.1 ppb) detected in soil sediments in Lake Nakuru with the previous levels from studies done by Nelson *et al*., (1998), average levels of chromium (67 ppb) and lead (22 ppb) chromium current levels are slightly lower and lead current levels are very high. However, the levels of metals detected in Lake Nakuru are generally lower than in the previous studies (SAPS, 2002). This could be due to the difference in seasons when the studies were done. The present higher average levels of lead in Lake Nakuru compared to the other lakes can be attributed to accumulation since the lake has no out-flow but has many inflows and also due to increase in the number of industries and population which in turn increases sewage discharge. The levels of all the metals under study were below the benchmark levels of the marine soil sediments (Table 5).

All the metals were detected in tissues and cadmium was detected only in the liver and kidney (Table 6) which agrees with studies done by Schafer *et al*.,(1999) that the liver and kidney accumulates cadmium. The highest mean concentration of cadmium was 57.29 µg/kg which is almost 40 times higher than what was found out by Kairu (1996) (1.3µg/kg) and Koeman *et al*. (1972) (1.35µg/kg). This can be attributed to bioaccumulation since the Lesser Flamingo can live for about 50 years. The Lesser

Flamingos are filter feeders, they feed through stiring up the lake sediments then filtering out their food and in the process can take in the metals in the lake sediments. The mean concentration of heavy metals were higher in the lake sediment than in the lake water (Table 5) and this is in agreement with other studies which have shown that sediments accumulate metals and are involved in the remobilisation of the trace metals in the aquatic systems under favourable conditions and in the interraction between sediment and water (Bols *et al*., 2001; Uzairu *et al*., 2009). There is also the possibility that the Lesser Flamingos consumes metals from the rivers that are discharging to the lakes as they drink water since they had considerable amount of metals (Table5), the Lesser Flamingos do not take the alkaline lake water but instead drink from the nearby fresh waters (Brown, 1973). Starvation was witnessed and lice from genus *Anatoecus ssp* were found on the Lesser Flamingos, all these are known to cause stress and lower the immunity. The presence of metals in the Lesser Flamingo tissues in combination with these external parasites and other unknown risk factors can lead to death of the birds. Absence of food in the digestive system also increase the absorbtion of metals.

5.2 Conclusions

a) The birds were stressed and under starvation as witnessed from empty crops and lack of fat reserves.

b) High fluoride concentration was detected in the lake water, sediments and the Lesser Flamingos tissues which could be an indication of contamination of the habitat which also affect the aquatic life (Lesser Flamingos).

c) Metals were detected in water, soil sediments and the Lesser Flamingo tissues but they were below the benchmark levels.

d) Lead in Lake Elementaita and Lake Nakuru waters and the bird’s tissues were above the recommended levels.

5.3 Recommendations

a) Much has not been done on the effect of fluoride on the Lesser Flamingos and more work is required in order to find out the toxic levels.

b) Further investigation is required in order to find out the actual cause of death of the Lesser Flamingos.

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APPENDICES

Appendix 1: Concentration of fluoride in water of lakes Nakuru, Elementaita, Oloidien and Crater

Sample site	Sample code	ppm
Lake Nakuru	LNK-S1	150
Lake Nakuru	LNK-S1	100
Lake Nakuru	LNK-S2	200
Lake Nakuru	LNK-S2	200
Lake Nakuru	LNK-S3	125
Lake Nakuru	LNK-S3	125
Lake Nakuru	LNK-S4	200
Lake Nakuru	LNK-S4	200
Lake Nakuru	LNK-S5	600
Lake Nakuru	LNK-S5	600
Lake Nakuru	LNK-S6	250
Lake Nakuru	LNK-S6	225
River Nderit (catchments)	LNK-S7	40
River Nderit (catchments)	LNK-S7	40
River Nderit (towards lake)	LNK-S8	30
River Nderit (towards lake)	LNK-S8	25
River Makalia	LNK-S9	5
River Makalia	LNK-S9	4
River Naishi	LNK-S10	5
River Naishi	LNK-S10	5
Sewerage drain (L. Nakuru)	LNK-S11	6
Sewerage drain (L. Nakuru)	LNK-S11	6
Sewerage drain (L. Nakuru)	LNK-S12	7
Sewerage drain (L. Nakuru)	LNK-S12	7
Lake Elementaita	LE-S1	100
Lake Elementaita	LE-S1	200
Lake Elementaita	LE-S2	200
Lake Elementaita	LE-S2	200
Lake Elementaita	LE-S3	60
Lake Elementaita	LE-S3	60
Lake Elementaita	LE-S4	55
Lake Elementaita	LE-S4	60
Lake Oloidien	LO-S1	28
Lake Oloidien	LO-S1	35
Lake Oloidien	LO-S2	30
Lake Oloidien	LO-S2	30
Crater Lake	CL-S1	80
Crater Lake	CL-S1	80
Crater Lake	CL-S2	30
Crater Lake	CL-S2	30

Appendix 2: Concentration of fluoride in soil sediments of lakes Nakuru, Elementaita, Oloidien and Crater

Sample site	Sample code	ppm
Lake Nakuru	LNK-S1	2.5
Lake Nakuru	LNK-S1	2
Lake Nakuru	LNK-S1	1.5
Lake Nakuru	LNK-S2	7
Lake Nakuru	LNK-S2	7.5
Lake Nakuru	LNK-S2	7.5
Lake Nakuru	LNK-S3	9
Lake Nakuru	LNK-S3	7
Lake Nakuru	LNK-S3	7
Lake Nakuru	LNK-S4	8
Lake Nakuru	LNK-S4	7.5
Lake Nakuru	LNK-S4	9.5
Lake Nakuru	LNK-S5	8
Lake Nakuru	LNK-S5	8
Lake Nakuru	LNK-S5	8
River Nderit (towards lake)	LNK-S8	0.8
River Nderit (towards lake)	LNK-S8	0.6
River Nderit (towards lake)	LNK-S8	0.7
River Makalia	LNK-S9	0.09
River Makalia	LNK-S9	0.09
River Makalia	LNK-S9	0.095
River Naishi	LNK-S10	0.2
River Naishi	LNK-S10	0.2
River Naishi	LNK-S10	0.1
Sewerage drain (L. Nakuru)	LNK-S11	0.45
Sewerage drain (L. Nakuru)	LNK-S11	0.75
Sewerage drain (L. Nakuru)	LNK-S11	0.95
Lake Elementaita	LE-S1	7.5
Lake Elementaita	LE-S1	8
Lake Elementaita	LE-S1	8
Lake Elementaita	LE-S2	8
Lake Elementaita	LE-S2	8
Lake Elementaita	LE-S2	5
Lake Elementaita	LE-S3	7
Lake Elementaita	LE-S3	7
Lake Elementaita	LE-S3	7
Lake Elementaita	LE-S4	6
Lake Elementaita	LE-S4	7
Lake Elementaita	LE-S4	6
Lake Oloidien	LO-S1	1
Lake Oloidien	LO-S1	1.5

Lake Oloidien	LO-S1	2
Lake Oloidien	LO-S2	2.5
Lake Oloidien	LO-S2	1.5
Lake Oloidien	LO-S2	2
Crater Lake	CL-S1	8
Crater Lake	CL-S1	9
Crater Lake	CL-S1	8
Crater Lake	CL-S2	8
Crater Lake	CL-S2	9.5
Crater Lake	CL-S2	9

Appendix 3: Concentration of fluoride in tissues of Lesser Flamingos

Animal Code	Tissue	ppm
2	Bone	100
2	Bone	100
2	Bone	100
4	Bone	200
4	Bone	100
4	Bone	100
5	Bone	100
5	Bone	100
5	Bone	100
6	Bone	180
6	Bone	190
6	Bone	100
2	Skin	0.65
2	Skin	0.7
2	Skin	-
4	Skin	3
4	Skin	3
4	Skin	3
5	Skin	1
5	Skin	2
5	Skin	1
6	Skin	6.5
6	Skin	7
6	Skin	6.5
2	Breast Muscle	0.06
2	Breast Muscle	0.3
2	Breast Muscle	0.09
4	Breast Muscle	0.3
4	Breast Muscle	0.2
4	Breast Muscle	0.5
5	Breast Muscle	0.6
5	Breast Muscle	0.6
5	Breast Muscle	0.6
6	Breast Muscle	0.6
6	Breast Muscle	0.5
6	Breast Muscle	0.5

Appendix 4: Concentration of metals in the waters of lakes Nakuru, Elementaita, Oloidien and Crater

Sampling site	Sample code	Pb (ppb)	Cd (ppb)	Cr (ppb)	As (ppb)
Lake Nakuru	LNK-S1	ND	ND	16.5	ND
Lake Nakuru	LNK-S1	ND	ND	14.9	ND
Lake Nakuru	LNK-S2	ND	ND	0.79	ND
Lake Nakuru	LNK-S2	ND	ND	1.26	ND
Lake Nakuru	LNK-S3	ND	ND	1.52	5.02
Lake Nakuru	LNK-S3	ND	ND	0.98	3.21
Lake Nakuru	LNK-S4	35	ND	ND	7.31
Lake Nakuru	LNK-S4	38	ND	ND	8.89
Lake Nakuru	LNK-S5	18	ND	7.56	0.802
Lake Nakuru	LNK-S5	15	ND	7	0.92
Lake Nakuru	LNK-S6	22	ND	6.5	0.96
Lake Nakuru	LNK-S6	16	ND	6	0.95
River Nderit (catchments)	LNK-S7	ND	ND	33.6	ND
River Nderit (catchments)	LNK-S7	ND	ND	36.2	ND
River Nderit (towards lake)	LNK-S8	ND	ND	40.2	0.14
River Nderit (towards lake)	LNK-S8	ND	ND	38.4	0.29
River Makalia	LNK-S9	ND	ND	ND	ND
River Makalia	LNK-S9	ND	ND	ND	ND
River Naishi	LNK-S10	4.4	ND	ND	2.18
River Naishi	LNK-S10	5.2	ND	ND	3.56
Sewerage drain	LNK-S11	3.1	ND	5.2	0.5
Sewerage drain	LNK-S11	2.98	ND	5.1	0.2
Sewerage drain	LNK-S12	ND	ND	3.12	0.19
Sewerage drain	LNK-S12	ND	ND	4.45	1.12
Lake Elementaita	LE-S1	25	ND	9.74	6.89
Lake Elementaita	LE-S1	28	ND	9.2	5.76
Lake Elementaita	LE-S2	15	ND	3.69	6.43
Lake Elementaita	LE-S2	13	ND	4.62	7.48
Lake Elementaita	LE-S3	2.1	ND	ND	12.9
Lake Elementaita	LE-S3	4.6	ND	ND	11.2
Lake Elementaita	LE-S4	13.1	ND	ND	13.8
Lake Elementaita	LE-S4	13.2	ND	0.098	13
Lake Oloidien	LO-S1	ND	ND	ND	20.1
Lake Oloidien	LO-S1	ND	ND	ND	22
Lake Oloidien	LO-S2	ND	ND	ND	1.83
Lake Oloidien	LO-S2	ND	ND	ND	1.53
Crater Lake	CL-S1	ND	ND	ND	1.11
Crater Lake	CL-S1	ND	ND	ND	1.09
Crater Lake	CL-S2	ND	ND	0.306	6.75
Crater Lake	CL-S2	ND	ND	0.54	8.43

Key:
ND - not detected
Pb – lead, cd – cadmium, cr – chromium, As - arsenic

Appendix 5: Concentration of metals in the soil sediments of lakes Nakuru, Elementaita, Oloidien and Crater

Sampling site	Sample code	Pb (ppb)	Cd (ppb)	Cr (ppb)	As (ppb)
Lake Nakuru	LNK-S1	240	52	31.1	ND
Lake Nakuru	LNK-S1	245	54	34	ND
Lake Nakuru	LNK-S1	239	52	36.2	ND
Lake Nakuru	LNK-S2	367	ND	48.1	799
Lake Nakuru	LNK-S2	401	0.96	48	801
Lake Nakuru	LNK-S2	350	1.7	48	804
Lake Nakuru	LNK-S3	563	137	80.7	551
Lake Nakuru	LNK-S3	560	129	80.4	548
Lake Nakuru	LNK-S3	560	136	79.8	550
Lake Nakuru	LNK-S4	556	113	70.8	233
Lake Nakuru	LNK-S4	490	113	76	234
Lake Nakuru	LNK-S4	570	108	68	233
Lake Nakuru	LNK-S5	439	86.7	54.3	190
Lake Nakuru	LNK-S5	436	84	53.8	186
Lake Nakuru	LNK-S5	436	83	58.6	187
River Nderit (towards lake)	LNK-S8	1291	107	69.5	264
River Nderit (towards lake)	LNK-S8	1280	107	69	268
River Nderit (towards lake)	LNK-S8	1199	104	71.01	262
River Makalia	LNK-S9	1028	68.6	126	162
River Makalia	LNK-S9	1035	69.9	125	160
River Makalia	LNK-S9	1030	65	126	160
River Naishi	LNK-S10	1150	69.8	101.9	172
River Naishi	LNK-S10	1042	79.2	105.6	169
River Naishi	LNK-S10	1102	75.4	103.5	169
Sewerage drain	LNK-S11	1758	ND	90.3	ND
Sewerage drain	LNK-S11	1729	ND	98	ND
Sewerage drain	LNK-S11	1774	ND	85.6	ND
Lake Elementaita	LE-S1	615	7.11	90.1	619
Lake Elementaita	LE-S1	588	7.09	87.9	619.1
Lake Elementaita	LE-S1	604	7.11	94	617.3
Lake Elementaita	LE-S2	546	109.1	64.5	478
Lake Elementaita	LE-S2	540	109.4	56	472
Lake Elementaita	LE-S2	580	107	60	475
Lake Elementaita	LE-S3	603	90.2	40.2	502
Lake Elementaita	LE-S3	550	89.9	34	504
Lake Elementaita	LE-S3	649	90.1	55	502.6
Lake Elementaita	LE-S4	520	52.3	72.5	450
Lake Elementaita	LE-S4	514	54	60	454
Lake Elementaita	LE-S4	499	54.3	64	451
Lake Oloidien	LO-S1	316	ND	53.2	529

Lake Oloidien	LO-S1	352	ND	54.2	534
Lake Oloidien	LO-S1	334	ND	54.2	530
Lake Oloidien	LO-S2	212	0.82	47.2	ND
Lake Oloidien	LO-S2	210	1.06	46	ND
Lake Oloidien	LO-S2	218	0.924	46.8	ND
Crater Lake	CL-S1	170	ND	180	184
Crater Lake	CL-S1	189	ND	182	190
Crater Lake	CL-S1	160	ND	179.8	186
Crater Lake	CL-S2	253	71.4	92.1	634
Crater Lake	CL-S2	249	69.89	90.6	632
Crater Lake	CL-S2	254	75.9	94.1	630

Key:
ND - not detected
Pb – lead, cd – cadmium, cr – chromium, As - arsenic

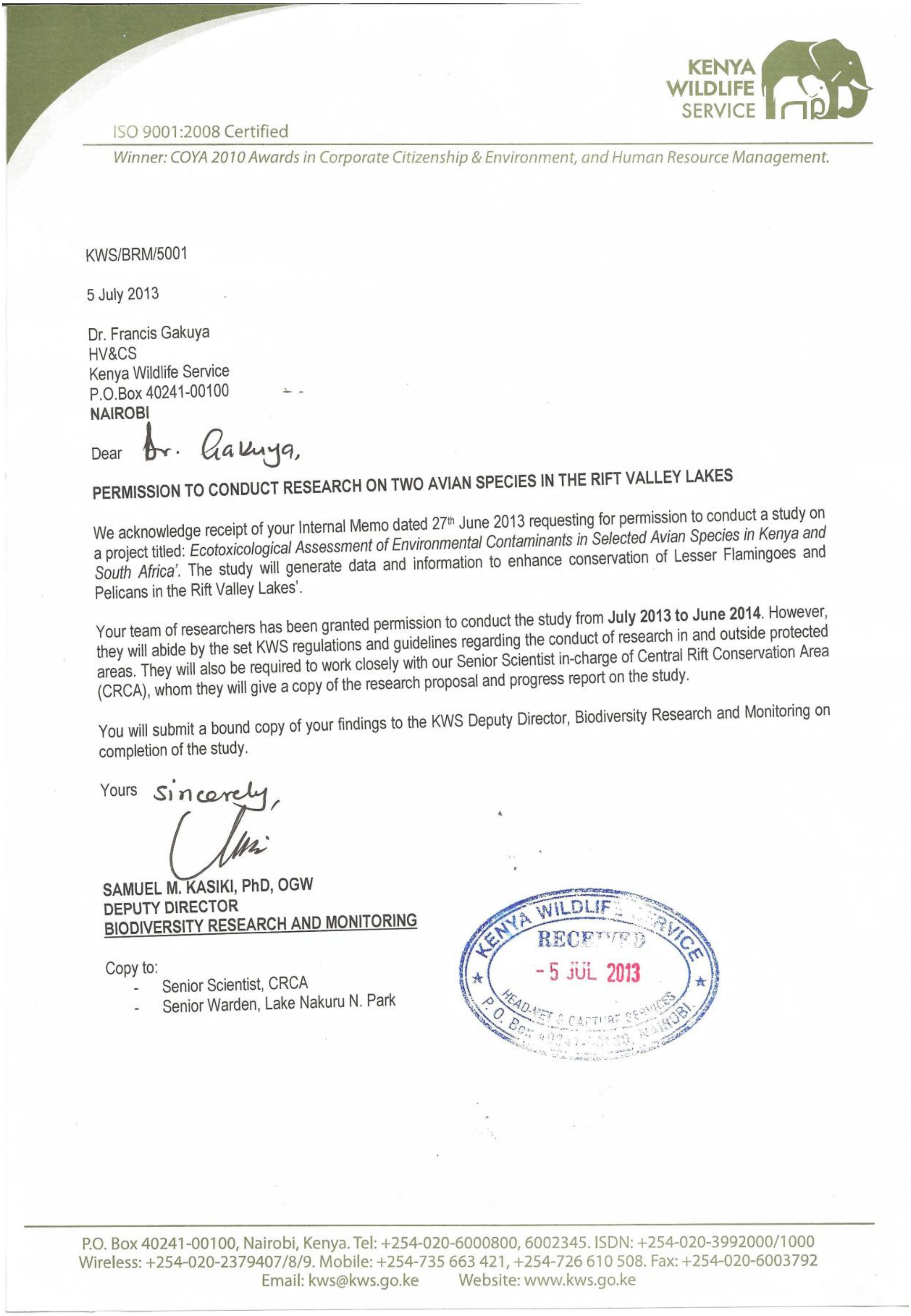
Appendix 6: Concentration of metals in tissues of Lesser Flamingos

Animal code	Tissue	Pb (ppb)	Cd (ppb)	Cr (ppb)	As (ppb)
1	Breast muscle	22	ND	1.29	6.27
2	Breast muscle	ND	ND	0.082	12.1
3	Breast muscle	ND	ND	0.653	12
4	Breast muscle	ND	ND	ND	7.18
5	Breast muscle	2.7	ND	4.41	10.7
6	Breast muscle	64	ND	1.51	14.3
7	Breast muscle	ND	ND	0.138	8.43
8	Breast muscle	ND	ND	1.1	10.6
1	Brain	0.41	ND	8.3	2.1
2	Brain	2.9	ND	20	6.35
3	Brain	2.9	ND	2.6	3
4	Brain	3.8	ND	13.5	9.6
5	Brain	4.7	ND	8.2	4.8
6	Brain	0.41	ND	4	4.7
7	Brain	ND	ND	16	6
8	Brain	ND	ND	12.6	5.4
1	Liver	12	19.5	11.4	25.8
2	Liver	32	56.3	54.8	12.1
3	Liver	14	88.7	5.69	25.8
4	Liver	19	88.3	22.5	16.9
5	Liver	8.5	72	15.4	15.3
6	Liver	18	16	10.9	13.5
7	Liver	25	38.6	5.25	8.85
8	Liver	4.9	78.9	21	17.1
1	Kidney	4.8	45.1	8.75	20.6
2	Kidney	2	ND	6.08	16.87
3	Kidney	ND	21.5	780	19.5
4	Kidney	31	116	105	18
5	Kidney	0.91	4.7	376	13.4
6	Kidney	60	63.6	30.5	13.6
7	Kidney	81	61.8	24.3	25.3
8	Kidney	17	71.8	23.7	25.7
1	Bone	190	ND	8.2	8.27
2	Bone	190	ND	14	7.24
3	Bone	120	ND	3.1	7.34
4	Bone	140	ND	9.4	5.2
5	Bone	170	ND	20	8.12
6	Bone	124	ND	17	11.1
7	Bone	99	ND	4.3	4.2
8	Bone	100	ND	11.9	3.07
1	Heart	3.8	ND	6.2	11.6
2	Heart	2.1	ND	9	11.4

3	Heart	0.34	ND	6.4	8.53
4	Heart	29	ND	14.1	7.28
5	Heart	0.64	ND	5.6	11.3
6	Heart	34	ND	4.3	9.59
7	Heart	60	ND	16.02	17
8	Heart	9	ND	9.3	6.77
1	Lungs	20	ND	5.22	17.2
2	Lungs	ND	ND	8.45	11.3
3	Lungs	13	ND	6.4	11.8
4	Lungs	19	ND	5.14	16.9
5	Lungs	16	ND	13.6	16.3
6	Lungs	24	ND	9.5	7.23
7	Lungs	17	ND	5.1	9.05
8	Lungs	2.2	ND	5.94	9.84

Key:
ND - not detected
Pb – lead, cd – cadmium, cr – chromium, As - arsenic

Appendix 7: Kenya Wildlife Service Permit



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KWS/BRM/5001

5 July 2013

Dr. Francis Gakuya
HV&CS
Kenya Wildlife Service
P.O.Box 40241-00100
NAIROBI

Dear Dr. Gakuya,

PERMISSION TO CONDUCT RESEARCH ON TWO AVIAN SPECIES IN THE RIFT VALLEY LAKES

We acknowledge receipt of your Internal Memo dated 27th June 2013 requesting for permission to conduct a study on a project titled: *Ecotoxicological Assessment of Environmental Contaminants in Selected Avian Species in Kenya and South Africa'*. The study will generate data and information to enhance conservation of Lesser Flamingoes and Pelicans in the Rift Valley Lakes'.

Your team of researchers has been granted permission to conduct the study from **July 2013 to June 2014**. However, they will abide by the set KWS regulations and guidelines regarding the conduct of research in and outside protected areas. They will also be required to work closely with our Senior Scientist in-charge of Central Rift Conservation Area (CRCA), whom they will give a copy of the research proposal and progress report on the study.

You will submit a bound copy of your findings to the KWS Deputy Director, Biodiversity Research and Monitoring on completion of the study.

Yours sincerely,

SAMUEL M. KASIKI, PhD, OGW
DEPUTY DIRECTOR
BIODIVERSITY RESEARCH AND MONITORING

RECEIVED
- 5 JUL 2013
HEAD-VET & CAPTURE SERVICES

Copy to:
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- Senior Warden, Lake Nakuru N. Park

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Appendix 8: pH of digested water samples for fluoride analysis

Sample code	Water pH
LNK-S1	5.98
LNK-S1	5.33
LNK-S2	5.66
LNK-S2	5.45
LNK-S3	5.99
LNK-S3	5.98
LNK-S4	5.65
LNK-S4	5.87
LNK-S5	6.3
LNK-S5	5.9
LNK-S6	5.5
LNK-S6	6.04
LNK-S7	5.2
LNK-S7	5
LNK-S8	5.56
LNK-S8	5.45
LNK-S9	5.32
LNK-S9	5.42
LNK-S10	5.42
LNK-S10	5.42
LNK-S11	6.4
LNK-S11	6.09
LNK-S12	6.1
LNK-S12	6.2
LE-S1	5.9
LE-S1	5.73
LE-S2	5.89
LE-S2	5.9
LE-S3	5.43
LE-S3	6.89
LE-S4	6.45
LE-S4	5.99
LO-S1	5.3
LO-S1	5.3
LO-S2	5.4
LO-S2	5.25
CL-S1	6.02
CL-S1	6.05
CL-S2	5.99
CL-S2	5.99

Appendix 9: pH of soil sediments and tissues after digestion for fluoride analysis

Sample /animal code	pH
LNK-S1	5.73
LNK-S1	5.64
LNK-S1	5.65
LNK-S2	5.69
LNK-S2	5.69
LNK-S2	5.69
LNK-S3	5.01
LNK-S3	5.67
LNK-S3	5.51
LNK-S4	5.7
LNK-S4	5.8
LNK-S4	5.63
LNK-S5	5.73
LNK-S5	5.09
LNK-S5	5.72
LNK-S6	5
LNK-S6	4.87
LNK-S6	5.93
LNK-S7	5.15
LNK-S7	5.74
LNK-S7	5.02
LNK-S8	4.79
LNK-S8	5.74
LNK-S8	5.77
LNK-S9	5.01
LNK-S9	4.97
LNK-S9	5.1
LNK-S10	5
LNK-S10	5.01
LNK-S10	5.28
LNK-S11	4.92
LNK-S11	4.73
LNK-S11	5.73
LO-S1	5.11
LO-S1	4.96
LO-S1	5.02
LO-S2	5.12
LO-S2	5.16
LO-S2	5.11
LE-S1	5.14
LE-S1	5.08
LE-S1	5.08

LE-S2	5.62
LE-S2	5.6
LE-S2	5.59
LE-S3	4.89
LE-S3	5.78
LE-S3	5.5
LE-S4	5.6
LE-S4	5.1
LE-S4	5.3
CL-S1	5.7
CL-S1	5.64
CL-S1	5.2
CL-S1	5.66
CL-S2	5.66
CL-S2	5.74
CL-S2	5.67
2	5.87
2	5.77
2	5.71
4	5.8
4	4.88
4	5.78
5	5.58
5	5.63
5	4.74
6	5.7
6	5.76
6	5.84
2	5.88
2	5
2	5.75
4	5.11
4	5.02
4	5.09
5	5.04
5	5.94
5	4.92
6	4.95
6	5.88
6	5.85
2	5.6
2	5.13
2	5.64
4	5.61
4	5.38

4	5.95
5	5.43
5	5.45
5	5.46
6	5.49
6	5.5
6	5.65