ASSESSMENT OF PRESENCE OF OESTROGEN E1, E2 AND EE2 IN LAKE KARIBA, ZAMBIA

LOZIWE NJOBVU

A DISSERTATION SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS IN HEALTH OF AQUATIC ANIMAL RESOURCES OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

2017

ABSTRACT

Oestrogens are hormones, naturally produced by mammals or synthetically produced as drugs used in hormone therapy or as contraceptives. In the recent past years, oestrogens have gained much recognition due to their detrimental effect to aquatic life, which ranges from induction of intersexuality to complete reproductive failure of fish. Their main source in the aquatic environment is attributed to human and livestock waste and residues of oestrogens are found in water downstream of waste water treatment plants (WWTP). As such, this research sought to investigate the presence of these hormones in Lake Kariba as well as identify the anthropogenic activities leading to their introduction. A structured questionnaire, was administered in order to identify anthropogenic activities that would lead to oestrogen introduction in Lake Kariba. Siavonga's harsh terrain precludes its involvement in intensive animal husbandry activities thereby leaving only human wastewater as the main source of oestrogens in the lake. The synthetic hormone 17α-ethinyl estradiol (EE2) was undetected in all of the samples collected evidently due to low usage of oestrogen containing contraceptives and high usage of pit latrines in the area. On the contrary, natural oestrogens were detected in all sites in the range of 0.38 ng/L to 6.68ng/L in water samples and 0.01 ng/g to 2.74 ng/g in sediment samples. Source of the detected oestrogens could be a result of waste water discharge into the lake as well from terrestrial and aquatic animals. Further investigations are required to identify other sources of oestrogen introductions in sparsely populated areas that recorded oestrogen levels as high as sites receiving waste water as well as the biological effects in aquatic animals.

DECLARATION

I, Loziwe Njobvu, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work and that has never been nor concurrently being submitted for a higher degree awards in any other institution.

_____ _____

Loziwe Njobvu Date

(M.Sc Candidate)

The above declaration is confirmed;

_____ _____

Prof. R. Mdegela Date

(Supervisor)

_____ _____

Prof. J. Lyche Date

(Supervisor)

_____ _____

Dr. K. Muzandu Date

(Supervisor)

COPYRIGHT

ACKNOWLEDGEMENTS

I extend my gratitude towards the TRAHESA project (TAN-13/0017) for awarding me the opportunity to pursue my post graduate studies through this program. Furthermore, my sincere gratitude is extended towards my supervisors; Prof. J. Lyche, Dr. K. Muzandu and Prof. R. Mdegela for the continuous guidance, support and encouragement towards this work. My special thanks goes to Prof. Ø. Evernsen, Prof. S. Mutoloki and Dr. H. Munang'andu for the motivation, technical, material and financial support in the successful analysis of my samples. My gratitude goes to my husband William Chilufya and children (Mangani, Musonda and Mitiyawo) for their support during the study program. I am indebted to my children who had to endure a motherless life during the period of my studies. In addition, I would like to express my sincere gratitude to my employer's the Ministry of Fisheries and Livestock particularly the Department of Fisheries for granting me study leave to pursue my studies and for the continuous support during the study period. My earnest gratitude goes to Mr. S. C. Msigala for his continuous support and guidance towards my research. And above all my greatest gratitude goes to the almighty God for according me the opportunity of a master's degree and seeing me through the entire program.

DEDICATION

I dedicate this work to my parents Elesani and Matilda Njobvu for their wise counsel which has enabled me to come this far. I also dedicate this work to my children Mangani, Musonda and Mitiyawo. May it be your source of inspiration in your future endeavours.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND ACRONYMS

µg	microgram
µg/L	microgram per litre
µl	microlitre
ANOVA	Analysis of Variance
cm	centimetre
conc.	Concentration
CSO	Central Statistical Office
DoF	Department of Fisheries
E1	Estrone
E2	Estradiol
E3	Estriol
EDCs	Endocrine Disrupting Compounds
EE2	Ethinyl-estradiol
ELISA	enzyme linked immunosorbent assay
GC/MS	Gas chromatography/mass spectroscopy
GPS	Global positioning system
HCl	Hydrochloric acid
HLB	hydrophilic lipophilic balance
HPLC	High performance liquid chromatography
HPLC/MS	High-performance liquid chromatography/mass spectroscopy
HRT	hormone replacement therapy
km	kilometer
km^2	square kilometer
km^3	cubic kilometer

mg	milligram
ml	millilitre
ml/min	milliliter per minute
MT	Metric ton
MW	Mega watts
ND	not detected
NDA	no data available
ng/g	nanogram per gram
OD	Optical Density
PNEC	Predicted No Effect Concentrations
SDH	Siavonga District Hospital
SWSC	Southern Water and Sewerage Company Limited
VTG	Vitellogenin
WWTP	Waste Water Treatment Plant
ZESCO	Zambia Electricity Supply company

CHAPTER ONE

1.0 INTRODUCTION

Lake Kariba is the world's largest man-made reservoir by volume (180 km^3) (Kolding *et al*., 2014) created primarily for hydropower production. It is over 223 km long and 40 km wide covering an area of 5,580 km^2 shared between Zambia (45%) and Zimbabwe (55%). Lake Kariba is a multi-use resource reservoir providing storage for the Kariba North Bank and Kariba South Bank power plants with capacities of 720 MW and 750 MW, respectively (World Bank, 2010). The lake has also a substantial fishing industry in form of commercial capture fisheries and cage farming. The inshore fishery exploits tilapia species (*Oreochromis niloticus*, *Oreochromis andersonii*) and other indigenous fish species like *(Hydrocynus vittatus*) caught largely by use of gillnets. In 2015, Lake Kariba contributed 13.5% (11,309 MT) and 25.5% (5,794 MT) from capture fisheries and aquaculture, respectively, towards the country's total fish production (Department of Fisheries, 2016). Fish is an excellent source of high quality animal protein and essential fatty acids which are much greater in fishes than in terrestrial animal-source foods (Beveridge *et al*., 2013). It is an important source of protein for the marginalised communities because of its relative affordability in comparison with other animal protein.

The biggest town along the shores of Lake Kariba on the Zambian side is Siavonga with a population of 18,638. Siavonga's population, was expanded by people displaced by the rising waters, when their area was flooded between 1958 and 1963 following the completion of the Kariba Dam. Tumbare (2008), states that the major sources of pollution in Lake Kariba are from: urban and industrial activities, mining activities (coal, copper, manganese and sulphide ores), agro-chemicals, oils and waste from boats on the Lake. A study conducted in 2010 by Ikenaka *et al*. (2010) showed significant increase in heavy metal concentration in Lake Kariba during the past 18 years.

Among many contaminants of recent global concern are emerging contaminants with endocrine disrupting effect on human and natural ecosystem. These contaminants have been termed as emerging because they do not fall under standard monitoring and regulatory programs but may be candidates for future regulation (Li and Migliaccio, 2010). Among them are oestrogens considered a group of steroid hormones with high potential of endocrine disruption of organisms in the aquatic ecosystem (Sim *et al*., 2011). Oestrogens exist in natural and synthetic form. Natural oestrogens estrone (E1), 17β-estradiol (E2) and estriol (E3) are predominantly female hormones, responsible for the maintenance of the health of reproductive tissues, breast, skin and brain (Manickum and John, 2014). Synthetic oestrogen 17α Ethinyl estradiol (EE2) is synthetic derivative of estradiol used as medication for endocrine related diseases and in contraceptives. EE2 is also used in livestock and aquaculture industry (Aris *et al*., 2014). Oestrogens are key regulators of physiological changes associated with reproduction in both sexes and regulate many other important physiological processes, including immune function and mineral homeostasis (Pinto *et al*., 2014).

A review of different publications by Adeel *et al*. (2016) summarizes the average daily excreted hormone per person indicated in table 1.

Table 1: Average steroid oestrogen excretion by humans (per person) µg/day

Person's	**E1**	**17β-E2**	**E3**	**EE2**
Pregnant women	787	277	9850	0
Menopausal, with HRT	31.5	59.2	90.7	0
Menstruating woman	9.32	6.14	17.4	0
Women	7	2.4	4.4	NDA
Menstruating females	3.5	8	4.8	NDA
Adult male	3.5	1.83	3.21	NDA
Menopausal, no HRT	2.93	1.49	3.9	0
Menopausal females	2.3	4	1	NDA
Males	1.6	3.9	1.5	
Female child	0.6	2.5	0.918	
Male child	0.63	0.54	0	

**NDA and HRT denote for no data available and Hormone Replacement Therapy respectively

Source: Adeel *et al*. (2016)

The main source of discharge of these oestrogens in the aquatic environment is through sewage discharge (Singhal *et al*., 2009).

Fish are good bio-indicators of environmental pollution monitoring and can play a significant role in assessing potential risks associated with contamination in the aquatic environment (Sethuraman *et al*., 2013). Oestrogens have been identified as causative agents of biochemical changes and loss of male characteristics in fish (Allen *et al*., 1999). Vitellogenin synthesis in male fish has been used as a biomarker to indicate exposure to oestrogenic compounds as shown in a study done by Hansen *et al*. (1998) where male trout exposed to lowest concentrations of 1 ng/L 17-β-estradiol led to induction of vitellogenin thereby providing evidence of oestrogens being endocrine disruptors. Predicted-no-effect concentrations (PNECs) for steroid oestrogens E1, E2, E3, and EE2 of aquatic organisms in surface water for more than 60 days are 6, 2, 60, and 0.1 ng/L respectively (Caldwell *et al*., 2012).

1.1 Problem statement and study justification

Purdom *et al*. (1994) and Kuster *et al*. (2004) among other researchers have shown wastewater being major contributors to the presence of endocrine disrupting oestrogens in aquatic environments. Specific studies have shown that exposure to oestrogens initiates production of vitellogenin in male fish (Panter *et al*., 1998), feminization of male fish causing intersexuality in wild fish populations (Jobling *et al*., 1998) and in extreme cases infertility in fish leading to reduced reproductive success (Nash *et al*., 2004).

Currently the status of endocrine disrupting oestrogens is unknown in Zambia. However, the situation on the ground in Siavonga points towards risks of introducing endocrine disrupting oestrogens in Lake Kariba.

1.2 Objectives

1.2.1 Main objective

To assess the presence of endocrine disrupting oestrogens from anthropogenic activities in Lake Kariba.

1.2.2 Specific objectives

The specific objectives for this study are;

i. To determine the presence of E1, E2 and EE2 oestrogen contamination in water and sediments in Lake Kariba;

ii. To describe the possible hotspots of contamination by oestrogens in Lake Kariba.

1.3 Research questions

This research was guided by the following questions.

i. What are the types and levels of concentration of these oestrogen contaminants if present?

ii. What are the possible sources of oestrogen contamination in Lake Kariba?

1.4 Hypotheses

$H_{o:}$ There is no significant difference in Estrone contamination in different locations of Lake Kariba

H_1: There is significant difference in oestrogen difference in Estrone contamination in different locations of Lake Kariba

H_o: There is no significant difference in total oestrogen contamination in different locations of Lake Kariba

H_1: There is significant difference in oestrogen difference in total oestrogen contamination in different locations of Lake Kariba

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Characteristics of oestrogens

Natural oestrogens E1, E2 and E3 play a key role in the physiology of humans and animals. Estradiol (E2) is the principal oestrogen found in all mammalian species during the reproductive years and is produced by the ovaries (Duff and DeAvila, 2005). Estriol (E3) is a weak oestrogen, which occur at very low levels in women who are not pregnant. In contrast, E3 is synthesized in very high quantities by the placenta during pregnancy. (Gustavo *et al.*, 2014). Estrone (E1), which also is a weak oestrogen is found in increased amounts in postmenopausal women (Duff and DeAvila, 2005). Synthetic oestrogen 17α-ethinyl estradiol (EE2) is potent oestrogen used in birth control pills (Bhandari *et al.*, 2016) and a derivative of the natural hormone E2 (Laurenson *et al.*, 2014). The molecular structures of the oestogens under discussion are outlined in Fig. 1 below:

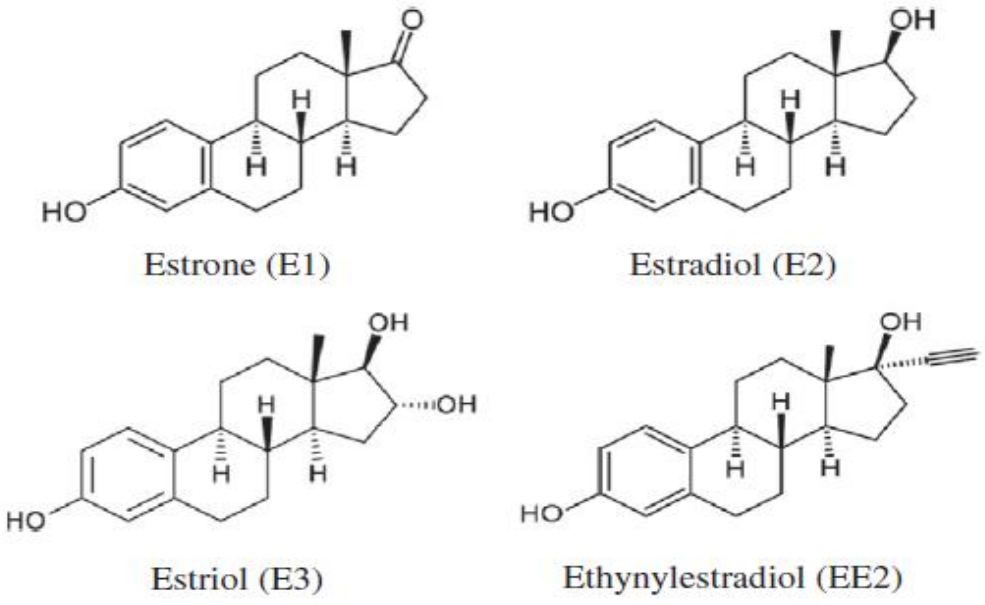

Figure 1: Chemical structures of E1, E2 and EE2

Source: Aris *et al*. (2014)

Corcoran *et al*. (2010) noted EE2 having a much lower water solubility than natural estradiol (E2) and being considerably more persistent in the aquatic environment. The lipophilic property of EE2 facilitates bio-accumulation in fish where an over 10,000-fold up-concentration after short-term exposure (10–21-day) have been reported. Consequently the potency of EE2 as an estrogenic chemical greatly increases the risk of adverse effects in the environment (Singhal *et al*., 2009). In the aquatic environment, these oestrogens exists as a mixture and may potentiate each other.

2.2 Sources of oestrogen contamination

A study of the occurrence of EE2 in the environment by Aris *et al*. (2014) identified major contributors as human urine, wastewater treatment plant effluent, livestock wastewater runoff from manure and sewage sludge. Studies conducted by Leet *et al*. (2012), which assessed the impact of cattle manure on fish populations, found hormone concentrations of <1 ng/L and evidence of reduced reproductive capacity in creek chubs (*Semotilus atromaculatus*). Findings from this study were characterized by fast somatic growth and males having undeveloped testes despite being at a maturity stage. In order to assess the contribution of wastewater to oestrogen contamination, Vadja *et al*. (2008) sampled white suckers (*Catostomus commersonii*) upstream and downstream a waste water treatment plant (WWTP) and showed gonadal intersex, altered sex ratios, reduced gonad size, disrupted ovarian and testicular histopathology as well as vitellogenin induction in white suckers sampled downstream as opposed to white suckers sampled upstream. Chemical analyses revealed a complex mixture of endocrine-active chemicals containing E2 and EE2 among others resulting in an estimated total oestrogen equivalence of up to 31 ng/L E2.

2.3 Effects of oestrogens

Oestrogens released into the aquatic ecosystem interact with hormonal systems of wildlife and humans and cause female-specific responses in males and juvenile organisms (Nekvapil *et al*., 2009). Woodling *et al*. (2006) found an 83% female sex ratio of white suckerfish collected downstream of sewage treatment facilities in greater Denver (USA) compared to 45% upstream. Furthermore, significant higher frequency of gonadal deformities such as intersex and delayed follicular maturation were found in the fish from the downstream location compared to upstream.

Studies on effects of the synthetic oestrogen EE2 on different wild fish species have been shown to induce feminization in fish, including induction of the female yolk precursor VTG in males, formation of a female reproductive duct in the testis and induction of intersex (Corcoran *et al*., 2010). These have been supported by experimental studies conducted by Morthorst *et al*. (2014) that showed that exposure of fertilized wild female eelpout to E2 concentrations ranging from 5.7 to 133 ng/L for 6 weeks in a flow through test system led to development of malformed to motionless larvae. Further studies carried out by Lei *et al*. (2013), exposed embryos of Japanese medaka to E1 of concentrations 5, 50, 500 and 5000 ng/L and observed death of more than 14% embryos in concentrations of 5 and 50 ng/L, which increased to about 28% and 31% deaths of embryos at 500 and 5000 ng/L, respectively.

Brian *et al*. (2007) conducted studies on Fathead Minnow and showed that a combination of oestrogen affect reproductive parameters even when the different oestrogens are present at low and individual concentrations which is considered safe. Exposure to the mixture induced significant reduction in the number of spawnings and egg production, and significantly higher VTG levels in male and females.

2.4 Oestrogen detection and quantification

Several methods have been used to detect and quantify oestrogens in the environment. Complicated but very sensitive and accurate analysis such as high-performance liquid chromatography (HPLC), high-performance liquid chromatography/mass spectroscopy (HPLC/MS), gas chromatography/mass spectroscopy (GC/MS) to mention a few have been used to detect and quantify oestrogens. These analysis are also complicated to perform, requiring expensive instruments and well-trained operators (Dai and Liu, 2017). Simpler, relatively fast and inexpensive immunoassay analysis such as enzyme-linked immunosorbent assay (ELISA) offers an alternative to chromatographic techniques (Singhal *et al*., 2009). ELISA is a plate-based assay technique involving enzyme-labelling of antigens or antibodies, catalysing the formation of a chromogenic product measured at 450nm optical density. ELISA assays are recommended by Pool (2008) for their simplicity, affordability and ability to screen a maximum of 43 samples in duplicate at a time.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study site

Lake Kariba lies between latitudes 16˚28´ and 18˚06´ south and longitudes 26˚40´ and 29°03´east. On the Zambian side, it is fed by seven rivers including the mighty Zambezi River and empties into the Zambezi River after power generation to the rest of the country over the north bank of the dam wall.

Sampling sites were purposively selected to include locations likely to be contaminated (shores of Siavonga town), areas of commercial fish farming, the nearest river feeding into the lake and an Island sparsely populated as a control thereby giving a representation of activities of Lake Kariba on the Zambian side.

Some sites on the shores of Lake Kariba were characterized by daily human activities ranging from swimming/bathing to washing of dishes and clothes. Other sites were discharge points of wastewater from the residential areas into the lake. Lake Kariba is the largest aquaculture site in Zambia mainly with commercial cage farms. The selected location for cage farming as allocated by the Department of Fisheries (DoF) currently contains 95 cages belonging to Yalelo and Lake Harvest fish farms. Yalelo is involved in sex reversal of fingerlings and the waste water is discharged in a canal connected to the lake. Lake Kariba on the Zambian side is fed by seven (7) rivers, Lufua River being the nearest to Siavonga town. The area around the inlet of Lufua River is of a rural set up with sparse human population. The point at which the river meets Lake Kariba is a breeding ground for tilapia fish species hence designated as an aqua-park by the DoF. The grounds are also a home to hippos and crocodiles. Lastly, Sampa Karumba Island, sparsely populated with little to no human activity sits on the boarders of Zambia and Zimbabwe on Lake Kariba.

3.2 Study design

During the study, qualitative data was collected from five (5) groups of key informants using a structured questionnaire (Appendix 1). The questionnaire aimed at establishing anthropogenic activities which could potentially contribute towards oestrogen contamination in the lake Kariba. The key informants included the local Government authority (Siavonga District Council), Southern Water and Sewerage Company Limited (SWSC), active lodges along the shores of the lake, Siavonga District Hospital (SDH), Kariba clinic and users of contraceptives.

A cross-sectional study was conducted to determine the presence and levels of oestrogen concentration in Lake Kariba. Water and sediment samples were collected from shorelines of Siavonga town, around the fish farm cages, an Island and Lufua River the nearest river to Siavonga feeding into Lake Kariba. The sampling stations were marked with a Global Positioning System (GPS) receiver and plotted as indicated in Fig. 2 below. Water parameters were recorded for each sampling point before sample collection (Appendix 2).

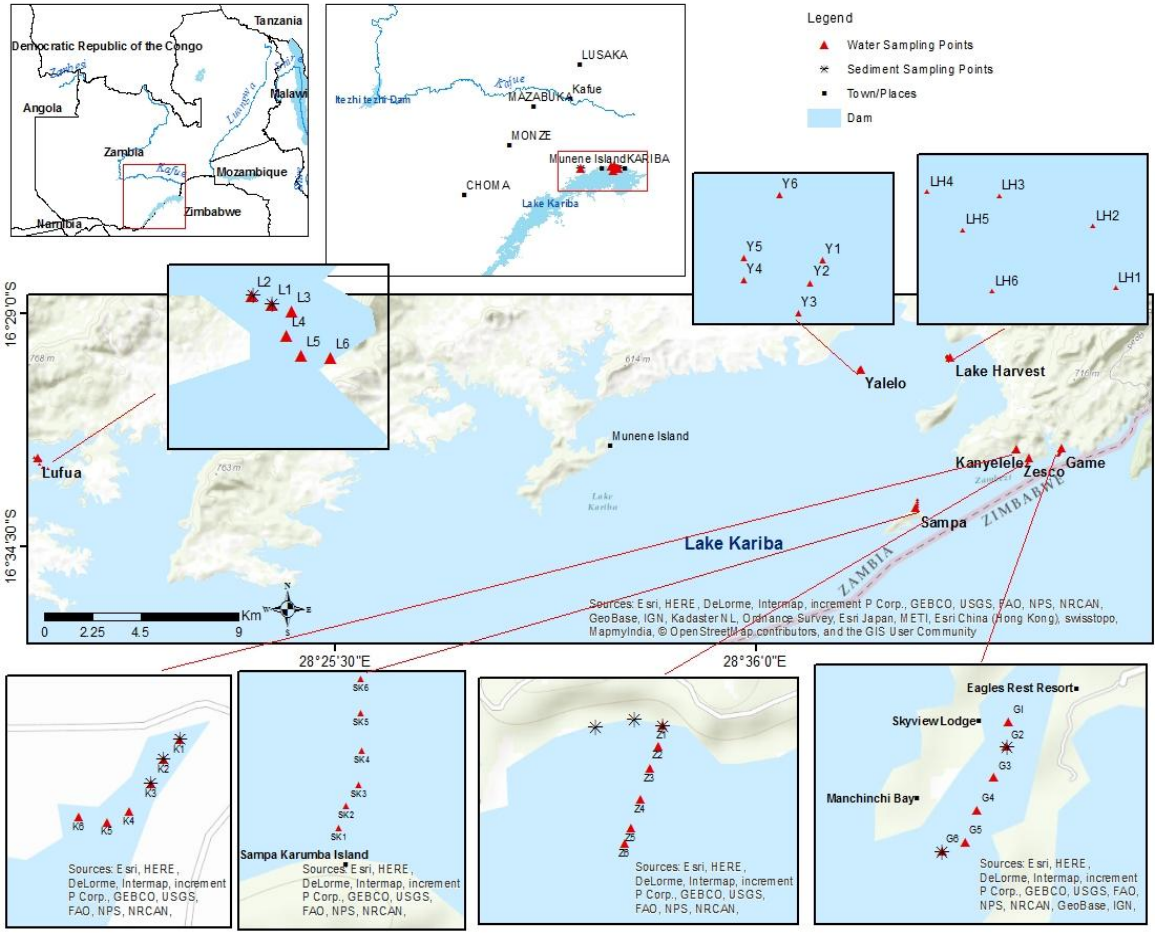

Figure 2: Map of Siavonga displaying sampling points

3.3 Sample size

The sampling stations were seven as indicated above representative of daily activities on Lake Kariba. Water and sediment matrices were identified ideal samples for this study. Sediments (known as sinks) act as environmental reservoirs thereby give an indication of levels of accumulation of contaminants in a water body. Each sampling station had six sampling points giving a total of 42 sampling points. Water samples were collected from all the sampling points whereas sediment samples were only collected at 10 points of the 42 points which were shallow. A total of 52 samples were collected but only 43 samples were analysed for presence of oestrogens. Sample size was determined by the maximum number of samples that can be analysed by an ELISA coated microplate.

3.4 Collection of samples

Samples were collected in five days towards the end of January 2017. The rains had commenced during this period with the temperature ranging between 23.9˚C-28.9˚C.

3.4.1 Collection of water samples

Plastic containers of 1.0L capacity were used for collecting water samples 20cm below the water surface directly and a water sampler was used to collect samples in places with visible raw sewage water and in Lufua River which had aquatic animals. The water sampled was immediately adjusted to pH 3.0 by addition of hydrochloric acid (HCl). The samples were stored in cool-boxes during sampling and frozen at -20°C the end of the sampling day.

3.4.2 Collection of sediment samples

Sediment samples were only collected in shallow waters and stored in plastic bags and placed together with the water samples in cool-boxes. At the end of the sampling, all samples were transported in a cool-box on ice to the laboratory for storage at 4ºC prior analysis. Samples were analysed within 10 days of sampling.

3.5 Extraction of hormones from samples

Extraction of oestrogens took place within three days of samples being transported to the laboratory. Both water and sediment samples were thawed at room temperature before commencing the extraction process.

3.5.1 Extraction of water sample

Hormones were extracted from water samples using Solid Phase Extraction (SPE) with Oasis® hydrophilic lipophilic balance (HLB) cartridges as described by (Hansen *et al*., 2011) with minor modifications as illustrated in Fig. 3.

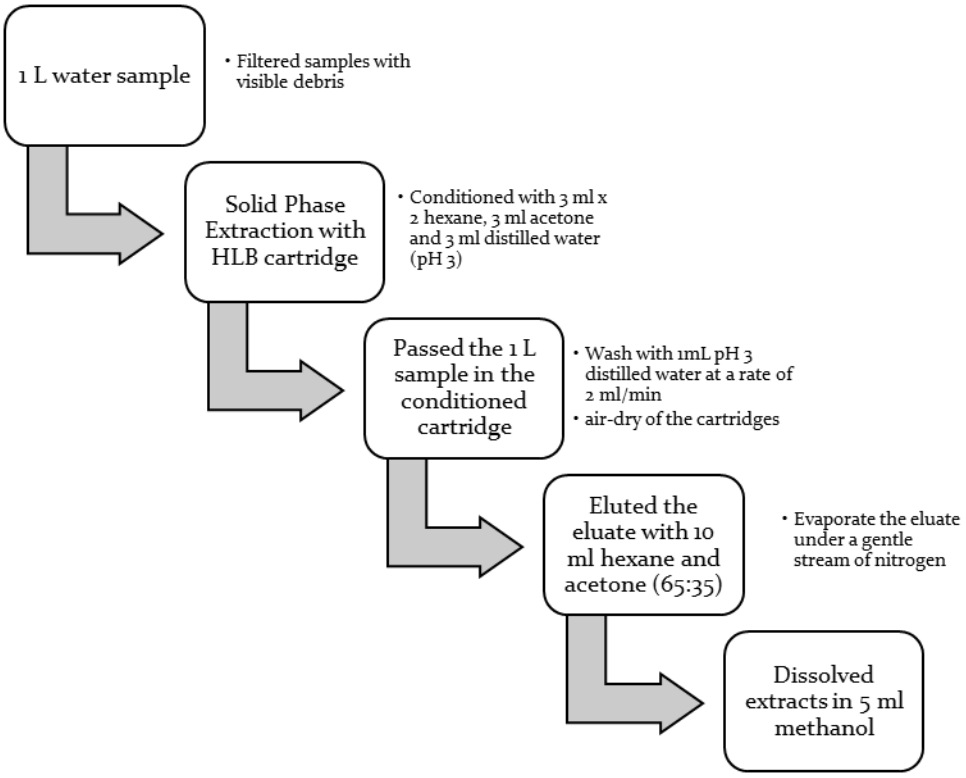

Figure 3: Solid Phase Extraction of water samples

Samples with debris were first filtered using filter papers to remove any visible debris. SPE was achieved using Oasis HLB 500 mg water cartridges. The SPE cartridges were first conditioned with 3 ml hexane twice, 3 ml acetone and finally with 3 ml distilled water with its pH adjusted to 3.0. The sample/filtrate was then passed through the cartridges using a vacuum pump at the rate of 6 ml/min. The cartridges were then washed with 1 ml of pH adjusted distilled water at a rate of 2 ml/min. This was followed by air-drying of the cartridges by allowing the continued vacuum suction until dry. The analyte was then eluted with 10 ml hexane and acetone (65:35) evaporated under a gentle stream of nitrogen and reconstituted with 5 ml methanol. The samples were stored in a freezer at -20˚C prior analysis.

3.5.2 Extraction of sediment samples

Figure 4 summarises the protocol by Chun *et al*. (2005) used to extract hormones from sediment samples.

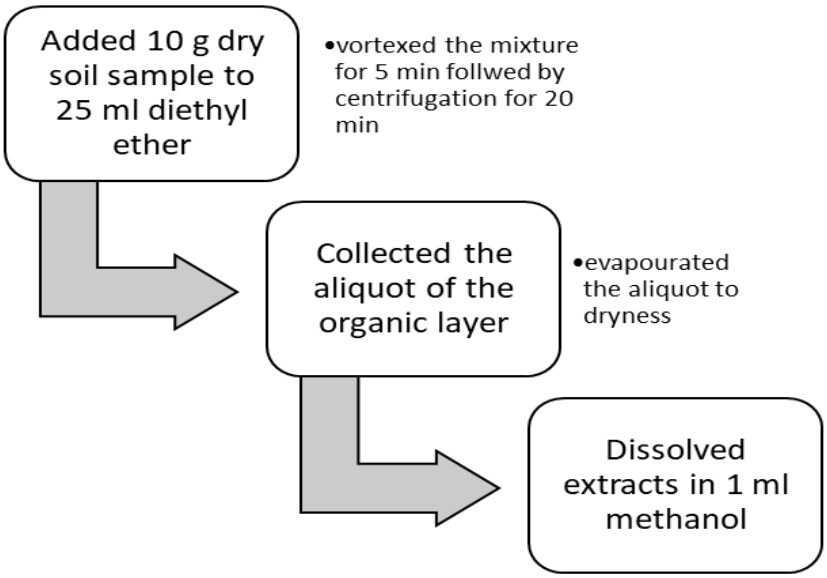

Figure 4: Sediment extraction method

3.6 Sample preparation and analysis

Samples were being analysed for E1, total oestrogen E1/E2/E3 and EE2 using Ecologiena® ELISA kits. The assays quantitative analysis range was between 0.05µg/L and 3µg/L.

3.6.1 Water samples preparation

Out of the total 42 water samples collected, only 31 were selected for analysis. Selection of samples was based on high probability of oestrogen concentration, therefore all samples collected along the shores of Siavonga town qualified for analysis. Samples were tested for pH to ensure they fell within the range of 5.0 to 8.0. From the selected samples, 0.5 ml of each sample solution was added to 4.5 ml distilled water to obtain a sample with a final methanol concentration of 10%. After dilutions, the samples were stored at 4˚C. Dilutions were carried out a day prior to ELISA analysis.

3.6.2 Sediment sample preparation

From the 1 ml final samples obtained during the extraction process illustrated in Fig. 4, 0.5 ml of each of the samples was diluted with 4.5 ml distilled water to obtain a methanol concentration of 10 % as recommended for ELISA analysis. The samples were stored at 4˚C.

3.6.3 Water and sediment ELISA analysis

Preceding analysis, samples and ELISA kits were thawed at room temperature for 30 min (According to Manufacturer's recommendations). The antigen-enzyme conjugate solution was prepared and 100 µl dispensed in all the wells of an uncoated micro plate. 100 µl of the 5 provided standards (wells 1-5), 31 water samples (wells 6-36) and 10 sediment samples (wells 37-46) were added in duplicate to the uncoated micro plate containing conjugate solution, mixed by filling and expelling the micropipette tips 10 times. Of the above mixture, 100 µl was dispensed into corresponding wells on the coated micro-plate which were incubated for 60 min at room temperature. After incubation, the unbound material was dispensed off and the micro-plate washed 3 times with the provided wash solution according to the manufacture's instruction. Colour solution of 100 µl was dispensed into each well of the micro-plate and incubated for 30 min at room temperature. Then 100 µl of stop solution was dispensed into each micro plate well to terminate the reaction.

The absorbance was measured at 450nm using the ELISA reader. This process was repeated for the other oestrogens being tested using respective ELISA kits.

3.7 Data analysis

Appendix 3 lists the results obtained from the analysis of the samples for oestrogen E1, E1/E2/E3 and EE2. To obtain actual concentrations of oestrogens, ELISA Optical Density (OD) results were log transformed using GraphPad Prism 7 and concentrations reported

as ng/L for water and ng/g for sediment samples. The correlation coefficients (r^2) for E1, E1/E2/E3 and EE2 were 0.994, 0.989 and 0.958 achieving almost perfect linearity. The standard curve detection range was 0-3 µg/L for total oestrogens (E1/E2/E3) and EE2 and 0-5 µg/L for E1. Data analysis was done using JMP Pro 13.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Questionnaire findings

4.1.1 Local Government authority and Southern Water and Sewerage Company Limited (SWSC)

Siavonga's population stands at 42,869 based on the Central Statistical Office (CSO) population summary report of 2010 whilst the urban part of Siavonga (Kariba) lies at 16,415 having 3,496 households (CSO, 2012). The Environmental planner of Siavonga elaborated on appropriate sewage disposal methods, which involved the use of honey sucker trucks to dislodge full septic tanks and conduct routine inspections for compliance. However, the Local Government handed over the sewage services to a utility company Southern Water and Sewerage Company Limited (SWSC). SWSC is mandated to provide water and sewerage services on behalf of the Local Authorities who are the shareholders.

Currently Siavonga SWSC has about 1,300 clients with septic tanks, soakaways and pit latrines for the purpose of onsite sanitation and dislodging full septic tanks. In cases of dislodging, a tanker vehicle is hired from Choma and empties either at the Kafue or Mazabuka treatment plants (Fig. 2). The company also conducts monthly testing of water for minerals and bacteria in order to provide clean safe water to the community. The majority of the Siavonga community is marginalised therefore unable to pay the stipulated fees for SWSC services, which may be a reason for observed sewage leakages in the community. The high number of unplanned settlements makes it difficult for SWSC to effectively execute its mandate in sewage disposal.

4.1.2 Lodges along the shores of Lake Kariba

Seven active lodges located on the shores of Lake Kariba were interviewed on methods of sewage disposal of their establishment. The structured interview revealed compliance by presence of septic tanks and soakaways in the respective establishments and using either SWSC or other private companies for dislodgement of full septic tanks. However, two lodges admitted to defaulting by allowing full septic tanks to overflow and old pipes bursting their waste into the Lake.

With the low water levels of the lake Kariba, visual analysis presented a safe location of the soakaways from the lake.

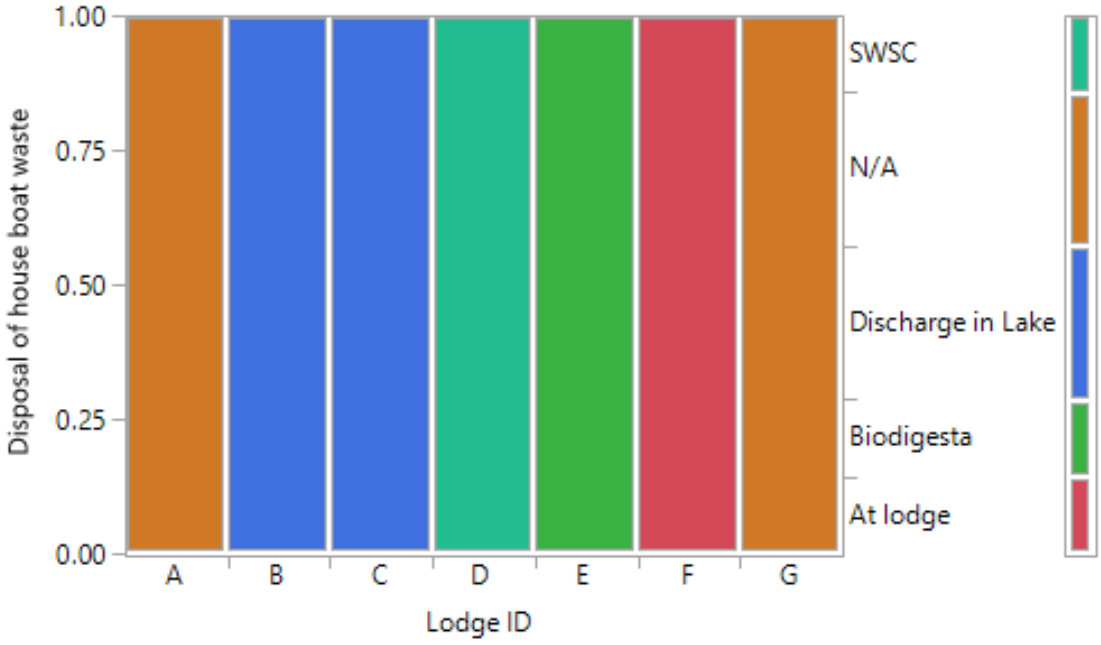

Figure 5: Disposal of houseboat waste by respective lodges

Being a tourist town, boat cruises are so often offered on the lake. Out of the seven interviewed lodges along Lake Kariba, five had houseboats with a seating capacity of approximately 40 people. Of these five boats, two of them dispose-off their raw waste on the lake around Munene Island (Fig. 2) a decent distance away from the lodge.

4.1.3 Siavonga District Hospital and clinic

Contraceptives administered by the two health facilities included oral pills, injections and implants. Of recent, the hospital noticed the demand skewing towards combined oral pill and Medroxyprogestrone injection as shown in Fig. 6. When asked about disposal of expired contraceptives, the clinic indicated returning expired drugs to the hospital where they undergo drug destruction by burning as instructed by the drug destruction protocol. However they further stated how scarce contraceptives expire in contrast to not meeting the demand. In this case, there may be no drug being disposed off into Lake Kariba.

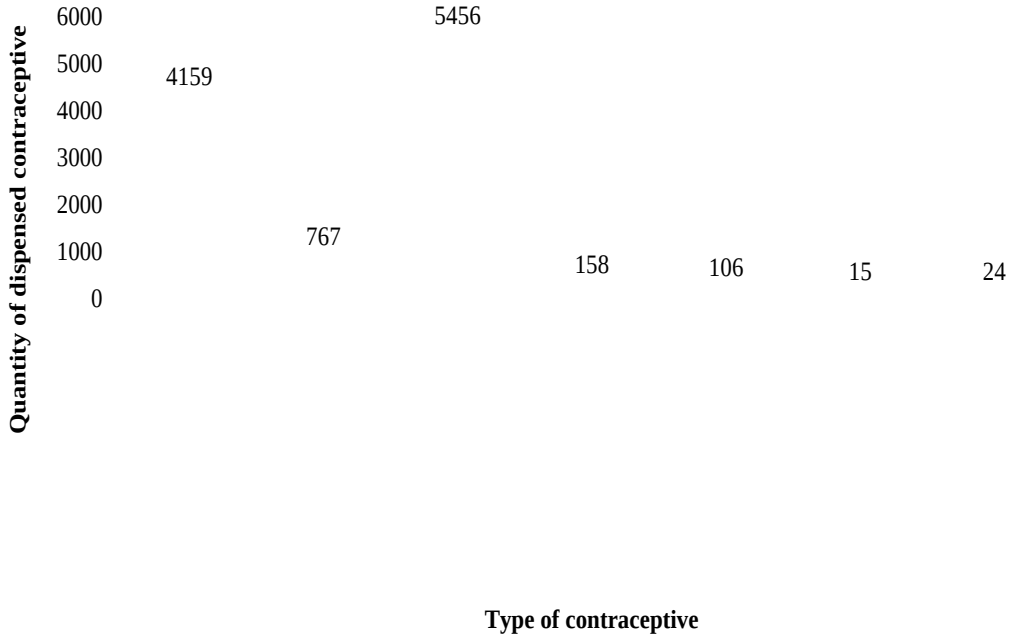

Figure 6: Quantity and types of contraceptives administered in Siavonga district

From the contraceptives administered by the hospital and the clinic, only combined oral contraceptives contain synthetic EE2 as part of an active ingredient. The rest of the contraceptives administered contain progesterone and/or progestin as the active hormone. This point toward low discharge levels of EE2 into the environment in Siavonga.

4.1.4 Users of contraceptives

Since the study targeted users of contraceptives, 72 respondents were interviewed mainly from the family planning clinic at Siavonga District Hospital (SDH). The majority of the respondents were between the ages of 21 to 25 years (Fig. 7). The most preferred form of contraceptive among the respondents was the Medroxyprogestrone injection, which was used by 51.4% (Fig. 6) of the respondents, corresponding with the data from the health facilities in Siavonga.

Figure 7: Contraceptive usage by age groups

Analysis of age groups of contraceptive users showed that 62.5% of the respondents were Kanyelele residents. Kanyelele stream where sediment and water samples were collected is used for various activities from providing drinking water to Kanyelele community, washing of clothes, bathing activity, being a harbour for fish traders, and kapenta rigs. Outflow of waste from leaking pit latrines have been observed to leach into the stream. The docking of the kapenta rigs in the stream has led to contamination of the stream with oils used to propel the rigs, which was evident in some sediment collected. With all these

domestic activities taking place in the stream, it is expected to have higher concentrations of other contaminants in addition to oestrogen hormones.

The survey recorded 81% usage of pit latrines as opposed to flush toilets and other methods. The dominant use of pit latrines confines oestrogens within the pit latrine with an exception of a few that were spotted leaking as a result of not being well constructed. Respondents cited sewage leakages coming from some pit latrines in Kanyelele compound discharging into Kanyelele stream leading into Lake Kariba as well as raw sewage gashing directly into the Lake from ZESCO houses along the lake. In this case, these two sited places qualify to be a potential sources of oestrogen contamination on Lake Kariba.

4.2 Analysis of oestrogen concentrations

Significant amount of total oestrogen E1/E2/E3 were detected in both water and sediment samples. E1 was only detected in water samples whilst synthetic EE2 was not detected in any of the analyzed samples. E1 was detected in 28 of 31 water samples ranging from no detection (ND) to concentrations of 5.63 ng/L (Fig. 8). The highest detection of E1 was recorded in water sample from Lufua River (5.63 ng/L) followed by Game (3.97 ng/L).

Total oestrogen E1/E2/E3 was detected in 27 water (ND to 6.68 ng/L) and 9 (ND to 2.74 ng/g) sediment samples. The highest concentrations of E1/E2/E3 were detected in water sample from Yalelo (6.68 ng/L), Kanyelele (6.44 ng/L) and Lufua River (5.63 ng/L) as shown in Fig. 9.

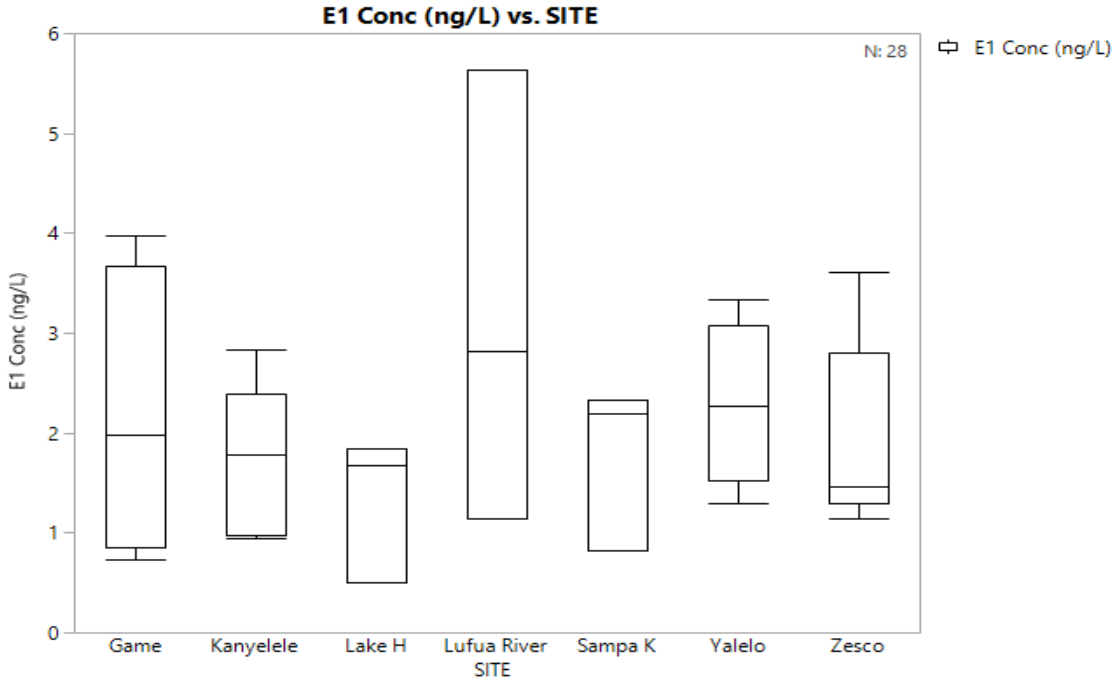

Figure 8: Box plot representation of E1 concentration (ng/L) per site

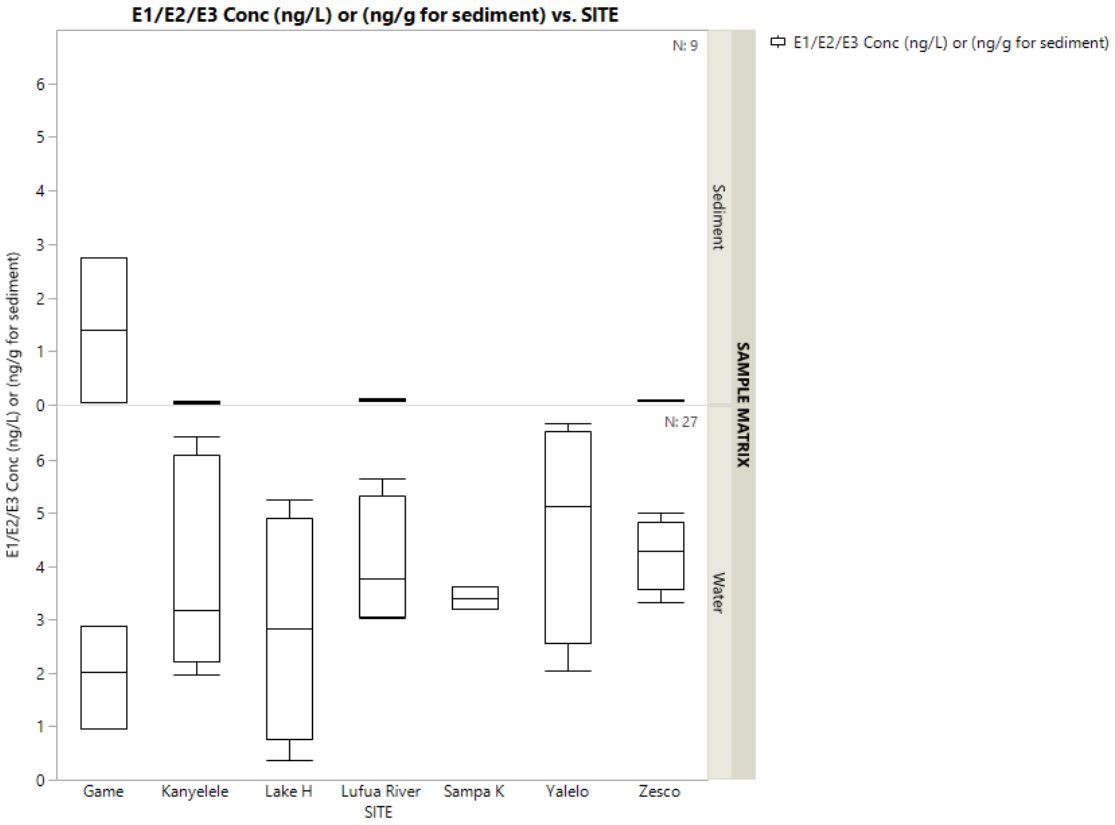

Figure 9: Box plot representation of E1/E2/E3 concentration (ng/L) per site in sediment (upper part) and water (lower part).

The Analysis of Variance (ANOVA) for E1 indicated no significant difference in mean concentration of E1 among all the locations at 5% significant level given the F ratio (0.8110) and probability (p= 0.5731). Similarly, ANOVA for E1/E2/E3 also showed no significant difference in mean concentrations of E1/E2/E3 in the sites given the F ratio (F= 0.8377) and probability level (p = 0.5510) at 5 % significance level of significance.

Table 2 below summarises the mean ± standard error of the recorded concentrations. Total oestrogen E1/E2/E3 concentrations were dominant compared to E1. The highest mean concentrations were recorded from Yalelo (4.74 ng/L) followed by Zesco (4.23 ng/L) and Lufua River (4.05 ng/L). Lake Harvest water samples had the lowest mean concentration of E1 at 1.33 ng/L while Lufua River had the highest at 3.19 ng/L. These results might be contradictory to the assumption that oestrogens in the water originate from human sources, because Lufua River is sparsely populated with no anthropogenic activity. However, the area where samples were collected is populated with hippos and crocodiles (aquatic animals) which may be the sources endogenous oestrogens detected in this area.

Table 2: Summary of average levels of oestrogen detected in water and sediment samples

Site	**Water samples**		**Sediment samples**
	E1 conc. (ng/l)	**E1/E2/E3 conc. (ng/L)**	**E1/E2/E3 conc. (ng/g)**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**
Game	2.17 ± 1.49	1.96 ± 0.96	1.40 ± 1.90
Kanyelele	1.76 ± 0.75	3.84 ± 1.92	0.04 ± 0.03
Lake H	1.34 ± 0.73	2.83 ± 2.14	
Lufua River	3.20 ± 2.27	4.05 ± 1.22	0.09 ± 0.03
Sampa K	1.78 ± 0.83	3.41 ± 0.29	
Yalelo	2.29 ± 0.83	4.75 ± 2.10	
Zesco	1.93 ± 0.99	4.23 ± 0.69	0.08 ± 0.01

Legend: E1- Estrone, E2- 17β-estradiol, E3- Estriol, E1/E2/E3- Total oestrogens, EE2- Ethynyl-estradiol

Absence of EE2 might be as a result of low usage of oestrogen containing contraceptives in Siavonga district (Fig. 6). This firmly supports results of ELISA analysis, which indicated absence of EE2 in Lake Kariba. Considering reported effects of EE2 on aquatic environment, its absence in Lake Kariba is a positive result and should be maintained.

On the other hand, presence of E1 and total oestrogen E1/E2/E3 does point towards contamination of the Lake with natural hormones whose main sources in the aquatic environment may be humans as well as wild animals (Jobling *et al*., 1998). This reinforces responses from respondents of the survey conducted prior to sampling that indicated concern with sewage contamination of Kanyelele stream and discharge of untreated effluent from Zambia Electricity Supply Company (ZESCO) flats flowing into the Lake. This was further supported by observations made on the lake during sampling indicating sewage contamination from the two sites, which were purposively selected for sampling. Oestrogen levels recorded for total oestrogen E1/E2/E3 were relatively higher than those for E1 which is expected since total oestrogen E1/E2/E3 was testing for a combination of the three natural oestrogens E1, E2 and E3.

Sediment samples recorded lower concentrations in comparison to water samples. This maybe an indication that oestrogen contamination is fairly recent on Lake Kariba. It might also be that oestrogen concentrations released into Lake Kariba are very low hence slow accumulation in the lake.

Oestrogen concentrations detected in this study from the water and sediment samples were compared to what has been reported by others worldwide as shown in Table 3. Studies conducted by Cargouët *et al*. (2004) found surface water and wastewater treatment plants (WWTPs) containing natural and synthetic oestrogens in the ranges of 1.0-3.2 ng/L and 2.7–17.6 ng/L, respectively. A review done by Ying *et al*. (2002)

showed that Dutch surface waters contained E1 of 0.3 ng/L and E2 below quantification limit of < 1 ng/L and Tiber river water in Italy having concentration of 0.11 E2 and 1.5 ng/L E1. Studies conducted by Lei *et al.* (2009) in three rivers in Tianjin area, China using a similar protocol as in this study, showed oestrogen concentrations were within the range of 4.29–49.8 ng/L, 2.51–21.2 ng/L and 1.64–24.4 ng/L for E1, E2 and EE2 in water samples and 1.03–21.4 ng/L, 0.71–9.70 ng/L and 0.93–7.67 ng/L for E1, E2 and EE2 in sediment samples. Generally, the concentrations detected in Kariba are much lower than the highest values recorded in other studies cited in Table 3. Estrone concentrations obtained in this study is below the PNEC at 6 ng/L. Total oestrogen E1/E2/E3 (6.68 ng/L) being a combination of three oestrogens, was above PNEC of E1 and E2 (2 ng/L) but lower than E3 PNEC (60 ng/L).

Table 3: Summary of oestrogen levels documented in other studies

Surface Water (ng/L)				
Location	**E1**	**E1/E2/E3 (E2)**	**EE2**	**Reference**
Beitang River, China	4.29–49.8	2.51–21.2	1.64–24.4	Lei *et al.* (2009)
Dutch waters	0.3	< 1	-	Ying *et al.* (2002)
Tiber River, Italy	1.5	0.11	-	Ying *et al.* (2002)
Kuilis and Eerste Rivers, South Africa	<1.1	0.8-4.7	-	Pool (2008)
Tamagara River, Japan	3.4-6.6	0.6-1.0	-	Isobe *et al.* (2003)
Lake Kasumigaura, Japan	0.2-0.8	ND	-	Isobe *et al.* (2003)
Seine and Oise Rivers, France	1.1-3.0	1.4-3.2	1.1-2.9	Cargouët *et al.* (2004)
Ngerengere River, Tanzania	2.58-18.84	0.03-9.3	0.07-0.84	Msigala *et al.* (2017)
Sediment (ng/g)				
Location	**E1**	**E1/E2/E3 (E2)**	**EE2**	**Reference**
Beitang River, China	1.03–21.4	0.71–9.70	0.93–7.67	Lei *et al.* (2009)
Tokyo Bay, Japan	0.05-3.60	ND-0.59	-	Isobe *et al.* (2006)

Legend: E1- Estrone, E2- 17β-estradiol, E3- Estriol, E1/E2/E3- Total oestrogens, EE2- Ethynyl-estradiol

Effects of oestrogen contaminants however small in quantity as shown by Hansen *et al.* (1998) mixed with other oestrogen compounds in environment may cause detrimental effects on fish. Estrogenic compounds do interact with each other and in combination they may have addictive or synergistic effects on biological systems (Thorpe *et al.*, 2003). Studies by Hansen *et al.* (1998) and Msigala *et al.* (2017) further demonstrate that even

the smallest concentration of oestrogen 1 ng/L and 5 ng/L respectively, have an effect on the aquatic environment by inducing vitellogenin synthesis. With the levels of oestrogen concentrations recorded in this study, it is therefore expected that fish in Kariba undergoes vitellogenin synthesis. However these levels recorded are too low to cause deleterious endocrine disruptions effect in other forms of life. However deleterious endocrine disruptions to other forms of aquatic life been reported in bullfrog tadpoles exposed to EE2 levels of 10 ng/L (Adeel *et al*., 2016).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Siavonga has a harsh terrain for farming as well as animal rearing therefore the locals shun agricultural activity. The findings of the study showed little to no evidence of oestrogen contamination by livestock and agricultural activity. Based on observations, it may be concluded that the main anthropogenic activity that could be a source for oestrogen contamination of Lake Kariba is sewage discharge and to a lesser extent existence of aquatic animals.

Natural oestrogens E1 were detected only in water samples. E1 concentrations were lower compared to total oestrogens E1/E2/E3 concentrations which were detected in both water and sediment samples. Generally concentrations recorded in Lake Kariba are lower than what has been reported in other studies. Sediment samples recorded much lower levels of total oestrogens E1/E2/E3 in comparison to water samples. The extraction methods used were able to detect presence of hormones at 10,000 enrichment, therefore they qualify to be adopted as standard methods for future oestrogen studies.

5.2 Recommendations

Further intensive research is needed to identify other sources of oestrogens not identified in this research. Since this is the first of this kind of study in Zambian natural waters, similar studies to be conducted in all the other major water bodies as a baseline study to oestrogen studies in Zambia are recommended. With the already dwindling fish stock in Lake Kariba, the DoF needs to revise its policy to address ways of reducing oestrogen contamination in collaboration with other relevant institutions. Laws can also be enacted to restrain spread and accumulation of these oestrogens to levels causing deleterious

endocrine disrupting effects on organisms. Communities residing along natural water bodies need to be sensitised on effects of oestrogen contamination on the aquatic environment and ways of reducing contamination.

REFERENCES

Adeel, M., Xiaoming, S., Yuanyuan, W., Dennis, F. and Yuesuo, Y. (2016). Environmental impact of estrogens on human, animal and plant life: A critical review. *Environment International* 99: 107–119.

Allen, Y., Matthiessen, P., Scott, A. P., Haworth, S., Feist, S. and Thain, J. E. (1999). "The Extent of Oestrogenic Contamination in the UK Estuarine and Marine Environments. *The Science of the Total Environment* 233: 5-20.

Aris, A. Z., Shamsuddin, A. S. and Praveena, S. M. (2014). Occurrence of 17α-ethynylestradiol (EE2) in the environment and effect on exposed biota: A review. *Environment International* 69: 104–119.

Beveridge, M. C. M., Thilsted, S. H., Phillips, M. J., Metian, M., Troell, M. and Hall, S. J. (2013). Meeting the food and nutrition needs of the poor: The role of fish and the opportunities and challenges emerging from the rise of aquaculturea. *Journal of Fish Biology* 83(4): 1067–1084.

Bhandari, R.K., Deem, S., Kassotis, C. and Nagel, S.C. (2015). Effects of the environmental estrogenic contaminants bisphenol A and 17α-ethinyl estradiol on sexual development and adult behaviors in aquatic wildlife species. *General and Comparative Endocrinology* 214: 195–219.

Brian, J. V., Harris, C. A., Scholze, M., Kortenkamp, A., Booy, P., Lamoree, M., Pojana, G., Jonkers, N., Marcomini, A. and Sumpter, J. P. (2007). Evidence of Estrogenic Mixture Effects on the Reproductive Performance of Fish. *Environmental Science and Technology* 41(1): 337–344.

Caldwell, D. J., Mastrocco, F., Anderson, P. D., Länge, R. and Sumpter, J. P. (2012). Predicted-no-effect concentrations for the steroid estrogens estrone Predicted-no-effect concentrations for the steroid estrogens estrone, 17β-estradiol, estriol, and 17α-ethinylestradiol. *Environmental Toxicology and Chemistry* Journal 31 (6): 1396–1406.

Cargouët, M., Perdiz, D., Mouatassim-Souali, A., Tamisier-Karolak, S. and Levi, Y. (2004). Assessment of river contamination by estrogenic compounds in Paris area (France). *Science of the Total Environment* 324: 55–66.

Central Statistical Office (2012). 2010 Census of population and housing population summary report; Population summary report. Lusaka, Zambia. 154pp.

Chun, S., Lee, J., Geyer, R. and White, D. C. (2005). Comparison of three extraction methods for 17β-estradiol in sand, bentonite, and organic-rich silt loam. *Journal of Environmental Science and Health* 40 (5): 731–740.

Corcoran, J., Winter, M. J. and Tyler, C. R. (2010). Pharmaceuticals in the aquatic environment: A critical review of the evidence for health effects in fish. *Critical reviews in toxicology* 40(4): 287–304.

Dai, Y. and Liu, C. (2017). Detection of 17 β-Estradiol in Environmental Samples and for Health Care Using a Single-Use, Cost-Effective Biosensor Based on Differential Pulse Voltammetry (DPV). *Biosensors* 7(15): 1-13.

Department of Fisheries (2016) 2015 Fisheries Statistics Annual Report, Lusaka, Zambia. 36pp.

Duff, B. and DeAvila, M. (2005). Presence/Absence of estrogen in the Racoon River Watershed and Des Moines water works treatment process, Des Moines aterworkslaboratory. [http://www.dmww.com/upl/documents/water-quality/lab-reports/lab-studies/presence-of-estrogen-in-the-raccoon-river-watershed.pdf]. Site visited on July 2016.

Gustavo, G., Alberto, F., Eugenia, A., Anabella, F., Martin, T. S. and Aloranti, C. V. (2014). Comprehensive assessment of estrogenic contamination of surface waters of the river basin suquia. *European Scientific Journal* 3: 1857–7881.

Hansen, M., Jacobsen, N. W., Nielsen, F. K., Björklund, E., Styrishave, B. and Halling-Sørensen, B. (2011). Determination of steroid hormones in blood by GC–MS/MS. *Analytical and Bioanalytical Chemistry* 400(10): 3409–3417.

Hansen, P. D., Dizer, H., Hock, B., Marx, A., Sherry, J., McMaster, M. and Blaise, C. (1998). Vitellogenin – a biomarker for endocrine disruptors. *TrAC Trends in Analytical Chemistry* 17(7): 448–451.

Ikenaka, Y., Nakayama, S. M. M., Muzandu, K., Choongo, K., Teraoka, H., Mizuno, N. and Ishizuka, M. (2010). Heavy metal contamination of soil and sediment in Zambia. *African Journal of Environmental Science and Technology* 4(11): 729-739.

Isobe, T., Serizawa, S., Horiguchi, T., Shibata, Y., Managaki, S., Takada, H., Morita, M. and Shiraishi, H. (2006). Horizontal distribution of steroid estrogens in surface sediments in Tokyo Bay. Environmental Pollution 144(2): 632–638.

Isobe, T., Shiraishi, H., Yasuda, M., Shinoda, A., Suzuki, H. and Morita, M. (2003). Determination of estrogens and their conjugates in water using solid-phase extraction followed by liquid chromatography-tandem mass spectrometry. *Journal of Chromatography A*. 984: 195–202.

Jobling, S., Nolan, M., Tyler, C. R., Brighty, G. and Sumpter, J. P. (1998). Widespread sexual disruption in wild fish. *Environmental Science and Technology* 32(17): 2498-2506.

Kolding, J., Jacobsen, N. S., Andersen, K. H. and van Zwieten, P. A. M. (2014). Maximizing fisheries yields while maintaining community structure. *Canadian Journal of Fisheries and Aquatic Sciences* 73(4): 644-655.

Kuster, M., Alda, M. J. L. and Barcelo, D. (2004). Analysis and distribution of estrogens and progestogens in sewage sludge, soils and sediments. *Trends in Analytical Chemistry* 23: 790-798.

Laurenson, J. P. Bloom, R. A., Page, S. and Sadrieh, N. (2014). Ethinyl Estradiol and Other Human Pharmaceutical Estrogens in the Aquatic Environment : A Review of Recent Risk Assessment Data. *American Association of Pharmaceutical Scientists Journal* 16(2): 299-310.

Leet, J. K., Lee, L. S., Gall, H. E., Goforth, R. R., Sassman, S., Gordon, D. A., Lazorchak, M. J., Smith, E. M., Javfert. T. C and Sepúlveda , M. S. (2012). Assessing Impacts of Land-Applied Manure from Concentrated Animal Feeding Operations on Fish Populations and Communities. *Environmental science and technology* 46: 13440−13447.

Lei, B., Huang, S., Zhou, Y., Wang, D. and Wang, Z. (2009). Levels of six estrogens in water and sediment from three rivers in Tianjin area , China. *Chemosphere* 76(1): 36–42.

Lei, B., Wen, Y., Wang, X., Zha, J., Li, W., Wang, Z., Suna, Y., Jia Kanga, K. and Wang, Y. (2013). Effects of estrone on the early life stages and expression of vitellogenin and estrogen receptor genes of Japanese medaka (Oryzias latipes). *Chemosphere* 93(6): 1104–1110.

Li, Y. and Migliaccio, K. (2010). Water Quality Concepts, Sampling, and Analyses. CRC Press, London, 344pp.

Manickum, T. and John, W. (2014). Fate and environmental risk assessment of endocrine disrupting compounds at the wastewater treatment works in Pietermaritzburg (South Africa). *Science of the Total Environment* 468: 584–597.

Morthorst, J. E., Brande-lavridsen, N., Korsgaard, B. and Bjerregaard, P. (2014.) 17 β - Estradiol causes abnormal development in embryos of the viviparous eelpout. *Environmental Science Technology* 48: 14668−14676.

Msigala, S. C., Mabiki, F. P., Styrishave, B. and Mdegela, R. H. (2017). Performance of wastewater stabilization ponds in treatment of endocrine disrupting estrogens in treatment of endocrine disrupting estrogens in Morogoro urban and peri-urban, Tanzania. *International Journal of Public Health and Epidemiology* 6(1): 305–317.

Nash, J. P., Kime, D. E., Van der Ven, L. T. M., Wester, P. W., Brion, F., Maack, G., Stahlschmidt-Allner, P. and Tyler, R. C. (2004). Long-term exposure to environmental concentrations of the pharmaceutical ethynylestradiol causes reproductive failure in fish. *Environmental Health Perspectives* 112(17): 1724-1733.

Nekvapil, T., Borkovcová, I., Smutná, M. and Svobodová, Z. (2009). Estrogenic Profile of the Svratka and Svitava Rivers in the Brno Area. *Acta Veterinaria Brno* 78(2): 313–317.

Panter, G.H., Thompson, R.S. and Sumpter, J.P. (1998). Adverse reproductive effects in male fathead minnows (*Pimephales promelas*) exposed to environmentally relevant concentrations of the natural oestrogens, oestradiol and oestrone. *Aquatic Toxicology* 42: 243–253.

Pinto, P.I.S., Estêvão, M.D. and Power, D.M. (2014). Effects of estrogens and estrogenic disrupting compounds on fish mineralized tissues. *Marine Drugs Journal*, 12(8): 4474–4494.

Pool, E. J. (2008). The estrogenicity of sewage effluent entering the Eerste-Kuils River catchment system, Water Research Commission Report No 1590/1/08, Western Cape, South Africa. 43pp.

Purdom, C. E., Hardiman, P. A., Bye, V. V. J., Eno, N. C., Tyler, C. R. and Sumpter, J. P. (1994). Estrogenic Effects of Effluents from Sewage Treatment Works. *Chemistry and Ecology* 8(4): 275–285.

Sethuraman, A., Kiros, S. and Tomass, Z. (2013). Residues of organochlorine pesticides in fishes from the Mumbai west coast of India. *Internation Journal of Pure and Applied Zoology* 1(1): 109–116.

Sim, W. J., Lee, J. W., Shin, S. K., Song, K. B. and Oh, J. E. (2011). Assessment of fates of estrogens in wastewater and sludge from various types of wastewater treatment plants. *Chemosphere* 82: 1448–1453.

Singhal, N., Song, Y., Johnson, A. and Swift, S. (2009). Estrogenic Endocrine Disrupting Compounds; Review and Analytical Procedures for GC-MS and ELISA Analyses, Auckland Regional Council, Technical Report No.005. 75pp.

Thorpe K. L., Cummings R. I., Hutchinson T. H., Scholze M., Brighty G., Sumpter J. P. and Tyler C. R. (2003). Relative potencies and combination effects of steroidal estrogens in fish. *Environmental Science and Technology 37(6)*: 1142-1149.

Tumbare, M. J. (2008). Managing Lake Kariba sustainably : Threats and challenges Managing Lake Kariba sustainably : threats and challenges Management of Environmental Quality*: An International Journal* 19 (6): 731-739.

Vadja, A. M., Barber, L. B., Gray, J. L., Lopez, E. M., Woodling, J. D. and Norris, D. O. (2008). Reproductive Disruption in Fish Downstream from an Estrogenic Wastewater Effluent. *Environmental Science and Technology* 42(9): 3407–3414.

World Bank (2010). The World Bank, The Zambezi River Basin; A Multi-Sector Investment Opportunities Analysis, The World Bank Water Resources Management Africa Region, Washington, 148pp.

Woodling, J. D., Lopez, E. M., Maldonado, T. A., Norris, D. O. and Vajda, A. M. (2006). Intersex and other reproductive disruption of fish in wastewater effluent dominated Colorado streams. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology* 144(1): 10–15.

Ying, G.G., Kookana, R.S. and Ru, Y.J. (2002). Occurrence and fate of hormone steroids in the environment. *Environment International* 28: 545–551.

APPENDIX

Appendix 1: Structured interviews with five (5) groups of key stakeholders in Siavonga

The Local Government (council)/ Southern Water and Sewerage Company Limited (SWSC):

1. What is the population of Siavonga town;

   Male_____ female_____

2. How many households are in Siavonga town

   _____

3. How many households are connected to the council sewage system?

   _____

4. Those not connected to council system, how do they dispose their sewage?

   _____

5. How many lodges are in Siavonga?

   _____

6. How many of these are located along the shores of the lake?

   _____

   _____

7. How many of these lodges are connected to the council sewage system?

   _____

   _____

8. Those not connected how do they dispose off their waste?

   _____

   _____

9. How is council sewage disposed off? (Take note of treatment given to sewage before disposal)?

   _____

   _____

10. What are the appropriate methods of sewage disposal? Are there monitoring measures by council to ensure appropriate disposal of sewage?

    _____

    _____

11. Location of the sewage treatment plant (observation to determine proximity to the lake)

    _____
    _____

12. What agricultural industries (livestock, vegetables) are present in Siavonga?

    _____
    _____

13. What challenges do you face in ensuring proper disposal of sewage? What intervention measures have you put in place to ensure proper disposal of sewage?

    _____
    _____

The commercial farmers:

1. How long have you been in the business?

   _____

2. To date, how many cages, ponds and hatcheries do you have?

   Cages _____ Ponds_____ Hatcheries_____

3. What is the source of your water (What kind of treatment does it undergo if any)?

   _____
   _____

4. What treatment do you administer to minimize disease vulnerability of the fish and at what stage(s)?

   _____
   _____

5. Do you practice sex reversal or use hormones to enhance growth of fish?

   _____
   _____

6. Do you feed your fish with any growth hormones? If yes which ones?

   _____
   _____

7. How and where is the waste water from the farm disposed off (See site)?

   _____
   _____

8. Do you have any concerns with pollution on the Lake?

   _____
   _____

9. If yes in 8, ask on possible sources of pollution and proposed intervention measures

   _____
   _____

The lodges around the lake:

1. How long has your establishment been in existence
   _____
   _____
2. What is the lodging capacity?
   _____
   _____
3. Which gender (M/F) comprises the higher percentage of clientele?
   _____
   _____
4. Do you have private sewage or connected to council?
   _____
   _____
5. Any treatment if yes what kind of treatment is given to the sewage?
   _____
   _____
6. How and where is the sewage disposed off?
   _____
   _____
7. Do you (as a lodge) have any concerns with pollution on Lake Kariba fishery?
   _____
   _____
8. If yes, what are the possible sources of pollution and proposed intervention measures?
   _____
   _____

Hospital/ clinics:

1. What kind/types of contraceptives do you administer?
   _____
   _____
2. Which are the most preferred contraceptives?
   _____
   _____
3. How much contraceptives do you administered on a monthly basis?
   _____
   _____
4. What are the ages of women receiving these contraceptives?
   _____
   _____

5. How and where do you dispose off expired contraceptives? (See disposal site)
   _____
   _____

Users of contraceptives (Women/girls):

1. How old are you?
   _____
   _____
2. Where do you live?
   _____
   _____
3. What is your occupation?
   _____
   _____
4. What contraceptive do you use?
   _____
   _____
5. How long have you been using this contraceptive?
   _____
   _____
6. What type of toilet do you have at home?
   _____
   _____

Appendix 2: Raw data on water parameters measured in different locations

Sampling site	**Sampa K**					
Site identity	Latitude	Longitude	Temp	pH	DO mg/l	Conductivity
SK1	16˚33'31.0"	028˚40'01.0"	28.4˚C	7.75	7.7	84.7
SK2	16˚33'30.2"	028˚40'00.0"	28.4˚C	7.74	7.9	85.7
SK3	16˚33'28.1"	028˚40'00.8"	28.4˚C	7.81	9.1	85.9
SK4	16˚33'26.5"	028˚40'00.7"	28.5˚C	7.79	8.7	85.9
SK5	16˚33'23.8"	028˚40'00.9"	28.6˚C	7.74	7.6	85.9
SK6	16˚33'20.3"	028˚40'00.8"	28.6˚C	7.83	6.9	85.9
Sampling Site	**Game Stream**					
Site identity	Latitude	Longitude	Temp	pH	DO mg/l	Conductivity
G1	16˚32'10.9"	028˚43'34.8"	29.1	7.88	4.9	92
G2	16˚32'12.6"	028˚43'33.8"	28.9˚C	7.94	7.4	86.7
G3**	16˚32'14.6"	028˚43'33.4"	28.9˚C	7.96	9.2	86.4
G4	16˚32'16.6"	028˚43'32.3"	28.8˚C	7.8	8.7	86.3
G5	16˚32'18.0"	028˚43'29.6"	28.7˚C	7.94	5.8	86.2
G6**	16˚32'17.9"	028˚43'26.7"	28.7˚C	8	9.3	86.1
Sampling Site	**Kanyelele Stream**					
Site identity	Latitude	Longitude	Temp	pH	DO mg/l	Conductivity
K1**	16˚32'08.8"	028˚42'27.9"	28.7˚C	7.5	4.4	7.02
K2**	16˚32'09.3"	028˚42'27.5"	28.9˚C	7.6	6.3	6.87
K3**	16˚32'10.2"	028˚42'26.9"	28.7˚C	7.81	7.3	106.5
K4	16˚32'10.8"	028˚42'26.3"	28.6˚C	7.63	7.5	90.2
K5	16˚32'11.4"	028˚42'25.8"	28.6˚C	7.73	8.3	90.2
K6	16˚32'11.7"	028˚42'24.7"	28.6˚C	7.8	7	90.7
Sampling Site	**Zesco Community**					
Site identity	Latitude	Longitude	Temp	pH	DO mg/l	Conductivity
Z1**	16˚32'20.3"	028˚42'48.4"	28.7˚C	7.8	5.7	86.3
Z2	16˚32'21.6"	028˚42'48.1"	28.8˚C	8	6.5	86.5
Z3	16˚32'24.5"	028˚42'48.2"	28.7˚C	7.9	10.1	86.1
Z4	16˚32'27.6"	028˚42'47.4"	28.5˚C	7.8	7.5	86
Z5	16˚32'31.7"	028˚42'45.8"	28.6˚C	8	11.9	86
Z6	16˚32'36.3"	028˚42'43.3"	28.6˚C	8	9.5	86
Sampling Site	**Lufua River**					
Site identity	Latitude	Longitude	Temp	pH	DO mg/l	Conductivity

L1**	16˚32'21.3"	028˚18'08.8"	23.9˚C	7.27	8.1	109.5
L2**	16˚32'20.1"	028˚18'05.7"	24.1˚C	7.33	6.3	109.8
L3	16˚32'21.9"	028˚18'13.1"	27.8˚C	7.07	5	104
L4	16˚32'25.9"	028˚18'18.4"	27.9˚C	7.11	4.6	101.9
L5	16˚32'27.8"	028˚18'21.4"	27.9˚C	7.14	4.6	99.4
L6	16˚32'27.7"	028˚18'25.2"	27.9˚C	7.16	5.1	99.3
Sampling Site	**Lake Harvest**					
Site identity	Latitude	Longitude	Temp	pH	DO mg/l	Conductivity
LH1	16˚30'01.3"	028˚40'50.6"	27.2˚C	7.47	5.5	85.3
LH2	16˚29'59.5"	028˚40'49.9"	27.2˚C	7.32	5.5	85.3
LH3	16˚29'58.6"	028˚40'47.0"	27.2˚C	7.36	8.5	85.3
LH4	16˚29'58.5"	028˚40'44.8"	27.2˚C	7.19	5.6	85.1
LH5	16˚29'59.6"	028˚40'45.9"	27.2˚C	7.19	5.6	85.1
LH6	16˚30'01.4"	028˚40'46.8"	27.3˚C	7.32	6.4	85.1
Sampling Site	**Yalelo**					
Site identity	Latitude	Longitude	Temp	pH	DO mg/l	Conductivity
Y1	16˚30'18.5"	028˚38'37.2"	27.2˚C	7.39	10.5	84.8
Y2	16˚30'19.4"	028˚38'36.7"	27.4˚C	7.44	8.2	84.9
Y3	16˚30'20.6"	028˚38'36.2"	27.4˚C	7.46	6.7	81.8
Y4	16˚30'19.3"	028˚38'33.9"	27.4˚C	7.37	6.3	84.9
Y5	16˚30'18.4"	028˚38'33.9"	27.4˚C	7.36	5.8	84.9
Y6	16˚30'15.9"	028˚38'35.4"	27.4˚C	7.42	5.1	85

Appendix 3: Absorbance and oestrogen concentrations of ELISA analysed samples

SAMPLE MATRIX	SITE	ELISA #	Ave Absorbance, 450nm			Conc (ng/L) or (ng/g for sediment)		
			E1	E1/E2/E3	EE2	E1	E1/E2/E3	EE2
Water	Game	1	1.33	1.21	1.31	1.19	2.02	ND
Water	Game	2	1.13	1.25	1.32	3.98	0.96	ND
Water	Game	3	1.21	1.17	1.35	2.76	2.89	ND
Water	Game	4	1.37	1.45	1.30	0.74	ND	ND
Water	Game	5	1.41	1.29	1.26	ND	ND	ND
Water	Kanyelele	6	1.25	1.00	1.28	2.25	5.97	ND
Water	Kanyelele	7	1.35	0.97	1.38	0.98	6.44	ND
Water	Kanyelele	8	1.35	1.13	1.32	0.94	3.61	ND
Water	Kanyelele	9	1.26	1.20	1.40	2.09	2.31	ND
Water	Kanyelele	10	1.21	1.22	1.71	2.83	1.96	ND
Water	Kanyelele	11	1.30	1.18	1.38	1.49	2.76	ND
Water	Lake H	12	1.28	1.26	1.24	1.84	0.38	ND
Water	Lake H	13	1.29	1.12	1.41	1.67	3.80	ND
Water	Lake H	14	1.39	1.22	1.64	0.50	1.87	ND
Water	Lake H	15	1.42	1.04	1.98	ND	5.26	ND
Water	Lufua River	16	1.43	1.17	1.49	ND	3.02	ND
Water	Lufua River	17	1.33	1.16	1.43	1.14	3.16	ND
Water	Lufua River	18	1.21	1.09	1.33	2.82	4.39	ND
Water	Lufua River	19	1.04	1.02	1.36	5.63	5.63	ND
Water	Sampa K	20	1.25	1.27	1.63	2.19	ND	ND
Water	Sampa K	21	1.24	1.13	1.41	2.33	3.62	ND
Water	Sampa K	22	1.36	1.16	1.46	0.83	3.21	ND
Water	Yalelo	23	1.25	0.96	1.61	2.24	6.69	ND
Water	Yalelo	24	1.32	1.10	1.51	1.29	4.14	ND
Water	Yalelo	25	1.24	1.21	1.34	2.31	2.05	ND
Water	Yalelo	26	1.17	0.99	1.40	3.33	6.11	ND
Water	Zesco	27	1.15	1.05	1.35	3.62	5.01	ND
Water	Zesco	28	1.26	1.28	1.26	2.00	ND	ND
Water	Zesco	29	1.33	1.10	1.22	1.14	4.25	ND
Water	Zesco	30	1.31	1.15	1.21	1.46	3.34	ND
Water	Zesco	31	1.31	1.09	1.41	1.45	4.33	ND
Sediment	Game	32	1.60	1.18	1.32	ND	2.75	ND
Sediment	Game	33	1.53	1.17	1.35	ND	0.06	ND
Sediment	Lufua River	34	1.48	1.01	1.40	ND	0.11	ND
Sediment	Lufua River	35	1.44	1.15	1.38	ND	0.07	ND
Sediment	Kanyelele	36	1.45	1.42	1.45	ND	ND	ND
Sediment	Kanyelele	37	1.55	1.26	1.26	ND	0.02	ND
Sediment	Kanyelele	38	1.55	1.16	1.35	ND	0.06	ND
Sediment	Zesco	39	1.54	1.13	1.32	ND	0.07	ND
Sediment	Zesco	40	1.56	1.09	1.30	ND	0.09	ND
Sediment	Zesco	41	1.56	1.13	1.56	ND	0.07	ND