USE OF FISH BIOMARKERS TO ASSESS MICRO- POLLUTANT EXPOSURE AND EFFECTS IN LAKE KANYABOLI, KENYA

BY

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DECLARATION

STUDENT'S DECLARATION

"I declare that this thesis hereby submitted to the University of Eldoret, for the degree of Master of Science in Environmental Studies (Environmental Biology) has not been submitted by me for a degree at this or any other University; that it is my work in design and execution, and that all materials contained herein has been duly acknowledged"

Signature……..Date…….

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SUPERVISORS' APPROVAL

This thesis has been submitted for examination with our approval as university supervisors'

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DEDICATION

To my wife Phelisters and my daughters, Whitney, Ashley and Alecsis

ABSTRACT

A lot of research in ecotoxicology is currently focused on identifying and developing suitable biomarkers for use in assessing environmental pollution. This study, set out to evaluate the use of a suite of biomarkers in *Clarias gariepinus* in assessing micro-pollutant exposure and effects in Lake Kanyaboli from November 2013 to April 2014. Fish were collected with the aid of a gill net of mesh size 4" and 5" whereas bottom sediment was collected by ErkMan crab sampler. The concentrations of Cadmium, Chromium, Zinc, Copper, and Lead in lake sediment and in the liver of *Clarias gariepinus* were determined using Flame Atomic Absorption Spectrophotometry (AAS). A necropsy-based health assessment index (HAI), the condition factor, hepatosomatic index (HSI), and splenosomatic index (SSI) were measured in fish from all the sampling stations. Fish were semi-quantitatively sampled from which an organ index was calculated to examine the micro- pollutant exposure and effects in fish from all the study stations. Metals concentrations were significantly ($F_{\alpha, v1, v2} = 7.16$; $p < 0.05$) higher in station 2 than at the other stations. The mean heavy metal concentration in *Clarias gariepinus* liver were Cd (nd-0.22±0.01), Cr (0.37 ± 0.01 - 1.67 ± 0.03), Zn (7.12 ± 0.03 - 13.40 ± 0.32), Cu (2.12 ± 0.12 - 4.16 ± 0.12) and Pb (1.63 ± 0.33 - 14.40 ± 0.58). Zn exhibited a significance difference between the stations ($F_{\alpha, v1, v2} = 7.79$; $p < 0.05$). Redundancy ordination analysis (RDA) revealed positive linear relation between heavy metals in sediments and fish liver except for Zinc concentration in sediments and fish liver which showed a negative relationship. The mean of the biomarkers were HAI (20.61 ± 1.01 - 47.43 ± 1.12), Condition factor (0.60 ± 0.03 - 0.91 ± 0.29), HSI (0.53 ± 0.03 - 0.67 ± 0.03), SSI (0.08 ± 0.00 - 0.12 ± 0.01) and Semi-quantitative histological assessment index (8.3 ± 0.41 - 17.63 ± 0.36). The multivariate analysis of the histology – based fish health assessment index (HBFHAI) showed that these biomarkers are being influenced by the concentration of heavy metals in sediments as well as fish liver and therefore qualified as biomarkers for assessing pollutant exposure and effects. It is concluded that histology-based fish health assessment index biomarkers can be used to assess the health of aquatic organisms and ecological health of Lake Kanyaboli and other aquatic habitats within the Lake Victoria Basin. However, a more comprehensive chemical analysis of water and sediments samples including the measurements of endocrine disrupting chemicals is recommended for future studies to further investigate possible causative agents regarding liver alterations identified.

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ABBREVIATIONSAND ACRONY

ANOVA	Analysis of Variance
BINU	Biological Indicators for National Use
CD	Circulatory Disturbances
CES	Critical Effect Size
CF	Condition Factor
DCCA	De trended Canonical Correspondence Analysis
DDT	Dichlorodiphenyltrichloroethane
DNA	Deoxyribonucleic Acid
Gok	Government of Kenya
HAI	Health Assessment Index
HPA	Health Protection Agency
HSI	Hepatosomatic Index
I	Inflammation
IMS	Industrial Methylated Spirit
IQ	Intelligent Quotient
KWS	Kenya Wildlife Service
LVBC	Lake Victoria Basin Commission
MDS	Multidimensional Scaling
OSHA	Occupational Safety and Health Administration
PAH	Polycyclic Aromatic Hydrocarbon
PC	Progressive Changes
PCB	Polychlorinated biphenyls
RC	Regressive Changes
RDA	Redundancy Analysis

RES	Reticulo- Endothelial System
RP	Reaction Patterns
SPSS	Statistical Program for Social Scientist
SQHI	Semi-Quantitative Histological Index
SSI	Splenosomatic Index
T	Tumour
TL	Total Length
UN	United Nations
UNEP	United Nations Environment Program
USEPA	Unites States Environment Protection Agency
WHO	World Health Organization

DEFINITION OF TERMS

Aneurysm: Circumscribed dilation of arterial blood vessels

Biological assessment: The use of biomonitoring data of samples of living organisms to evaluate the condition or health of a place (e.g., a stream, wetland or a lake)

Biological monitoring: Sampling the biota of a place (e.g., a stream, lake or a wetland)

Exophthalmus: Protrusion of the eyeball due to fluid, gas, tissue reaction

Histology: Science dealing with the structural forms of normal tissue with the aid of light microscopy

Histopathology: Science dealing with abnormal manifestation of diseased tissues

Quantitative histological assessment: Refers to cell/tissue identification, description and the identification of changes within a cell structure

Semi-quantitative histological assessment: Refers to the quantification, measurement of identified tissue/organ damage and the degree of pathological importance of alterations

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CHAPTER ONE

INTRODUCTION

1.1 Background Information

As a result of intensification of agriculture, rapid industrialization and urbanization, increasing quantities of man-produced pollutants have been discharged into the environment. When these pollutants enter water bodies they can have direct or indirect impacts on the biota of aquatic systems. They often interfere with the normal functioning of an organisms and its ability to live in harmony with the environment (Adams *et al.*, 1990). The changes they cause in behavior, growth, and reproduction of an organism will eventually result in undesirable effects at higher biological organization levels. Therefore, there is a great need for sensitive and reliable methods to assess the impacts of pollution to the aquatic environment.

Pollution of aquatic ecosystems has primarily been monitored by periodic sampling of water and comparison of its physical and chemical characteristics (USEPA, 2005; Groom *et al*., 2006). However, there are limitations with this approach that reduces its usefulness for environmental management. The most important limitation is that results relates to the time when sampling was done, yet there may be great variability in the pollutant level due to the discontinuity of pollutant discharge. Chemical analysis provides little information on the impact of compounds that are easily metabolized and excreted by organisms (Wernersson, 2012). There may also be a large range of pollutants within a lake depending on the environment through which the water drains making it impossible to measure all of these to define their impacts on the lacustrine health. Chemical analysis

are unable to quantify bioavailability of the chemicals to the biological receptor and do not provide any indication of deleterious effects of contaminants on the biota (Norin *et al.,* 2013). The chemical methods are also gauged as "difficult to perform" and sometimes require extensive sample clean up (USEPA, 1986) and are relatively expensive (Norin *et al.,* 2013).

Most of the available data on harmful effects of pollutants on organisms have been obtained from mortality-based acute standard toxicity tests in the laboratory (Kaiser, 2001). Although they provide important information on how organisms respond to environmental stressors, ecotoxicological tests mainly inform about acute and not sub-lethal or chronic effects (Hela *et al.,* 2005). Polluted sites in nature generally consist of a mixture of pollutants, most Eco toxicological studies focus on exposure and effects of single compounds (Yang, 1994). Another limitation of laboratory tests relates to the bioavailability of toxicants: unlike the concentration of toxicants of controlled toxicity tests, physico-chemical properties such as water flow and pH of natural ecosystems may mediate bioavailability (De Zwart, 2005). In general, physico-chemical processes (e.g. ionization, dissolution, precipitation, complexation and partitioning) reduce the concentration of toxicants that is actually experienced by the biota. These processes depend on individual properties of the toxicants and on the abiotic characteristic of the ecosystems (De Zwart, 2005). Therefore, although laboratory studies provide invaluable preliminary information on the effects of environmental stressors, further studies in natural habitat are needed to increase ecological realism.

1.2 Biomarkers

The development of biomarkers in the late 1980s provides enormous possibilities for using biological responses to assess environmental exposure and effects. The most common usage of the term biomarker has been for biochemical, cellular, physiological or histological indicators of either exposure to or the effects of xenobiotic chemicals at the sub-organismal or organismal level (Bernet *et al*., 1999; Hanson *et al*., 2006; Nikalje *et al*., 2012).

Effects of pollutants are usually expressed first at molecular/biochemical level. Changes at these levels can induce structural and functional changes at a higher level, such as hormonal regulation, immune system and metabolism in an organism. These changes may finally impair the growth, reproduction and survival ability of the organism (Adams *et al*., 1990). A variety of changes observable at molecular, biochemical, cellular, or physiological levels in individuals have been studied as biomarkers for investigating the present or past exposure of the individual to pollutants (Hanson and Larson, 2011).

The use of biomarkers in environmental monitoring has several advantages (Hanson and Stark, 2012). Biomarkers at molecular and biochemical level respond quickly to changes in environment. The rapid response can offer early warning signals of environmental deterioration and potential effects of toxicants at sites. Because of these properties, the use of biomarkers strengthens monitoring and assessment of the extent and nature of environmental degradation. Measurements of biomarkers provide scientific evidence for a link between toxicants exposure and relevant biological effects at an individual, a population or at community level. Because changes in biomarkers are often characteristics of exposure to a particular type of pollutant(s), biomarkers help to

establish a cause-and-effect relationship between environmental exposure and effects. Biomarkers can indicate the exposure of organisms to toxic chemicals that do not bioaccumulate or are rapidly metabolized and eliminated, such as polycyclic aromatic hydrocarbons (PAHs). The changes in biomarkers are the integrated consequences of exposure to the parent compounds as well as their metabolites. The biomarkers also reflect the integrated effects of exposure to complex mixture of contaminants and other environmental factors such as water temperature, water velocity, sediment, oxygen, and food availability. They present the cumulative effects of these factors on the target organisms.

Biomarkers of aquatic pollution can be subdivided into three classes (Van der Oost *et al*., 2003). Biomarkers of exposure, covering the detection and measurement of an exogenous substance or its metabolite or the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured in a compartment within an organism. Biomarkers of exposure can be used to confirm and assess the exposure of individuals or population to a particular substance (group), providing a link between external exposure and internal dosimetry. Biomarker of effect, including measurable biochemical, physiological or other alteration within tissues or body fluids of organisms that can be recognized as associated with an established or possible health impairment or disease. Biomarkers of effect can be used to document either preclinical alteration or adverse health effect due to the external exposure and absorption of a chemical. Biomarker of susceptibility, indicating the inherent or acquired ability of an organism to respond to the challenge of exposure to a specific xenobiotic substance, including genetic factor and changes in receptors which alter the susceptibility of an organism to the exposure.

Biomarkers of susceptibility help to elucidate variation in the degree of response to toxicant exposure observed between different individuals. The bioaccumulation of certain persistent environmental contaminants in animal tissue may be considered to be a biomarker of exposure to those chemicals (WHO, 1993).

Due to the complexity of natural systems, single parameters do not appropriately reflect the effects of multiple stressors on the integrity of aquatic systems. An adequate set of endpoints is required to determine the biological significance of stress and the underlying cause or mechanistic basis of observed effects (Madanire-Mwoyo *et al.*, 2012b). Environmental monitoring programs should therefore, include a variety of chemical, physical and biological indicators, with each being used in their respective roles as environmental stressors(xenobiotics), exposure response (biomarkers) and effect response (bioindicators) (Xenopoulus and Lodge, 2006). Bioindicators have the advantage that they show a long term response (chronic) to intermittent pollution, they respond to all toxicants they are exposed to, and biological assessment are more rapid and comparatively less expensive than chemical analyses (Van der Oost *et al*., 2003).

Great effort has been devoted towards the selection of appropriate biomonitor and/or indicator organisms (Salanki *et al.,* 2003). It is reported that organisms vary widely in their sensitivity to different pollutants, and that no single species or monitoring systems is the most sensitive or suitable for the detection of all possible toxic pollutant (Forbes and Forbes, 1994). At organismal level, fish are widely used as sentinel species for biological evaluation to quantify ecological changes that result from the combination of physical, chemical and biological stressors (Oberdoff and Hughes, 1992) because they have some

particular features and advantages as indicators of freshwater ecosystem health (Streit, 2008).

Despite some limitation related to mobility, fish are considered to be most useful organisms for biomonitoring environmental pollution (Van der Oost *et al.*, 2003) because they are located at the top of the food chain, are highly visible resources, and are known to accumulate toxicants (Streit, 2008). In addition, they are in direct contact with pollutants in the water via their gills and their body surface. Fish are excellent indicators of aquatic health because they live in water all their life, differ in their tolerance to amount and type of pollution, are easy to collect with the right equipment, live for several years, are easy to identify in the field, represent a broad spectrum of community tolerance from very sensitive to highly tolerant and respond to chemical, physical and biological degradation in characteristic response pattern (USEPA, 2004).

According to Harris, (1995) fish possess three main attributes which make them useful for environmental programs: a) fish are sensitive to most forms of human disturbances; b) fish are useful for monitoring at all levels of biological organization and c) fish monitoring programs have a favourable cost- benefit ratio. Because of the aforementioned features of fish, several approaches have been used over the past years to evaluate the effect of stress on the health of fish population (Adams *et al*., 1993). The use of biomarkers, to provide biological responses to assess environmental exposure and effects for fish population in the field, is one such approach. Biomarkers can be sensitive indicators of sub-cellular stress in organisms exposed over short and longer periods to a range of pollutants (Adams *et al.,* 2000). Several international studies (Valavanidis *et al*., 2006; Sole *et al*., 2006; Richardson *et al*., 2010) have shown that biomarkers are useful

tool in the monitoring of aquatic biotic “health” and therefore give an indirect measure of possible environmental pollution or degradation.

In an environmental context, biomarkers potentially offer a more sensitive, rapid, cost-effective option for surveillance monitoring, as indicated by results of a small number of trials which has taken place in recent years (Langston *et al*., 2014). Applied carefully, such assays may be particularly useful “early warning” systems to inform management decision in situation where there is insufficient time to wait for response at the community level to filter through before action is taken. Equally, they can be used to measure change in the system, including improvements resulting from remedial action, over relatively short time- scale. (Langston *et al.,* 2014).

In Kenya, the application of biomarker as a monitoring and assessment “tool” is in its infancy compared to America and Europe and as such there is scarcity of reliable long term biological effect data relevant to Kenya’s tropical climate as compared to the temperate climates. It is therefore important to develop a biomarker to assess the level of micro-pollutants in the environment as well as monitor their effects on aquatic biota and thus help to control pollution of aquatic ecosystems. Scientifically this can be determined by assessing biological effect of environmental pollution using a suite of biomarkers in wild fish at several areas impacted by different anthropogenic activities. Fish are known to have the ability to concentrate chemicals, and different pollutants in their muscle, gills and different organs such as liver and kidney (Mahino *et al*., 2014).

The use of biomarkers may provide a more precautionary and effective monitoring of environmental quality impacts, with a clear link to management measures. Thus, as well

as using these techniques to assess bioavailability and toxic effects of particular pollutant groups at individual sites, there is a great deal of interest within Kenya as to their potential role in providing a risk-screening approach across sites, and also in surveillance monitoring, to assess the overall stress imposed on communities through exposure to toxicants. Output from such monitoring and assessment could trigger further investigations at high risk sites thus helping to direct management responses.

The assessment of the environmental quality as well as the health of aquatic biota using biomarkers is extremely important, not only for understanding of contamination effect on the ecological equilibrium of the area, but also for the social and economic implication *vis- a- vis* the local communities that depend on the natural resources, thus the focus of this study.

In Lake Victoria Basin, Kenya, efforts to develop biomonitoring tools for aquatic resources have yielded promising results. Macro-invertebrate based indices of biotic integrity have been developed for a number of rivers and streams in the upper reaches (Masese *et al*., 2009a; Raburu *et al*., 2009a; Aura *et al*., 2010) and in lower reaches (Kobingi *et al*., 2009). Studies on entire riverine ecosystem include Raburu (2003) and Raburu *et al*. (2009) based on macro invertebrates and Raburu and Masese (2012) based on fish. Omukoto (2007) developed a fish-based index of biotic integrity (IBI) for the satellites lakes. This study takes a slightly different approach and focuses on biomarkers and not community assemblages.

1.3 Statement of the Problem

Yala swamp, where the Lake Kanyaboli is located, has been affected by human development such as reclamation since 1960s leading to approximately 2,300 ha of the swamp being drained (Gok, 1987). Currently, approximately 4,600 ha of the swamp has been reclaimed and is undergoing agricultural development to produce cotton, rice and horticultural products (Omukoto, 2007). The rice farming and other agricultural sectors are agrochemicals intensive industries, the use of agrochemicals in the catchment can lead to their introduction into Lake Kanyaboli directly, as drift during application, deposition and as runoff during rainy season (Mwamburi, 2009). The consequences of this could be pollution of Lake Kanyaboli with effluents from these agro-industrial activities (Ochieng, 2011), thus threatens the integrity of Lake Kanyaboli ecosystem health and community (Adoga, 2011). Any deterioration in environmental health of Lake Kanyaboli may lead to the consequent decline in aquatic biodiversity.

The use of agrochemicals poses a great challenge to the country to develop appropriate bioassessment techniques, cheap and suitable pollution indicators to support pollution abatements methods and policies and ensure optimal agricultural productivity, quality water to the Lake and for community use while improving the integrity of the already threatened biodiversity in the lake. Currently, information on agrochemical residues in Lake Kanyaboli and their effect on the aquatic organisms are fragmentary and inadequate. In addition, in Kenyan context, little is known about biomarker response of our endemic fish species in field assessment and biomonitoring of micro-pollutants. There is therefore need to generate data on major pollutants in the lake and there effect on

aquatic fauna for proper management of the lakes water quality, conservation of biodiversity and the sustainability of the Lake Victoria Basin ecosystems.

Knowledge of the type of species occurring in the lake and how they are affected by changes in the environment is important in understanding of the fate of the organism in the aquatic environment therefore, assessing changes in aquatic organism using a suit of biomarkers may provide indication of changes in environmental quality in lentic systems. Thus the knowledge of biomarker response will reflect the nature and quality of the aquatic environment.

1.4 Justification.

Lake Kanyaboli and the adjoining giant Yala Swamp is home and a living museum to many fish species and wildlife. This fact is supported by the reality that prior to the introduction of Nile perch in Lake Victoria for export market, the lake was dominated by over 300 fish species and currently, Lake Victoria only support three fisheries (Chemoiwa *et al*., 2013). Some of the endemic fish species that have been virtually lost from Lake Victoria still thrive in Lake Kanyaboli. Thus the conservation of the remaining endemic species is of utmost importance (Owiyo *et al*., 2014). To conserve the remaining population, it is important not only to investigate their species composition, but also study their response to human induced factors such as pollutant load.

Although some research has been done in Lake Kanyaboli, including speciation studies on some heavy metals in selected sites around the lake, including the Yala river mouth (Ochieng *et al.,* 2009) and analysis of Mn, Co, Cr, Cu, Ni, Pb and Zn to determine the background geochemical contribution (Lalah *et al.,* 2008), none has directly correlated heavy metals in sediment and fish, Further, no studies have addressed the potential effect

of the recent agro-industrial activities along the lake with respect to these heavy metals. Thus no reproducible fish health indices are available for monitoring health of aquatic organisms in Lake Kanyaboli. The histology-based fish health assessment indices developed for this lake will provide useful tool for ecological and environmental risk assessment of other satellite lakes within the region; for cost effective biomonitoring and assessment of the anthropogenic activities degrading structural and functional integrity and therefore, the health of water bodies within the Lake Victoria Basin, for rapid assessment, classification of environmental quality, policy formulation and implementation.

To evaluate the adverse effects of pollutant in the aquatic environment, it is imperative to use organisms that can respond differently to varying pollution levels. In this regard, species richness measures or abundance measure of some key species of interest such as fish and macro invertebrates have been used (Elias *et al*., 2014). However, this approach hardly conveys the interaction of cause and effect in the big picture of the Lake's degradation (Wepener *et al*., 2011). The value and effectiveness of using Kenya's endemic fish species as an assessment and monitoring tool to reflect health status of an aquatic system as well as aquatic biota, by analyzing a suit of biomarkers is relatively uninvestigated. Thus, in Kenyan context, little is known about biomarker response of our endemic fish species in field assessment and biomonitoring of micro-pollutants.

This work set out to close the knowledge gap that exists on biological effects of chemical pollution on the aquatic organism through biomarker studies. Measurements in benthic fish, in particular African catfish (*Clarias gariepinus*) of biological responses (biomarkers) related to exposure, and effect of pollutants and measurement of

concentration of contaminants in sediment of Lake Kanyaboli is a useful approach that links the bioavailability of pollutants and intrinsic toxicity in target organs (Van der Oost *et al.*, 2003).

1.5 Overall Objective of the Study

The overall objective of this study was to evaluate the use of a suite of biomarkers in wild African catfish (*Clarias gariepinus)* in assessing pollutant exposure and effects in Lake Kanyaboli.

1.5.1 Specific Objectives

1. To determine levels of selected heavy metals in sediments and liver of *Clarias gariepinus* in Lake Kanyaboli.
2. To establish the health status of *Clarias gariepinus* in Lake Kanyaboli using fish Health Assessment Index (HAI).
3. To determine the biometric indices (CF, HSI and SSI) of *Clarias gariepinus* in Lake Kanyaboli.
4. To determine histological alterations in the liver of *Clarias gariepinus* in Lake Kanyaboli using histological index.

1.6 Null hypotheses

1. The levels of the selected heavy metals in sediments and liver of *Clarias gariepinus* do not differ significantly among the sites in Lake Kanyaboli.
2. Pollutant load has no significant effect on the health of *Clarias gariepinus* in lake Kanyaboli

3. Pollutant load has no significant effect on the biometric indices of *Clarias gariepinus* in Lake Kanyaboli.
4. There is no significant histological difference in the *Clarias gariepinus* in Lake Kanyaboli.

1.7 The scope and limitation of the study

The study set out to generate data and information necessary for assessing fish health and thus by extension the ecological condition of Lake Kanyaboli. Due to financial constraints, only selected heavy metals were analyzed, other chemical pollutants were not analyzed and therefore no one knows their concentration and the magnitude of the problem they may cause in the entire aquatic environment. The study also targeted *C. gariepinus* to evaluate any histological alteration in the liver of fish only as a result of chemical pollution.

CHAPTER TWO

LITERATURE REVIEW

2.1.1 Sediment quality as an indicator of aquatic pollution

The study of sediments quality by evaluating the concentration of pollutants is necessary as it helps assess the potential toxicity of the system (Chakravarty and Patgiri, 2009). Numerous studies have been undertaken to investigate environmental health, by studying the concentration of pollutants in the sediment (Niewoudt *et al*., 2009; Varol, 2011). Pollutants in aquatic ecosystems generally exist in low levels in water (Őztürk *et al.*, 2009) and may accumulate in the sediment (Praveena *et al.,* 2008; Őztürk *et al* 2009). Sediments have a long residence time in the aquatic systems; therefore they are ideal for the assessment of pollutant levels (Saha *et al*., 2001; Oyoo-Okoth *et al*., 2010; Varol, 2011). Sediments are important source of organic and inorganic pollutants, due to their variable physical and chemical properties (Praveena *et al*., 2008). They play a functional role in the mobilization of contaminants in the aquatic systems under favourable conditions (Őztürk *et al*., 2009). In lacustrine communities, the population is directly and indirectly exposed to sediment and so the pollutants, and is at risk of contamination (Miller *et al*., 2004).

2.1.2 Bioaccumulation and Effects of heavy metal on fish

Fish provide high quality animal protein, vitamins, minerals and omega-3 fatty acids which have been associated with health benefits due to their cardio-protective effect on human beings (Gamal and Shamery, 2010; Nzeve *et al*., 2014). Despite these benefits, there are health risks related to fish consumption, due to potential adverse effects of

heavy metal contamination. According to Jarup (2003), heavy metals are well known environmental pollutants that cause serious health hazards to human beings. Anthropogenic activities continuously increase the amount of heavy metals in the environment, especially in the aquatic ecosystem. As the metal levels increase in aquatic ecosystems, they raise the concern of metal bioaccumulation through the food chain and related human hazards (Pheiffer *et al*., 2014).

It has been found that cadmium, copper, chromium, mercury, nickel and zinc accumulate in the heart, muscle, liver, gills and kidney of fish (Farombi *et al*., 2007; Van Dyk *et al*., 2007). Pollutants often show their effects on the histological level (Roberts, 1989; Hinton and Lauren, 1990). Histological changes have been seen in fish tissues exposed to heavy metals; for example the liver of *Oreochromis mossambicus* exposed to Cd and Zn showed hyaline droplet degeneration, congested blood vessels, cellular swelling and increased vacuolation associated with lipid accumulation (Van Dyk *et al*., 2007). Gills are often damaged or changed with exposure to toxicants. The number of cells in the primary lamellae of the gills increased (hypertrophy) in mosquito fish (*Gambusia holbrooki*) that had been exposed to inorganic mercury (Jagoe *et al*., 1996). Secondary lamellae aneurisms (telangiecstasia) and hypertrophy of the epithelial cells have been found in tiger fish (*Hydrocynus vittatus*) exposed to Cu (ATSDR, 2012).

2.2 Fish Health Assessment Index (HAI)

Several approaches have been used over the past years to evaluate the stress on the health of fish populations. However, most of them are constrained by both the time required for analysis and cost and cannot be applied to field studies. As an alternative, an empirical necropsy-based system of organ and tissue indices to provide a fish health and condition

assessment for fish population has been developed as described (Goede 1988; Goede and Barton 1990). Adams *et al*. (1993) improved on this method by developing a quantitative Health Assessment Index (HAI) to minimize the limitation of the necropsy-based system.

According to Schmitt *et al*. (2004), the Health Assessment Index (HAI) is a systematic method to identify external and internal lesions or abnormalities of each fish during a field necropsy. The HAI comprises the evaluation of the external condition of fish (any aberration of the skin, fins opercula and eyes) as well as internal organs and assigning values based on the degree of severity or damage observed. Abnormal conditions can assume values of 10, 20 or 30 depending on the severity of the condition, while 0 represent normal condition (Adams *et al*., 1993).

According to Adams *et al,* (1993) the HAI has been successfully tested in the United States of America in the pulp polluted Tennessee River basin (North Carolina, Tennessee, Alabama, Kentucky), the Hartwell Reservoir (Georgia, South Carolina) contaminated with polychlorinated Diphenyls, and in the Pigeon River (Tennessee, North Carolina) that receives effluents from a bleached Kraft mill. Lohner *et al.* (2001) used the HAI to assess the possible effects of exposure to elevated selenium levels of Sunfish population. Kovacs *et al*. (2002) however compared the HAI with other Community-based approaches to assess the biological status of fish in a river receiving pulp paper mill effluent in Canada. Schmitt *et al*. (2004; 2005) determined the effect of selected environmental contaminants on fish in Rio Grande Basin using the following fish species, *Cyprinus carpio, Micropterus salmoides*, *Micropterus dolomieui*, and *Ictalurus furcatus*. Hinck *et al.* (2007) also used the same index on three fish species to determine spatial

trends in accumulative contaminants, health indicators and reproductive biomarkers in fish from Colorado River.

While HAI is admittedly an effective biomonitoring tool, but it has limitations which include subjectivity and repeatability according to Pheiffer *et al*. (2014). Individual researchers may interpret observations differently and this may influence the end results of the HAI. Moreover, huge leaps between values, for example 10 and 20 may be misconstrued for a large physiological difference of disease (Modanire-Moyo *et al.*, 2012b). Another drawback of this method is that infection can occur in fish with no manifestation of disease symptoms or the infection may be difficult to identify because some stages of disease are invisible in many cases (Luus-Powell *et al.,* 2005). Sometimes, the clinical stage with the accompanying symptoms is too short to be observed because of the rapid mortality following the incubation stage, or environmental stressors may be sufficiently severe that fish die before observable changes in the structure or appearance appear. Moreover, there can be microscopic or histological structural changes without gross manifestation. Many of the limitation described above are however out-weighed by the widespread advantages of using HAI as a biomarker of fish health as well as environmental health (Watson *et al*., 2012). The HAI is a simple and inexpensive means of rapidly assessing the general health status in field situations.

While the development and incorporation of fish Health Assessment Index (HAI) into regular monitoring of general fish health as well as environmental health in other parts of the world is advancing, most African countries (except South Africa) are lagging behind. In South Africa, the concept has been tested and adapted for local condition through studies on the Olifants River system (Avenant-Oldewage *et al*. 1995). Crafford and

Avenant-Oldewage (2001) used the HAI to assess the health of Vaal River systems using C.*gariepinus* while Jooste *et al*. (2005b) compared the HAI of *C.gariepinus* and *O. mossambicus* as indicator species in lower Ga-selati River (Limpopo Province).

2.3 Fish Biometric indices as biomarkers

Fish biometric indices have been used extensively in fish health and population assessment as a first level screen to determine possible pollutant exposure. Biometric indices generally express organ weight as a percentage of total body weight. These indices reflect the status of organ systems, which may change in size due to environmental factors and stressors.

Biometric indices are useful indicators of general organ and fish health; however those indices should be interpreted with caution. These parameters are not sensitive or specific, and may be affected by non-pollutant factors (Van der Oost *et al*., 2003). Organo-somatic indices serve as an initial screening biomarker to indicate exposure and effects (Mayer *et al*., 1992). Condition factor (CF), hepato-somatic index (HSI) and Spleno-somatic index (SSI) biometrics were used in this study.

2.3.1 Condition factor (CF)

Various types of the condition factor have been calculated, and most of them were derived from the constant in the length-weight formula with n= 3 i.e. $K = Weight/Length^3$ (Le Cren, 1951). A variety of factors have been found to introduce changes in the condition factor, such as food availability, species competition, ambient temperature as well as exposure to xenobiotics (Adams and McLean, 1985). According to Adams *et al*. (1993) a value of one is indicative of a very good health status. A negative correlation

exists between disease and condition in fishes (MŐller, 1985). According to Blackwell *et al.* (2000) plump fish may be an indicator of favourable environmental conditions (e.g. habitat condition, ample prey availability), whereas thin fish may indicate less favorable environmental conditions.

A variety of factors have been found to introduce changes in the condition factor, such as changes in food availability, metabolism and changes in the gonadal status (Chellappa *et al*., 1995). Condition factor also varies greatly among fish taxa owing to their differential architecture, but condition indices can only vary from location to location with a species

The determination of condition factor is a useful tool when used as a first level screen to determine possible contaminant exposures (Van der Oost *et al*., 2003). Filipovic and Rasper, (2003) reported that Zn and Cd entering aquatic ecosystems cause enzymatic and hormonal disorders, DNA damages, defectiveness in the blood oxygen carrying capacity and electrolyte losses in fish which in overall result in alteration in condition factor. However, a limitation to using condition factor is that the results obtained do not provide evidence of the factor responsible for causing a change in the condition of the fish.

2.3.2 Hepato-somatic index (HSI)

The hepato-somatic index (HSI) is the weight of the liver expressed as a percentage of body weight (Schmitt *et al*., 2000). Hepatosomatic index provides an indication on the metabolic load on an animal. In a poor environment, fish usually have a smaller liver (with less energy reserved in the liver). According to Munshi and Dutta (1996), the normal HSI values are species-specific, but the usual range for osteichthyes is estimated

to be between 1 to 2%. Value lower than 1% indicates possible atrophy of hepatocytes while values higher than 2% indicate possible hypertrophy of hepatocytes.

Various bio-structural alterations have been observed in livers of fish. Hepatocyte coagulative necrosis, hepatocyte regenerative foci, and neoplasia appear to be associated with toxic agents (Yang, 2004). They involve an increase in cell number (hyperplasia) for the overgrowth of hepatocyte. An increase in cell size (hypertrophy) has also been observed in fish in lakes contaminated with polycyclic aromatic hydrocarbons (PAHs) or polychlorinated biphenyls (PCBs) due to proliferation of organelles (Van Dyk *et al*., 2012). Hepato-somatic index has been reported to decrease in fish exposed to high concentrations of cadmium and zinc (McHugh *et al.,* 2013).

2.3.3 Spleno-Somatic Index (SSI)

The spleen is a major hematopoietic organ in fish which stores red blood cells and disintegrates old blood cells. Because the spleen is a haemotopoietic organ, spleen size is considered a useful diagnostic factor. Dysfunction of the spleen could have effects at the whole organism level (Ackermann, 2008). Histological data show cellular changes occurring in the spleen with exposure to contaminants, supporting the use of the spleno-somatic indices (SSI) as a relevant indicator of spleen dysfunction (Ackermann, 2008). An increase, enlargement or swelling of the spleen, on the other hand, indicates disease or immune system problem (Goede and Barton, 1990). Changes have been observed in the spleen- somatic index of fish exposed to contaminants (Johansen *et al*. 1994; Stepanova *et al*., 1998).

SSI are not only responsive to pollution, they can also be affected by certain endogenous and exogenous factors such as, species of the fish, gender, age, sex, gonadal development, growth rate and seasonal changes (Yang, 2004).

2.4 Fish liver histology as a biomarker indicative tool

Macroscopic signs of toxicity are almost preceded by changes at tissue, cellular or molecular levels (Segnor and Braunbeck, 1990). According to Hinton and Lauren (1990) histological alteration is defined as any contaminant-induced physiological or biochemical change in an organism that leads to the formation of a lesion in cells, tissues or organisms.

Histology can be used as an indicator or biomarker of the effects of anthropogenic contaminants (Nikalje *et al*., 2012). Histological changes in animal tissues are powerful indicators of prior exposure to environment stressors and are the net result of adverse biochemical and physiological changes in an organism (Hinton and Lauren, 1990; Palanismay *et al*., 2011). It is a useful method to detect and assess the degree of pollution, to reveal the overall health of a population or ecosystems (Bernet *et al*., 1999; Velmuragen *et al.*, 2007; Mohamed, 2009).

Histological assessments have been widely used to evaluate the health of fish (Van Dyk *et al*., 2012). One of the great advantages of using histological assessment in environmental monitoring is that it allows examining specific target organs. Furthermore, the alterations found in these organs are normally easier to identify than functional alterations, and serve as warning signs of deteriorating animal health (Hinton and Lauren, 1990; Camargo and Martinez, 2007). Such alterations which occur in fish living in

polluted environments are in most cases described as pollutant- associated rather than pollutant-induced (Schwaiger *et al*., 1997). For field assessment, histology provides a rapid method of detecting adverse acute and chronic effects of exposure in various tissues (Hinton and Lauren 1990).

The use of histology as a biomarker of effect, however, does pose some difficulties and problems. Hinton and Lauren (1990) cited the ability to detect alterations depends on the investigator expertise in proper fixation, processing and staining of preparations and experience in different alteration in different tissues.

Incorporation of quantitative method is essential to the continued development of histopathological indices of pollution exposure, and to the interpretation of histological response (Newman and Jagoe, 1996). Bernet *et al*. (1999) proposed a histopathological assessment protocol which leads to standardized quantification thus allowing the comparison between different studies. The histopathological assessment protocol was successfully applied in various studies (Bernet *et al*., 1999; Bernet *et al*., 2004; Schmidt *et al*., 1999; Schmidt- Posthaus *et al*., 2001).

Fish are relatively sensitive to changes in their surrounding environment. Early toxic effects of pollution may, however, be evident on cellular or tissue level before significant changes can be identified in the behavior and/or external appearance of the individual (Newman and Jagoe, 1996).

Fish liver's macroscopic structure is an integrator of biochemical functions which, when altered, may produce biomarkers of prior exposure to toxicants. The liver plays a key role in xenobiotic metabolism and excretion, digestion and storage, and production of yolk

protein. Alterations in structure are therefore expected under certain toxic conditions (Hinton and Lauren, 1990). According to Gingerich (1982) damage to hepatic parenchymal tissue has been the most frequently reported pathological effects in fishes exposed to various chemical agents. The primary characteristics of this response include vacuolation of parenchymal cells and increased degenerative changes of hepatocytes that results in focal or zonal necrosis.

Histopathological characteristics of specific organ express condition and represent time integrated endogenous and exogenous impacts on the organism stemming from alterations at lower levels of biological organization (Swee *et al*., 1996). Therefore, histological changes occur earlier than reproductive changes and are more sensitive than growth or reproductive parameters. Histopathological changes in animal tissue are powerful indicator of prior exposure to environmental stressors and are the net results of adverse biochemical and physiological changes in an organism. Whereas biochemical and physiological techniques are also powerful methods of identifying pathological change, histopathological changes allow the identification of specific target organs, cells, and organelles that have been affected *in vivo*. For field assessment, histopathology is often the easiest method of assessing both short and long-term toxic effects (Hinton and Lauren, 1990). As an integrative parameter, histological response provides a better evaluation of an organism health than a single biochemical parameter (Swee *et al*., 1996).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Description of the study area

Lake Kanyaboli lies between latitude $0^{\circ}04'$N and $0^{\circ}02'$N and longitudes $33^{\circ}59'$E and $34^{\circ}17'$E. The lake is located in the northeastern extreme of Yala swamp at an elevation of 1156m above sea level. It is a small shallow Lake with an area of approximately 10.5 km^2 and a mean depth of 2.7 m (Fig. 3.1) (Aloo, 2003). The catchment area of Lake Kanyaboli is approximately 160 km^2 with a mean annual temperature varying between 16 $^{\circ}$C and 19 $^{\circ}$C throughout the year (Anyona, 1999) and a mean evaporation rate of 1800 mm and 2000 mm per year. The catchment receives an annual average rainfall of 760 mm. Rainfall pattern is bimodal with two wet seasons from March to May, and a short rainy season occurs between August and December. Generally, the rainfall pattern is erratic and unreliable, particularly in the period of August to December.

The lake is drained by Hwiro River to the north and Yala River to the south and is separated from Lake Victoria by a sand bar through which the Yala River cuts in many deltaic outflows into the lake. Yala River is a perennial, has tributaries originating from the highlands part of Rift-valley, passes through agricultural area and discharges some of its water through a canal into the lake. Hwiro River is also perennial and carries effluents from domestic wastes and agricultural activities. The lake has no surface outlet and therefore there is potential for accumulation of contaminants in sediment, water and biota. A thick papyrus swamp surrounds the Lake, with characteristic floating papyrus island.

Some of the activities that threaten the lake's ecosystems include the ongoing swamp reclamation and conversion, over- fishing, fishing using illegal gears (Aloo, 2003) and inflow of rivers from upper catchment where tea, maize and cattle farming is practiced. The effluents which get into the lake vary in composition and quantity and contain contaminants such as heavy metals. Some of the main sources of heavy metals into the lake include natural geochemical processes (Ochieng *et al.*, 2009) and usage of agrochemicals such as fertilizers and pesticides in the catchment (Mwamburi, 2009).

The natural geochemical and erosion processes, wash off during rainfall as well as anthropogenic disturbances in the upper catchment of the Yala river influence the heavy metals load in the Lake. The lake Kanyaboli catchment has an estimated average population of 333 persons per Km^{-2} (KNBS, 2010). The southern part of the lake is exposed to human activities including domestic waste disposal, intensive rice farming and use of fertilizers and pesticides which would influence heavy metals concentration and distribution in the lake.

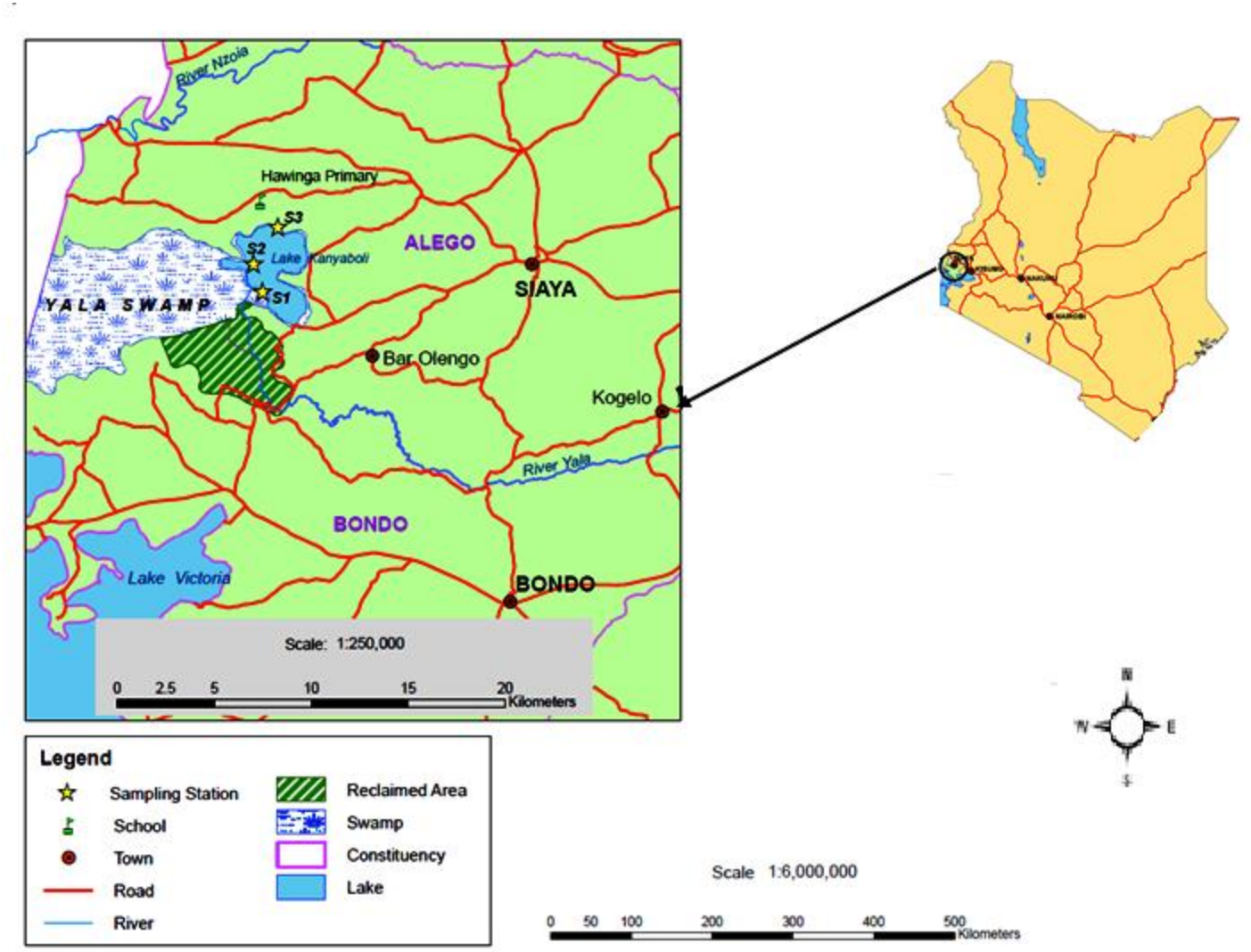

Figure 3.1 Lake Kanyaboli showing the three sampling stations (Source: Author, 2016)

3.2 Site selection and Sampling

The sampling period in this study was carried out from September, 2013 to March, 2014 which captured the short rainy season and the dry season and hence there were surface runoff carrying contaminants into the lake.

The sampling stations (Fig. 3.1) were purposively chosen based on the possible routes of heavy metals residues into the lake and included undisturbed area which served as a control. Areas with intensive agricultural activities and river mouth represented disturbed areas due to the potent influx of contaminants into the aquatic ecosystem.

The sampling station 1 (Kadenge discharge inlet) for fish and sediments on the lake represented the discharge point for the rice farm which forms a major agricultural activity around the lake. Station 2 was at Yala River mouth and represented upstream processes, anthropogenic activities and possible contamination sources from the river catchment as they channel used surface water into the lake. Station 3 (Gangu beach) characterized by minimal anthropogenic activities; mostly fishing and beach activities, served as the least disturbed area hence control site.

The fish fauna of Lake Kanyaboli are unique, being composed of fish species that populated Lake Victoria before the introduction of the *Lates niloticus* (Mbuta) (Mavuti, 1989). Fish particularly the benthivorous *Clarias gariepinus* (Mumi), is likely to be impacted by these contaminants and therefore provide a good biological indicator (Mwamburi, 2009).

The African catfish, *Clarias gariepinus* (Plate 3.1) was chosen as the model fish species for this study. This fish species is hardy, occurring even in most polluted waters.

However, tissue and organ anomalies resulting from environmental stress can be observed in this fish species (Crafford and Avenant- Oldewage 2009; Ramollo, 2009). This makes it a good indicator of chronic environmental stress, enabling it to reflect cumulative effects of both past and recent environmental quality conditions. *Clarias gariepinus* makes for an ideal test organism with which to conduct biomarker investigations in Lake Kanyaboli because of its availability and because they are apex predators. In addition, they are relatively poor swimmers that spend most of the time at the bottom of lakes and rivers (Wong *et al.*, 2011). They are bottom dwellers and do most of their feeding there. They are also obligate air breathers which mean they do spend some times on the surface.

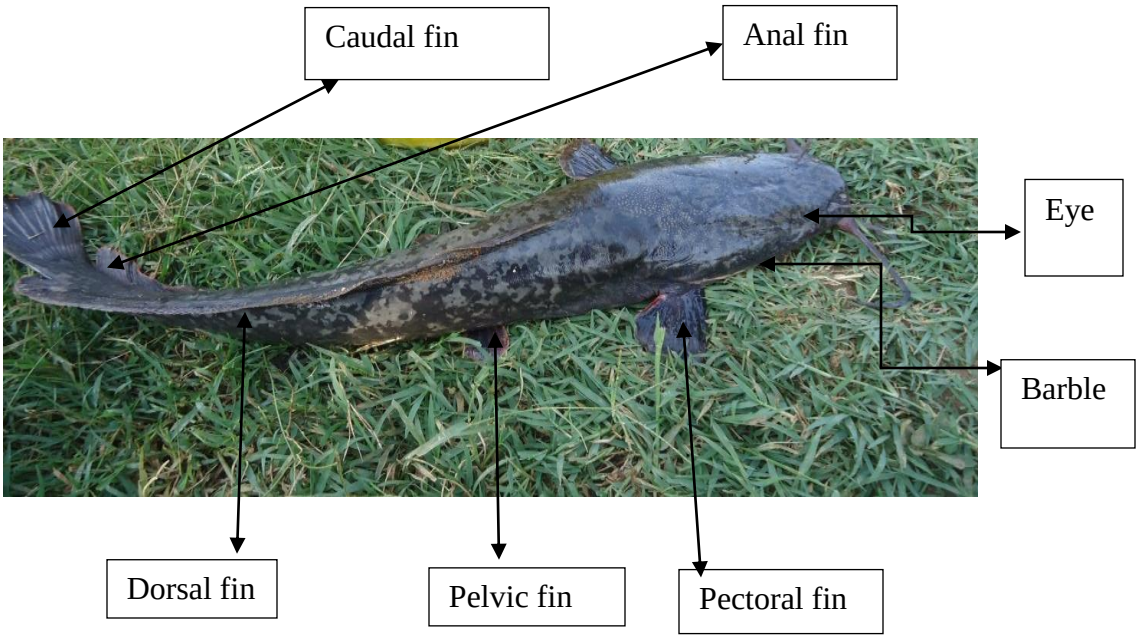

Plate 3. 1 African catfish, *Clarias gariepinus* sampled from Lake Kanyaboli showing different parts (Source: Author, 2016)

3.4 Determination of concentration of heavy metals in lake sediments and fish liver Samples

3.4.1 Sampling and handling Procedure

Sediments were collected at the bottom of the lakes using pre-cleaned Ekman Grab sampler (15 X 15 cm) in triplicates. A polypropylene spatula was used to transfer 10 g sediments sub-sample into acid rinsed polypropylene bottles, labeled and then placed in a cooler box.

Clarias gariepinus were caught from all the three study stations using a gill net of 4' and 5' mesh sizes. Fish samples obtained were immediately washed with the lake water at the point of collection, kept in pre-cleaned polythene bags, sealed, labeled and kept in ice boxes for transportation to the laboratory at Dominion Farms Ltd. In the laboratory, total length (cm) and weight (g) were recorded. The whole fish samples were wrapped separately with aluminium foil paper, put in polythene bag, labeled and placed in ice boxes for transportation to the department of geology and mines laboratory Nairobi. The samples were transported to the laboratory in the evening of the day of sampling.

3.4.2 Storage and preparation of samples

Once in the laboratory, the whole fish and 10 g of lake sediments samples were stored in separate freezers at -20 $^{\circ}$C until analyzed. The deep frozen samples were thawed at ambient laboratory temperature overnight. The liver of fish was removed using sterile surgical blade. Drying trays were made using foil papers where 0.600 g of liver of the fish and 2 g of sediments were placed in the oven and dried for 72 hours at 70 $^{\circ}$C.

3.5 Extraction and analysis of heavy metals in sediments

All glassware were washed in nitric acid solution and rinsed with distilled water. All reagents used during analysis were of analytical grade.

Ten grams of sediment was dried in the oven at 70 $^{\circ}$C for 72 hours and ground into fine powder. Two grams of each sample was weighed into a 100 ml beaker. This was digested using the procedure recommended by Gupta *et al.* (2008) which involves addition of 5 ml of an acid mixture of concentrated nitric acid, sulphuric acid and perchloric acid at a ratio 6: 3: 1. This was shaken well and allowed to stand for 5 minutes. The samples was then digested on a hot plate starting at 70 $^{\circ}$C through to 120 $^{\circ}$C until volume reduced to approximately 1ml and production of floating suspended white fumes of SO_3 was clearly observed. This was allowed to cool to room temperature and 20 ml of 5% HCL acid solution added. This was then heated on a hot plate at about 75°C for 15 minutes and then allowed to cool. The samples were then filtered through Whatman number 541 filter paper pore size (125 microns) into a 100 ml volumetric flask and topped up to volume with 5% HCL and preserved in the cold room at 4 $^{\circ}$C awaiting analysis by AAS. A blank comprising of the reagents in the proportion as for the sample but containing no sample was prepared likewise.

3.6 Extraction and analysis of heavy metals in fish liver

The fish livers were digested according to Gupta *et al*. (2008). An acid mixture of two parts concentrated nitric acid and one part perchloric acid (2:1) was prepared. One gram (1.0 g) dried liver material was weighed and put in a 100 ml digestion tube. 5 ml of the acid mixture was added and the sample was placed on a hot plate. The samples were

heated at 60 °C for 15 minutes. The heat was then increased to 120 °C and digested for 75 minutes or until the sample cleared. The tube was removed from the hot plate when the sample was clear. The sample was then cooled and sufficient distilled water added to bring the solution to 100 ml and preserved in the cold room at 4 °C awaiting analysis by AAS. A blank comprising of the reagents in the proportion as for the samples but containing no sample material was prepared likewise.

The concentration of elements (Cd, Cr, Cu, Zn and Pb) in the lake sediments and fish liver was measured using atomic absorption spectrophotometer (AAS) Analyst 800 (Parkin Elmer Instrument, USA) with an acetylene flame (Cu and Zn) and argon non-flame (Cd, Cr and Pb), after preparation of calibration standards through serial dilution. The overall recovery rates (Mean ± SD) for Cd, Cr, Cu, Zn and Pb were 103± 8.3, 91± 3.0, 89± 5.6, 91± 2.3 and 90±3.5 respectively. The detection limit for Cd, Cr, Cu, Zn and Pb was 0.02, 0.05, 0.04, 0.10 and 0.04 µg g^{-1} respectively.

3.7. Determination of Fish Health Assessment Index (HAI)

3.7.1. Sampling and handling procedures

At each site, *C. gariepinus* were collected by means of gill net mesh size 4’ and 5’. The nets were deployed from early morning, and checked two hourly to minimize the amount of time fish spent in the net in order to reduce any imposed stress. Only living fish size 250 - 550 g were selected, kept in a 100 L plastic tank and immediately transported to the Dominion Farm Ltd laboratory for liver collection and necropsy analysis. A total of 105 *C. gariepinus* specimens were collected in Lake Kanyaboli between September, 2013 and March, 2014.

At the laboratory, fish were kept in large holding tanks filled with water from each site to minimize stress. Fish were weighed and total length (TL in cm) was measured. In addition, the sex of each fish specimen was also recorded. Total length was measured to the nearest 0.1 cm, using a measuring board. The total wet weight was measured to the nearest 0.1 g with an electronic weighing balance (Scanvaegt salter, model 323; Avery Weigh-Tronx Ltd, West Midlands, UK). Examination of external organs and tissues (eyes, gills, fins, skin and opercula) and internal examination of liver and spleen were performed as described by Jooste *et al.* (2005b) and recorded on HAI data sheet (Appendix A) adopted from Adams *et al.* (1993). This was done to identify any possible abnormalities. All the internal organs were assessed with the help of a colour chart developed by Watson (2001) (Fig. 3.2).

The liver and spleen were then removed weighed and their weight recorded after which the liver were placed in 10 % neutrally buffered formalin to preserve them for histological analysis at the laboratory. Designated characters were assigned to the organs as indicated in the HAI as shown in table 3.1

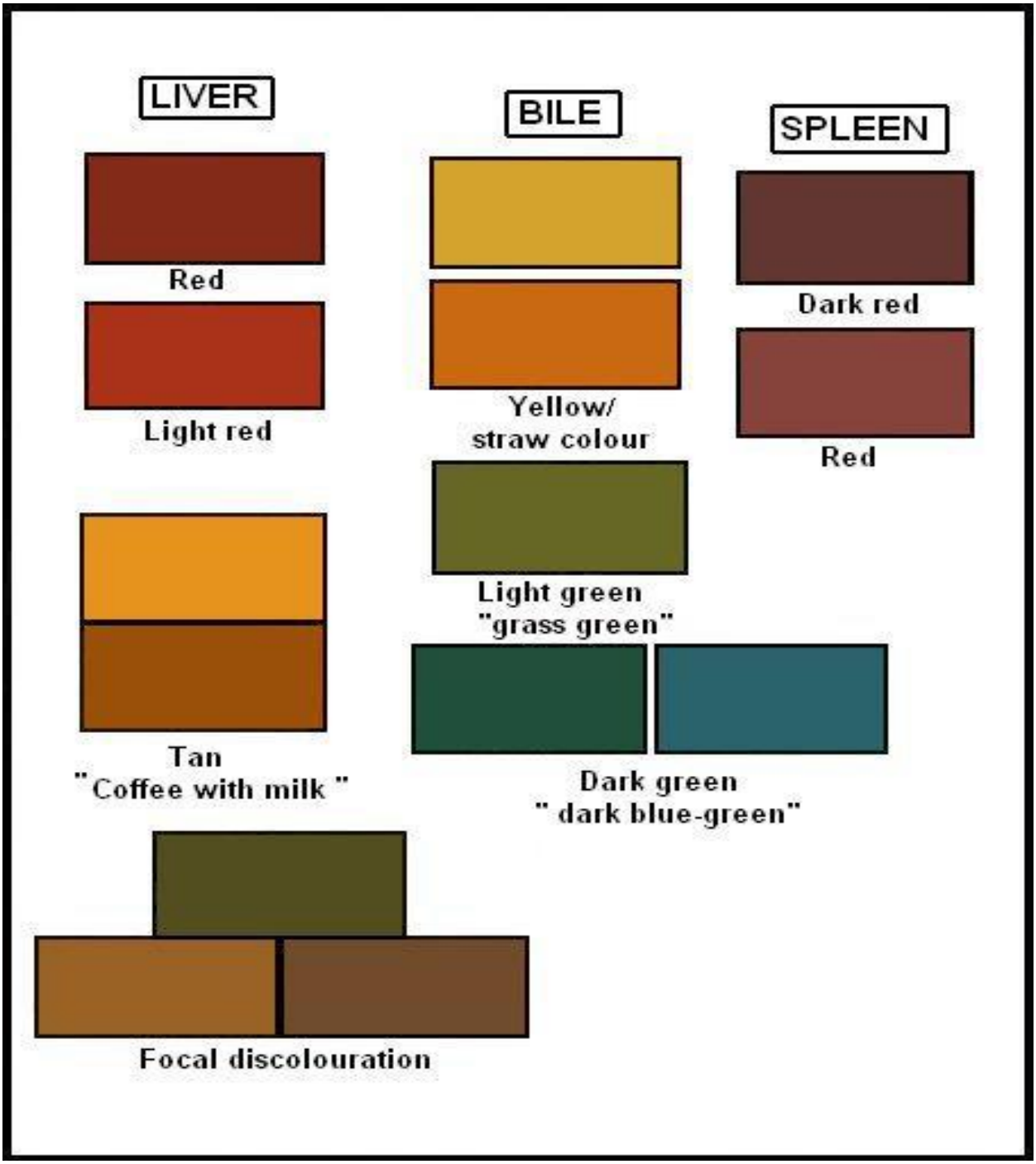

Figure 3.2: Color chart used to compare the colour of liver and spleen of *C.gariepinus* in Lake Kanyaboli (Source: Watson, 2001)

Table 3. 4: Fish health variables with assigned characters showing the norm and deviation from the norm in necropsy based system used to assess fish health in Lake Kanyaboli (Adapted from Jooste *et al*. 2005b)

Variables **External Variables**	**Variable condition**	**Originalfield designation**	**Adopted value for the HAI**
Length	Total length in millimetres	mm	-
Weight	Weight in gram	g	-
Eye	Normal	N	0
	Exopthalmia	E1/E2	30
	Haemorrhagic	H1/H2	30
	Blind	B1/B2	30
	Missing	M1/M2	30
	Other	OT	30
Fins	No active erosion & previous erosion healed over	0	0
	Mild active erosion with no bleeding	1	10
	Severe active erosion with haemorrhage/ secondary infection	2	20
Skin	Normal, no aberrations	0	0
	Mild skin aberrations	1	10
	Moderate skin aberration	2	20
	Severe skin aberration	3	30
Opercula	Normal/ no shortening	0	0

	Mild/ slight shortening	1	10
	Severe shortening	2	20
Gills	Normal	N	0
	Frayed	F	30
	Clubbed	C	30
	Marginate	M	30
	Pale	P	30
	Other	O	30
Internal variables (necropsy)			
Spleen	Black	B	0
	Red	R	0
	Granular	G	0
	Nodular	NO	30
	Enlarge	E	30
	Others	OT	30
Liver	Red	A	0
	Light red	B	30
	"Fatty" liver "coffee with cream" colour	C	30
	Nodules in liver	D	30
	Focal discolouration	E	30
	General discolouration	F	30
	Others	OT	30
Parasites	No observed parasites	0	0
	Few observed parasites	1	10

3.7.2 Calculation of the Health Assessment Index (HAI)

Original field designation of all variables from the autopsy based system were substituted with comparable numerical values into HAI (Table 3.1). All the variables of the HAI were presented with a value ranging from 0-30, depending on the condition of the organ tested. Any abnormality in the eyes, gills, liver and spleen were given a value of 30. Other variables (Skin, fin, opercula) abnormalities were rated 10, 20 or 30 depending on the degree of abnormality, with the greatest abnormality ranked as 30.

To calculate an index value for each fish within a sample, numerical values for all variables were summed. The HAI for sample population were calculated by adding all individual fish health index values and dividing it by the total number of fish examined. The variables used in the calculation of HAI index included length, weight, eye, fins, skin, opercula, gills, spleen and liver. The standard deviation for each sample was calculated as proposed by Adams *et al.* (1993).

$$SD = \frac{\sum_{i-1}^{N}(vi - X^2)}{N - I}$$

Where: N = number of fish per site

X = average index for each site

vi = index value for fish i.

The coefficient of variation (CV) was calculated as proposed by Adams *et el* (1993) :

$$CV = 100 \times SD/X$$

Where SD = standard deviation

X = average index for each site

3.8 Determination of Fish biometrical Indices

3.8.1 Condition Factor (CF): Length- weight relationship

Fish were weighed and measured upon arrival in the Dominion Farm Ltd laboratory for the calculation of condition factor (CF). The condition of fish, based on the analysis of length-weight data, indicates the condition of the fish in a habitat. The CF was determined for the different fish samples to ascertain any difference in health of the fish between the different sampling sites. The condition factor (CF) for each fish was calculated according to (Barnham, 1998; Williams, 2000).

$$CF = \frac{W \times 10^5}{L^3}$$

Where; W = Weight in g,

L = Body length in mm

3.8.2 Hepato- somatic Index

The whole liver was removed and weighed at the Dominion Farm Ltd Laboratory for calculation of the hepato-somatic index (HSI). The HSI was determined by expressing the liver weight as a percentage of the total body weight (Schmit *et al*., 2001) using the formula.

$$HSI = \frac{Liver\ weight(g)}{Total\ body\ weight(g)} \times 100$$

3.8.3 Spleno-somatic Index

The whole spleen was removed and weighed at the Dominion Farm Ltd laboratory for calculation of the spleno-somatic index (SSI). The SSI was determined by expressing the spleen weight as a percentage of the total body weight (Schmit et al., 2001) using the formula;

$$SSI = \frac{Spleen\ weight(g)}{Total\ body\ weight(g)} \times 100$$

3.9 Tissue preparation and histological analysis

Processing fish tissue for histology involved fixation (during sampling), dehydration, clearing, infiltration, imbedding, sectioning, staining and lastly mounting (Gabe, 1976; Humason, 1979).

3.9.1 Fixation of *Clarias gariepinus* Liver

The fixation process preserves tissue structures by converting the semi-fluid consistency of cells to a semi-solid consistency. Fixation was achieved using 10% neutral buffered formalin (37% formaldehyde (100mL), sodium phosphate monobasic (4g), sodium phosphate dibasic (6g), distilled water (900 mL) according to Fowler (2011). The liver were removed from the fixative after 48 hours to prevent brittleness and excessive hardening, and were placed in 70% industrial methylated spirits (IMS; 90%; Sigma) to remove excess fixative, and then stored at room temperature.

3.9.2 Wax impregnation, embedding and sectioning of the liver

The stored liver was removed from the 70% IMS and was placed in a labeled plastic tissue processing cassette (VWR, UK) and stored in 70% IMS before wax impregnation. Impregnation of the tissue with wax was carried out using a Shandon Hypercenter XP autoembedder. The autoembedder ran an 18 hour cycle to dehydrate the tissue with increasing concentration of ethanol (30%-50%-70%-80%-90%-100%-100%) for specific time period.

The liver was then washed in three baths of xylene which is miscible in both IMS and paraffin wax and allows wax to penetrate the tissue when it is washed twice in the wax bath (Table 3.2)

Table 3. 5: Steps and time taken by auto embedder during tissue processing

Step	Solutions	Immersion Time
1	30% ethanol	1 hour
2	50% ethanol	1 h 20 min
3	70% ethanol	1 h 20 min
4	80% ethanol	1 h 20 min
5	90% ethanol	1 h 20 min
6	100% ethanol	1 h 20 min
7	100% ethanol	1 h 20 min
8	Xylene	1 h 35 min
9	Xylene	1 h 35 min
10	Xylene	1 h 35 min
11	Paraffin wax	2 h
12	Paraffin wax	2 h

Immediately after wax impregnation, the liver was embedded in blocks of paraffin wax (2 cm x 3 cm x 1 cm) using metal embedding moulds and a wax dispenser (Electro

thermal Engineering Ltd, Uk), oriented correctly so that sectioning would provide a transverse cross section of the liver. Each liver was embedded in one paraffin block. Prior to sectioning, paraffin blocks were chilled on ice for 1 hour. This procedure hardened the wax and improved sectioning. Sectioning was carried out using a rotary microtome (Reicther-Jung 2050), with a fresh section of microtome blade (Shandon LP MX 35 Premium) for each section to minimize tearing, blocks were first trimmed such that the tissue was exposed and the area of excess wax to be sectioned was reduced. Sections were cut at 5 µm as a ribbon of section and then floated onto the surface of a bath of 30% IMS for up to 1 minute to reduce static charge and folding, and then transferred to a water bath maintained at 45 $^{\circ}$C to stretch the section, for up to 1 minute. Each section was floated onto a clean slide; one slide contained between 3 and 5 sections from each liver, and for each liver three glass slides were prepared. This provided a representative sample of sections through the liver. Slides were left overnight on a hotplate maintained at 40 $^{\circ}$C to allow section to adhere to them.

To distinguish cell types and structures under light microscope, sections were stained with hematoxylin (Purple/blue; Kobian Kenya Ltd), which binds to nuclear material, with an eosin counter stain (red; Kobian Kenya Ltd) to demonstrate clearly many different tissue structures, its versatility and its comparative simplicity of use according to guideline by Bancroft and Stevens (1982) and mounted with Entellan TM (Kobian Kenya Ltd).

3.9.4 Histology assessment

Histological sections were analyzed by light microscope (Zeiss Axioskop 40 microscope; Carl Zeiss Ltd, Germany) and digital images were obtained using Olympus DP 70 ccD camera (Olympus Optical, UK).

A qualitative assessment of the liver was undertaken, to identify histological changes (Van Dyk *et al*., 2009a; Pieterse *et al.*, 2010). The changes found were categorized into circulatory disturbances (CD), regressive changes (RC), progressive changes (PC), inflammation (I) and tumours (T) (Bernet *et al*., 1999).

3.9.5 Quantitative assessment

The five reaction patterns identified in the qualitative assessment were used in the semi-quantitative histological assessment where a numerical value was given for each alteration (Marchand *et al*., 2008; Van Dyk *et al*., 2009b). The protocol adapted from Bernet *et al*., (1999) and modified by Van Dyk *et al*. (2009a) was applied (Table 3.3). An important factor was allocated to represent the severity of the alteration according to Marchand *et al*. (2008) and Van Dyk *et al.* (2009a) as follows:

1 = Reversible alteration as exposure ends
2= Reversible alteration if the stressor is neutralized or removed
3= Alterations is irreversible
A score value was given on the frequency of the occurrence of the alterations according to Marchand *et al*. (2008) and Van Dyk *et al*. (2009a) as follows:
0 = Absent
2= Mild
4= Moderate
6= Severe

Table3. 6:Summary of the systematic procedure followed during this study while analyzing sections, specifying the functional units and cell examined as well as the importance factor for each alteration observed (Adapted from Bernet *et al.*, (1999) and modified by Van Dyk *et al.* (2009a).

Reaction pattern	Functional Unit	Alteration	Importance factor	Score value
		Liver		
RP I: CD	Blood vessels	Haemorrhage/hyperaemia/ aneurysm	1	1
		Intercellular oedema	1	2
RP 2: RC	Liver tissue	Architectural & structural alteration	1	1
		Plasma alteration	1	2
		Deposits	1	3
		Nuclear alteration	2	4
		Atrophy	2	5
		Necrosis	3	6
		Vacuolar degeneration		
RP 3: PC	Liver tissue	Hypertrophy	1	1
		Hyperplasia	2	2
RP 4: I	Including macrophage	Exudate	1	1
		Activation of RES	1	2
		Infiltration	2	3
RP 5: T	Whole section	Benign tumours	2	1
		Malignant tumours	3	2

Using importance factors and score values, the following indices were calculated

Reaction Index of an organ ($I_{org\ rp}$)

$$I_{org\ rp} = \Sigma_{alt}\left(a_{org\ rp\ alt} \times w_{org\ rp\ alt}\right)$$

Where: org = organ (constant)
rp = reaction pattern (constant)
alt = alteration
a = score value
w = importance factor

The quality of lesions in an organ is expressed by the reaction index. It is calculated by the sum of the multiplied importance factors and score values of the histological changes corresponding reaction pattern. The sum of the five reaction indices of an organ is equivalent to the organ index. Respective reaction indices of an organ from different individuals can be compared.

Organ index (I_{org})

$$I_{org} = \sum_{rp}\sum_{alt}\left(a_{org\ rp\ alt} \times w_{org\ rp\ alt}\right)$$

Where: org = organ (constant)
rp = reaction pattern
alt = alteration
a = score value
w = importance factor

This index represents the degree of damage to an organ. It's the sum of the multiplied importance factor and score values of all changes found within examined organ. A high index indicates a high degree of damage. Calculation of the organ index allowed a comparison between the degrees of damage of the same organ in different individual.

To classify the mean organ index value of each sampling site according to the degree of histological response, the results were evaluated according to a classification system (Van Dyk *et al*. 2009a; b) based on the scoring scheme by Zimmerli *et al*. (2007).

- Class 1 (Index< 10) normal tissue structure with slight histological alteration
- Class 2 (Index 10-25) normal tissue structure with moderate histological alterations
- Class 3 (Index 26-35) pronounced alteration of organ tissue
- Class 4 (Index > 35) severe alteration of organ tissue

3.10 Data management and analysis

Information in the hard copy datasheets was transcribed into an electronic spreadsheet using Microsoft Excel for both storage and processing. Descriptive statistics i.e. means and standard deviations were calculated with Ms. Excel. Analysis of variance was the statistical method applied to the data after log transformation to increase normality and homogeneity of variance. SPSS 17 and Statistica 10 were used as the analysis software. The purpose of analysis of variance was to test the differences in means (for groups or variables) for statistical significance. This was accomplished by analysis of variance, that is, by partitioning the total variance into the component that is due to random error and the components that are due to the differences between means. These latter variance components were then tested for statistical significance, and if significant, the null hypothesis of no difference means was rejected.

Analysis of variance was used to determine significance differences between values from heavy metal concentration in sediments and fish liver. Tukey's multiple comparison tests

were conducted to compare the mean values of heavy metal in sediments and fish liver between different sites. The means were deemed significantly different when $p < 0.05$ and insignificant when $p > 0.05$ at 95% confidence interval.

To determine the linear relationship between heavy metal in either sediment and fish liver as the grouping variables, redundancy analysis (RDA) was used in the CANOCO for Windows Package (ter Braak…). Prior to RDA, DE trended Canonical Correspondence Analysis (DCCA) was performed in order to determine the length of gradient of the dataset (extent of species turnover). In this case, a linear method was used because the length of gradient was less than 4 (Lepš and Šmilauer, 2003). Redundancy analysis (RDA) is a type of constrained ordination that assesses how much of the variation in one set of variables (in this case fish liver heavy metal concentration) can be explained by the variation in another set of variables (i.e. lake sediment heavy metal concentration).

Multidimensional Scaling (MDS) analysis was carried out using primer statistical package (Paliy and Shanker, 2016) and interpreted in relation to type and intensity of stress or pollution. A suite of indices (HAI, CF, HSI, SSI and SQHI) were calculated for each sample at each site. Performance of the indices was assessed using Kruskal Wallis to assess the difference in index value between sites (Carried out using SPSS 17). For variables with a significant test results, Mann-Whitney U test, controlling Type I error across tests by means of the Bonferroni adjustments

Pearson Product moment correlation was carried out using Statistica 10 in order to assess the strength of correlation between different indices in different study stations with different impact. Pearson product moment correlation was also used to explore any

relationship between indices and the heavy metal concentrations. Only percentage r values are given and not p-values to avoid Type 1 error due to multiple comparisons.

CHAPTER FOUR

RESULTS

4.1 The concentration of heavy metals in sediments

Concentrations of heavy metals found in sediments in Lake Kanyaboli are summarized in Table 4.1. It is evident that the sediment in Lake Kanyaboli is contaminated with Cr, Cu, Pb and Zn and the concentrations of these metals vary. Metal concentration in the three stations exhibited significant ($F_{\alpha, v1, v2} = 12.531$; $p < 0.05$). The concentration (µg g^{-1} dw) of the heavy metals in the sediment range from 0.49 to 0.77 for Cd, 4.26 to 7.48 for Cr, 79.61 to 87.09 for Zn, 14.03 to 20.43 for Cu and 14.38 to 155.56 for Pb. Metal concentration was significantly ($F_{\alpha, v1, v2} = 7.16$; $p < 0.05$) higher in station 2 than at the other stations.

Table 4. 3: The concentration of heavy metals in sediment (µg g-1 dw.) n = 45, (ẍ ± SE) In Lake Kanyaboli during the study period. Values are given as means of triplicate ±SE.

METALS	STATIONS		
	1	**2**	**3**
Cd	0.77±0.02	0.60±0.02	0.49±0.02
Cr	7.10±0.58	7.48±0.042	4.26±0.015
Zn	81.23±1.11	87.09±1.01	79.61±0.57
Cu	20.43±0.55	18.61±0.040	14.03±0.05
Pb	14.38±0.96	155.56±0.21	19.83±0.21

4.2 The concentration of heavy metals in the liver of *Clarias gariepinus*

Concentrations of heavy metals found in the liver of *C. gariepinus* in Lake Kanyaboli are summarized in the Table 4.2. All the target heavy metals were found to be present except

cadmium in the liver of *C. gariepinus* in all the stations. Although metal concentrations in liver were low, they exhibited significant levels ($F_{\alpha, v1, v2} = 17.484$; $p < 0.05$).The concentration ranged from nd to 0.22 for Cd, 0.37 to1.67 for Cr, 7.12 to 13.40 for Zn, 2.12 to 4.16 for Cu and 1.63 to 14.40 for Pb. The result on Table 4.2 revealed relatively high concentration of zinc and lead. The Zn content was significantly high as compared to other elements ($F_{\alpha, v1, v2} = 7.79$ $p < 0.05$). The highest mean concentration recorded was for Zn, Pb, Cu, Cr and the minimum was observed for Cd.

Table 4. 4: The concentration of heavy metals in *C.gariepinus* liver (µg g^{-1} ww.) n = 45, (ẍ ± SE) in Lake Kanyaboli during the study period. Values are given as means of triplicate ± SE.

Metals	Station 1	Station 2	Station 3
Cd	0.22 ± 0.01	0.16 ± 0.02	Nd
Cr	0.82 ± 0.34	1.67 ± 0.03	0.37 ± 0.01
Zn	13.40 ± 0.32	8.50 ± 0.02	7.12 ± 0.03
Cu	2.88 ± 0.51	4.16 ± 0.12	2.12 ± 0.12
Pb	5.40 ± 0.64	14.40 ± 0.58	1.63 ± 0.33

SE- Standard Error, WW- Wet weight, nd- not detected

4.3 Linear relationship of heavy metals in Lake Sediments and fish liver

Redundancy ordination analysis (RDA) was done to assess the relationship the concentration of heavy metals in sediments on the liver of *C.gariepinus.* In all cases, they depicted a positive linear relationship except for ZnF – ZnS which showed a negative relationship as shown in Fig. 4.1.

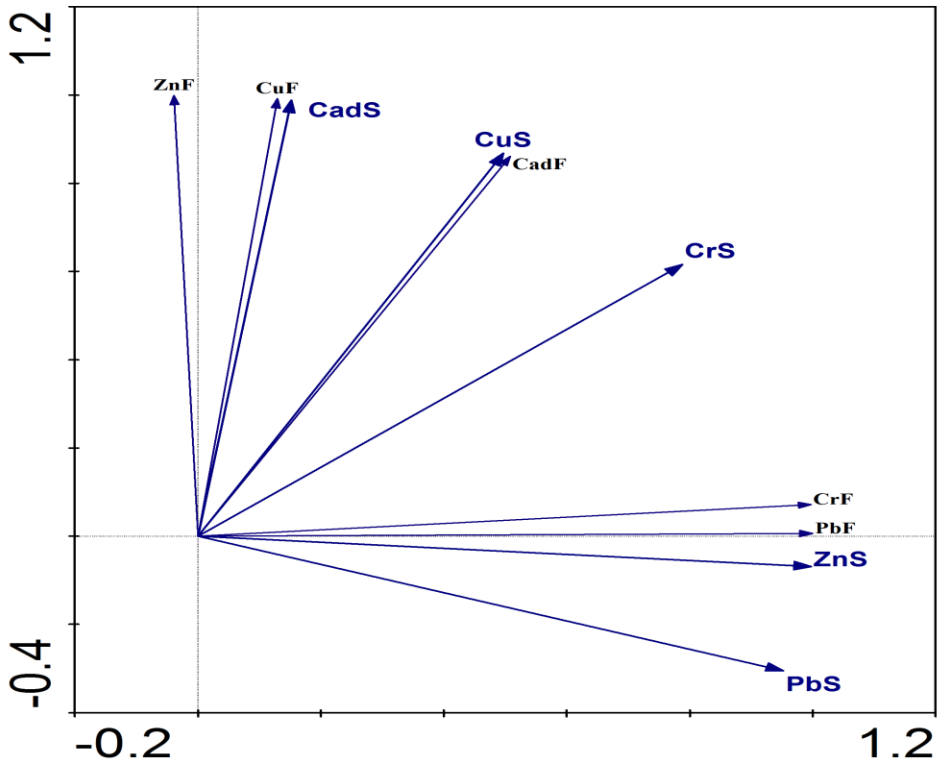

Figure 4.1. RDA analysis of the relationship between heavy metal in sediment and fish liver

Key: F- Heavy metal concentration in fish, S- Heavy metal concentration in sediments

4.4 Health Assessment Index (HAI)

Table 4.3 provides a summary of the Health Assessment Index for *Clarias gariepinus* from the three sampling stations in Lake Kanyaboli. The HAI results from the three sampling stations showed that *C.gariepinus* was affected in terms of necropsy- related anomalies. The coefficient of variation in HAI values for the *Clarias gariepinus* were highest for fish sampled from station 3 (90.35), lowest at station 1 (46.41) and intermediate at station 2 (63.76) (Table 4.3). The condition of the gills and liver were primarily responsible for influencing the HAI of *C. gariepinus* at all the stations.

Table 4.3: Health Assessment Index (HAI) values for *Clarias gariepinus* from the three sampling stations in Lake Kanyaboli during the study period

Sampling Sites	Health Assessment IndexValue	Standard deviation	Coefficientof variation
Station 1	47.43	22.01	46.41
Station 2	39.46	25.16	63.76
Station 3	28.61	25.85	90.35

Clarias gariepinus collected from Station 1 had the highest mean HAI value of 47.43. Gills, skin, fins and liver presented important anomalies in the *Clarias gariepinus* at this station. Changes in the gills and liver also contributed to a large extent, to higher overall HAI values of *Clarias gariepinus* sample population at station 2. The greatest number of ectoparasites was observed in *Clarias gariepinus* station 3, while the least number of endoparasites was observed in station 2.

Many of the abnormal liver recorded in Station 1 and Station 2 were nodules, focal discoloration or coffee-cream colour and fatty deposits (Plate 4.1, 4.2 and 4.3).

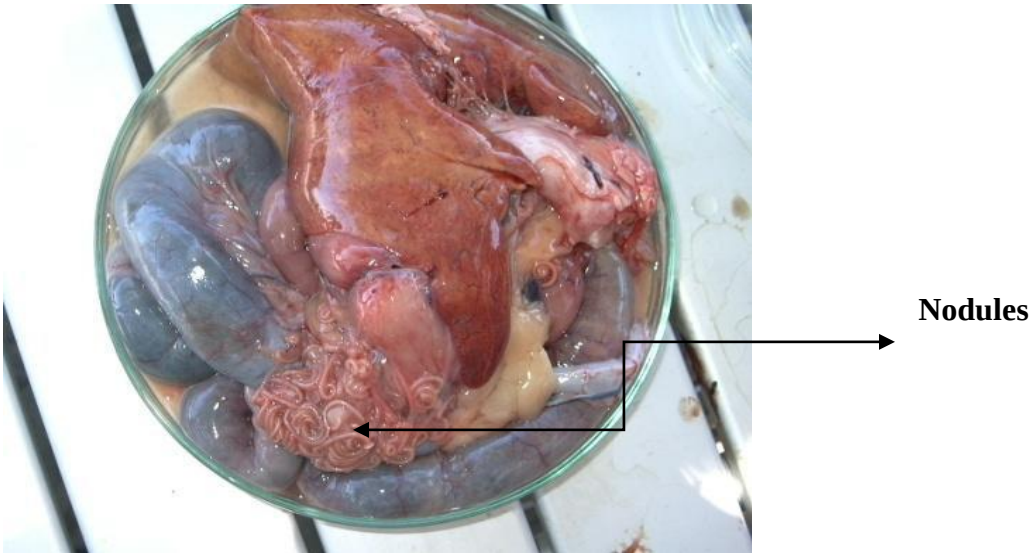

Plate 4. 1 The liver of *Clarias gariepinus* showing the nodules (source: Author, 2016)

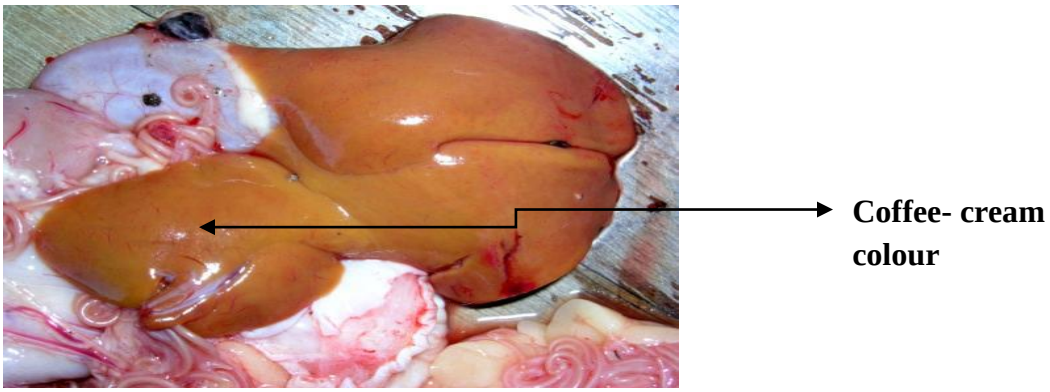

Plate 4. 2 The liver of Clarias gariepinus showing "Tan" with coffee-cream colour

(Source: Author, 2016)

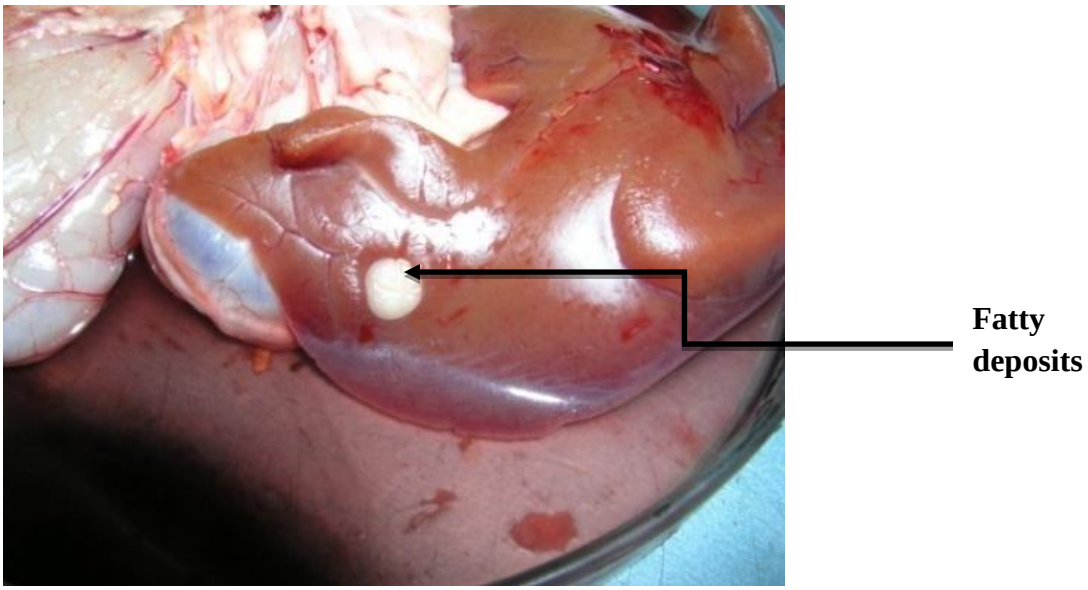

Plate 4. 3 Liver of Clarias gariepinus showing fatty deposits (Source: Author, 2016)

Anomalies of the spleen in *C. gariepinus* were only observed in 6.9% and 7.4% of the fish from Station 1 and Station 2, respectively. There were no anomalies of the fins, spleen in the *C. gariepinus* from station 3.

There were significant differences in the mean HAI of *C.gariepinus* among stations ($p < 0.05$). Mann-Whitney U- tests revealed significant differences in station 1 and 3 ($p < 0.05$) and station 2 and 3 ($p < 0.05$). But there were no significant differences in the HAI values between station 1 and 2.

4.5 The biometric indices of fish in Lake Kanyaboli

As discussed in section 2.4, the biometric indices calculated for this study includes the condition factor (K), hepato-somatic index (HSI), and spleno-somatic index (SSI). Table 4.4 summarizes the average values of the biometric indices as calculated for the sampling stations.

4.5.1 Condition Factor (K)

The condition factor (K) of the *Clarias gariepinus* is shown in Table 4.5. Mean K values for *C. gariepinus* were 0.60 at station 1, 0.71 at station 2 and 0.91 at station 3. There was a significant difference in the mean condition factor among sites ($p < 0.05$).

4.5.2 Hepato-somatic Index (HSI)

Hepato- somatic index of *C. gariepinus* from station 1 and station 2 recorded almost similar values of 0.66 and 0.67 respectively whereas station 3 recorded the lowest value of 0.53 (Table 4.4). There was a significant difference in the mean HSI among stations ($P < 0.05$).

4.5.3 Spleno-somatic index (SSI)

The SSI values for *C. gariepinus* were generally low throughout all the sampling stations ranging from 0.08 to 0.12 (Table 4.4). There was a significant difference in the mean SSI among stations ($P < 0.05$)

Table 4. 4: Biometric indices of *Clarias gariepinus* from Lake Kanyaboli during the study period

Sampling Stations	Condition Factor (K)	HIS	SSI
Station 1	0.60±0.03	0.66±0.05	0.10±0.01
Station 2	0.71±0.17	0.67±0.03	0.12±0.01
Station 3	0.91±0.29	0.53±0.03	0.08±0.00

4.6 Histology results

4.6.1Qualitative histology results

The percentage prevalence of the histological alterations identified in the target organ of *C. gariepinus* is presented in Table 4.5. Regressive alterations in the liver included intra vascular haemolysis, disconnection between hepatocytes and necrosis (plate 4.4, 4.5 and 4.6) were noted in fish across all the three stations while vascular degeneration was observed in fish from station 2 only (plate 4.5). Vacuolization was identified in the liver as round, empty space within the cell cytoplasm and the contents of the cells were most probably dissolved as a result of H&E staining technique, while necrosis was identified as having a darkly stained eosinophilic cytoplasm and usually having a pyknotic nucleus. Fish in station 2 had the most histopathological alterations.

Progressive changes were also identified in fish across the three sampling sites in the form of hypertrophy of hepatocytes (plate 4.4, 4.5, 4.6). Fish from station 2 had the most hypertrophy of hepatocytes (Table 4.5).

Table 4. 5: Percentage prevalence of histopathological alterations identified in the target organ of *C.gariepinus* from Lake Kanyaboli during the study period

	Sampling sites		
Alterations	**1**	**2**	**3**
	n=37	**n=35**	**n=33**
Liver			
Intra vascular haemolysis	73	68.6	54.5
Vascular degeneration	-	77.1	-
Vacuolation	67.6	42.9	36.4
Disconnection between hepatocytes	40.5	45.7	33.4
Necrosis	54.1	60.0	30.3
Hypertrophy	29.7	34.3	24.3

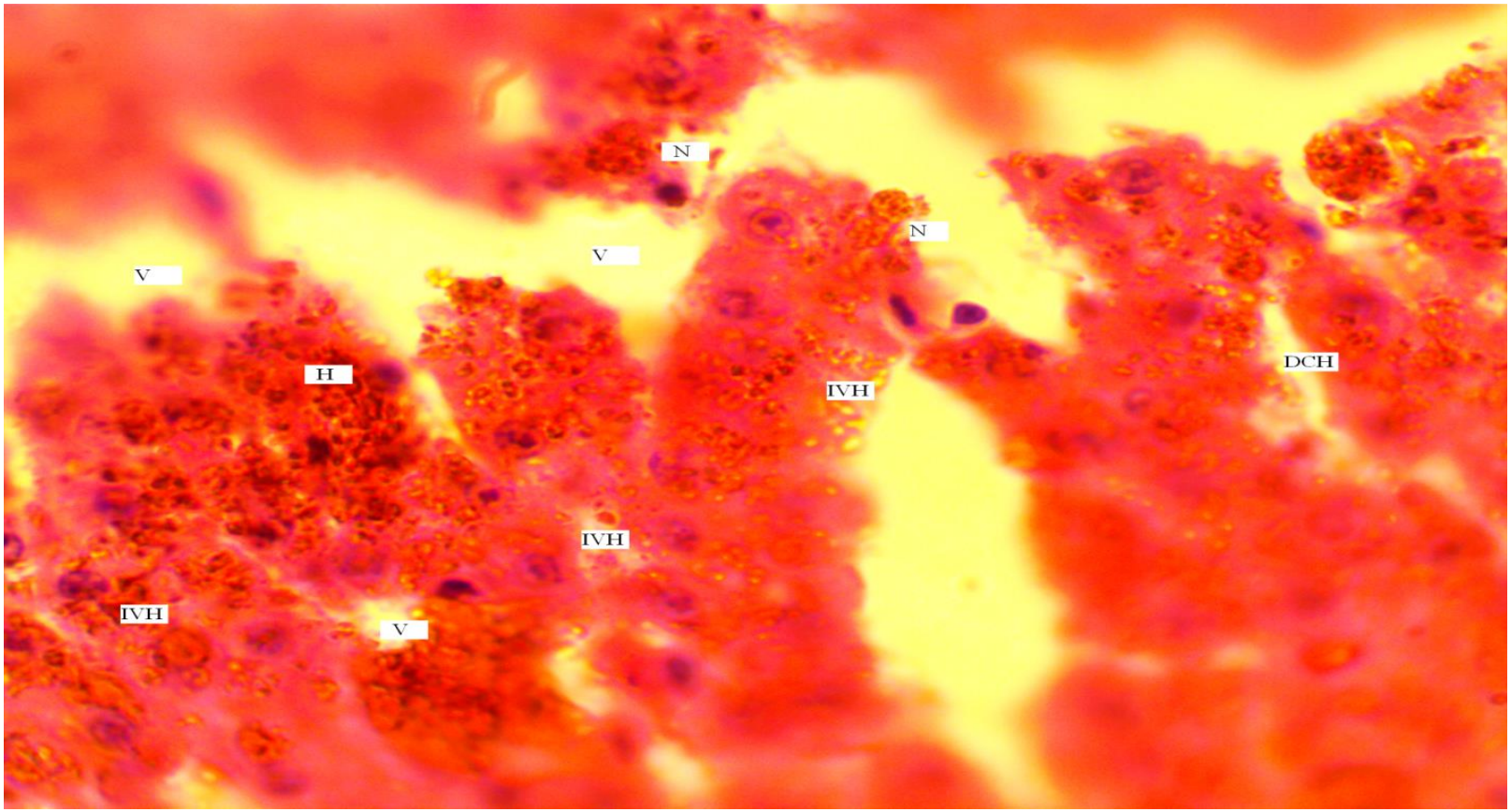

Plate 4. 4 Photomicrograph of *C. gariepinus* liver section (5 µm) stained with H&E showing (N) necrosis; (V) vacuolization; (H) hypertrophy; (IVH) intra vascular haemolysis; (DCH) Disconnection between hepatocytes during the study period (Source: Author, 2016)

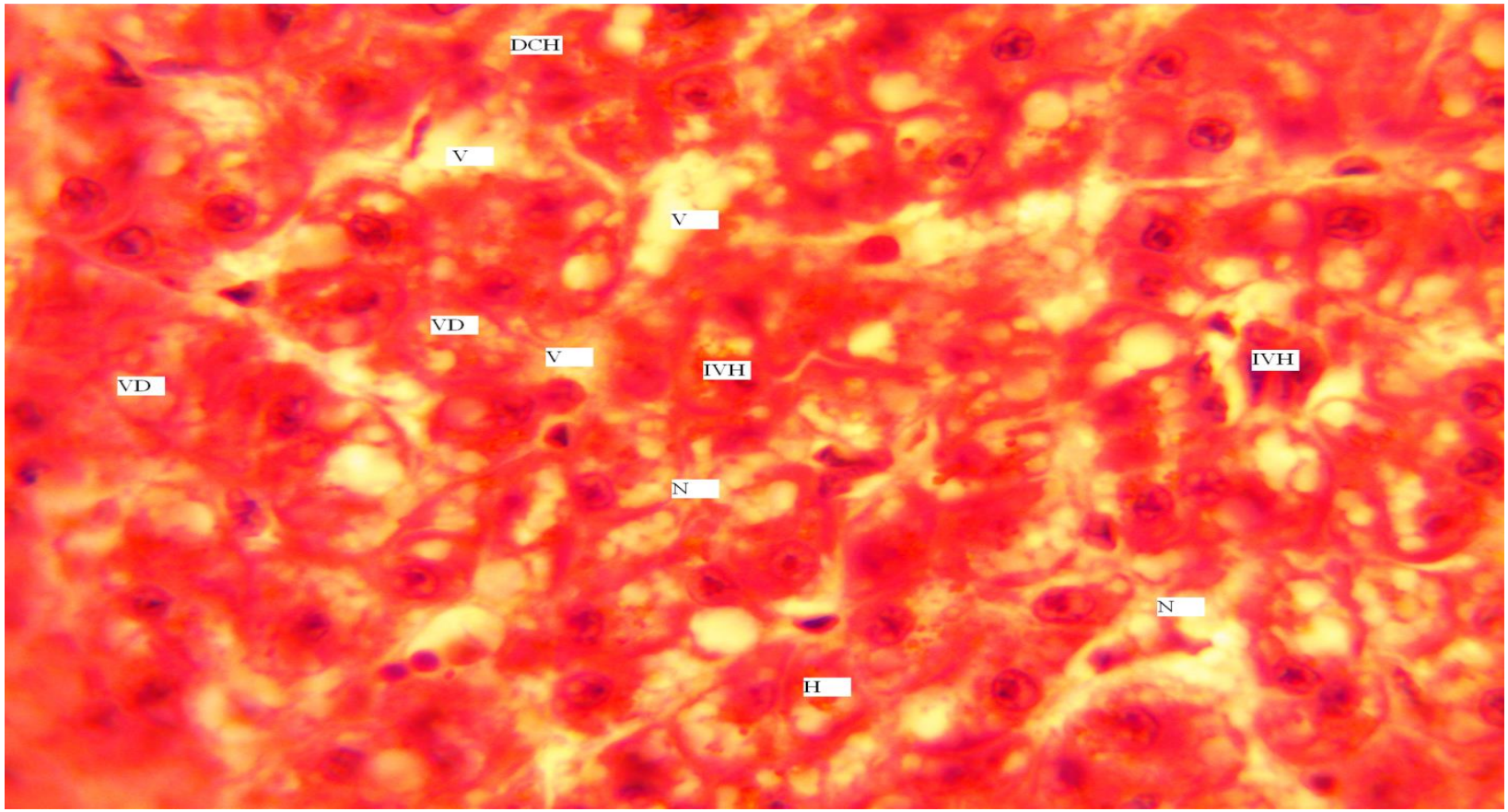

Plate 4. 5 Photomicrograph of *C. gariepinus* liver section (5 µm) stained with H&E showing (N) necrosis; (V) vacuolization; (H) hypertrophy; (IVH) intra vascular haemolysis; (DCH) Disconnection between hepatocytes; (VD) vascular degeneration during the study period (Source: Author, 2016)

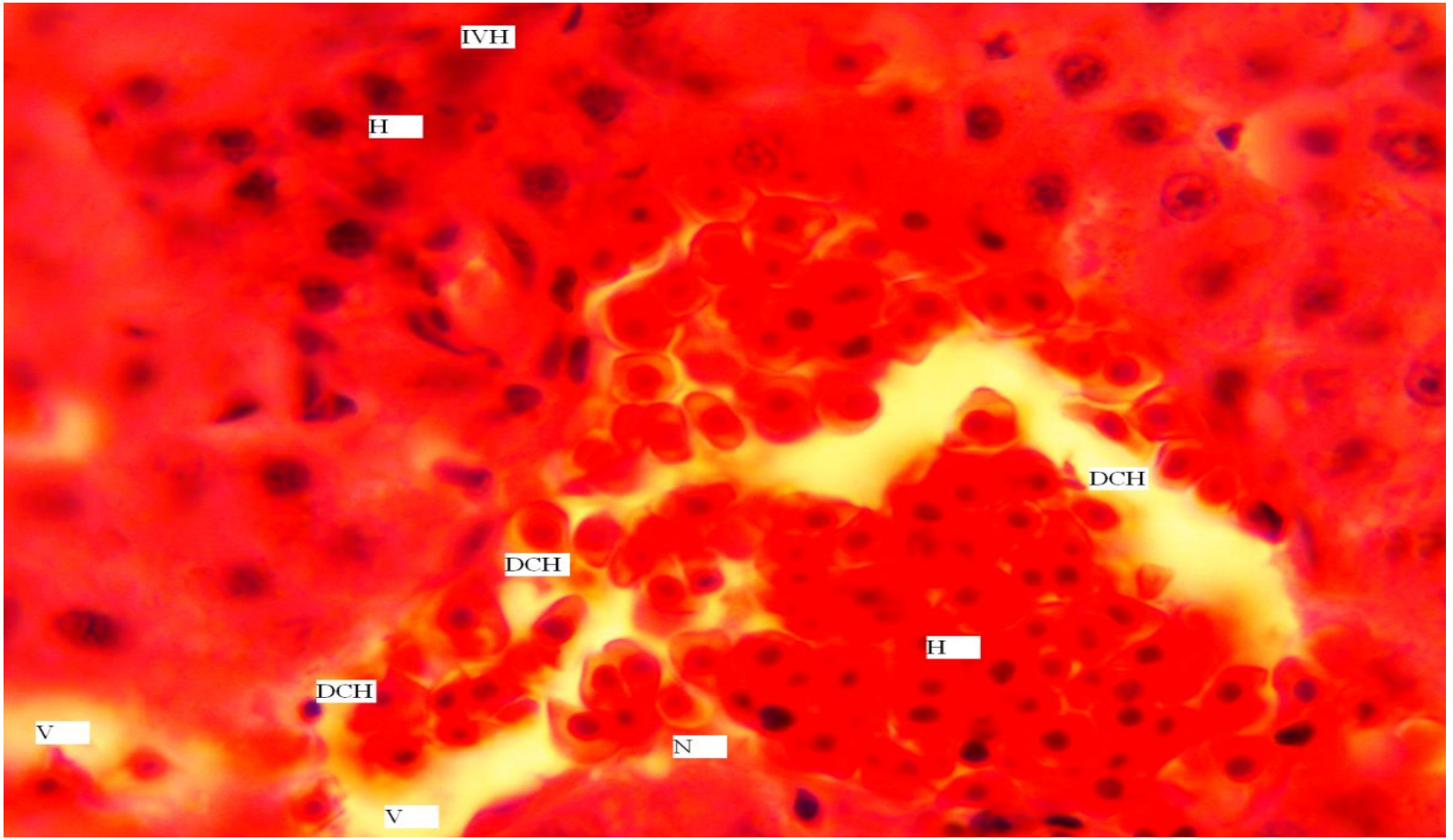

Plate 4. 6 Photomicrograph of *C. gariepinus* liver section (5 µm) stained with H&E showing (N) necrosis; (V) vacuolization; (H) hypertrophy; (IVH) intra vascular haemolysis; (DCH) Disconnection between hepatocytes during the study period (Source: Author, 2016)

4.6.2 Semi - quantitative histological assessment

Table 4.6 summarizes the semi-quantitative results obtained from the quantitative histopathological assessment tool. The reaction index (I org rp) gives an indication of the quality of the lesion in the liver organ, and the organ index (I org) gives an indication of the degree of damage. The results obtained from the quantitative analysis of the liver signify regressive changes (RC) and progressive changes (PC) only. A mean organ index of 13.43± 1.01, 17.63± 0.36 and 8.3± 0.41 were calculated for station 1, 2, and 3 respectively. The semi-quantitative assessment showed that the mean organ index (liver index) of the stations fall within the requirements of class 1 (normal tissue structure with slight histological alterations), observed in a reference station (Station 3) and class 2 (normal tissue structure with moderate histological alterations) observed in station 1 and 2 respectively (Table 4.6). There were significant differences in the mean of semi-quantitative histological index among stations ($p < 0.05$). Mann-Whitney U- tests revealed significant differences in station 1and 2, 1and 3 as well as 2 and 3 ($p < 0.05$).

Table 4.6: Mean liver index for semi-quantitative histology of *Clarias gariepinus* from sampling stations in Lake Kanyaboli during the study period (RP = Reaction Pattern, CD = Circulatory Disturbances, RC = Regressive Changes, PC = Progressive Changes, I = Inflammation and T = Tumour)

		LIVER I org rp	
RP	**Station 1**	**Station 2**	**Station 3**
CD	0.00	0.00	0.00
RC	10.60	16.21	7.5
PC	2.83	1.42	0.8
I	0.00	0.00	0.00
T	0.00	0.00	0.00
I org	**13.43**	**17.63**	**8.3**

4.7 Correlation Analysis of Histology-Based Fish Health Assessment Index biomarkers in Lake Kanyaboli during the study period

The Pearson product moment correlation analysis revealed no correlation among the biomarkers. There was only a weak correlation between semi-quantitative histological index and health assessment index (r = 34.25; Table 4.7). The MDS plots reflected results found in the correlation (Figure 4.2).

Table 4.7: Pearson product moment correlation with percentage r between Semi-quantitative histological index, health assessment index, condition factor, Hepatosomatic index, and splenosomatic index of *Clarias gariepinus* in Lake Kanyaboli.

	SQHI	HAI	CF	HSI	SSI
SQHI					
HAI	34.25				
CF	11.05	-7.79			
HSI	15.18	13.33	-4.65		
SSI	15.11	17.28	-12.46	13.08	

KEY: SQHI- Semi-Quantitative Histological Index, HAI- Health Assessment Index, CF- Condition Factor, HSI- Hepatosomatic Index, SSI- Splenosomatic Index

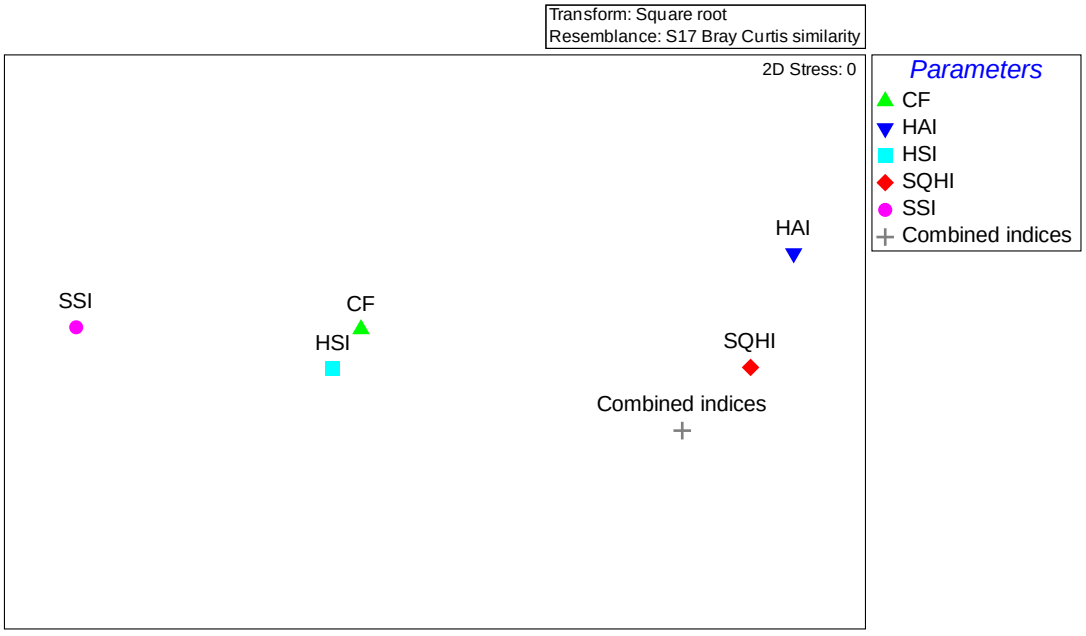

Figure 4.2. Multidimensional scaling of the association SQHI, HAI, CF, HSI and SSI in Lake Kanyaboli during the study period

4.6 Multidimensional Scaling and Correlation Analysis of Histology-Based Fish Health Assessment Index and Heavy metal in sediment and Fish liver of Lake Kanyaboli

Multidimensional Scaling (MDS) was used to assess the association of biomarkers with the concentration of heavy metals in sediments as well as the fish liver (Fig.4.3). Zn concentration in sediments and Pb in sediments showed positive associations with the Health Assessment Index (HAI) while the concentrations of Pb, Zn and Cu in fish showed a positive association with the Semi-Quantitative Histological Index. In addition, there was a positive association between Semi-Quantitative Histological Index (SQHI) with the concentration of Cu, Cr, Zn and Pb in sediments (Fig. 4.3). The Hepatosomatic index (HSI) showed a close association with the concentration of Cr in fish and Cad in sediments. The Spleno-Somatic Index showed a close association with the concentration of Cad in fish.

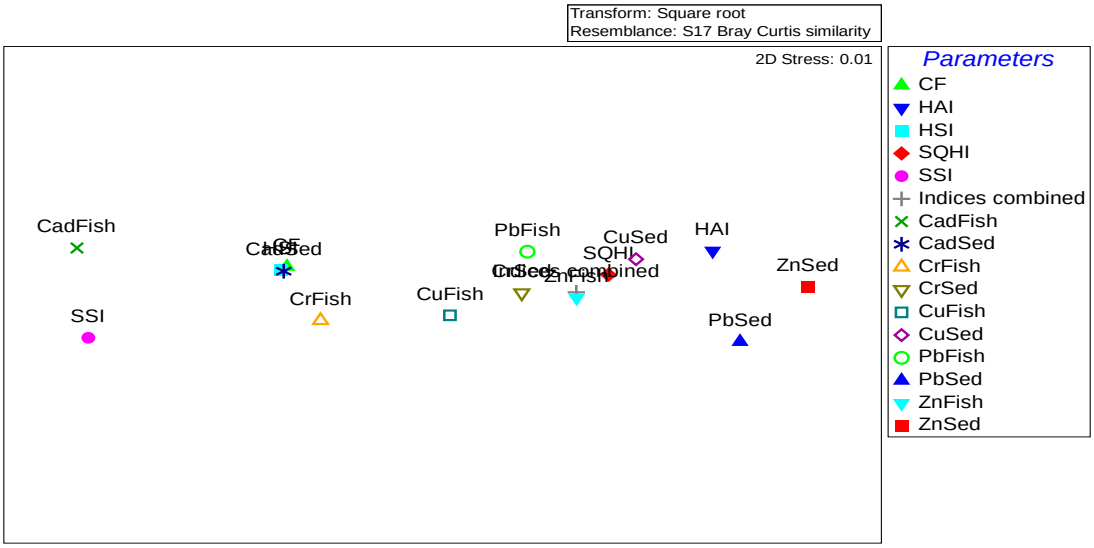

Figure 4.3. Multidimensinal scaling of the association between SQHI, HAI, CF, HSI SSI and the concentration of heavy metals in sediments and fish liver in Lake Kanyaboli during the study period

Pearson product moment correlation was done on the association between semi-quantitative histological index, health assessment index, condition factor, hepato-somatic index, spleno-somatic index and the levels of heavy metal in sediment and fish liver. The results revealed a positive association between semi-quantitative histological index with heavy metals both in the lake sediments (r = 58.41 for Cd - SQHI, 73.09 for Cr - SQHI, 54.26 for Zn - SQHI and 67.91 for Cu - SQHI) and fish liver (r = 60.41 for Cd - SQHI, 49.09 for Cr - SQHI, 47.40 for Zn -SQHI, 61.96 for Cu - SQHI and 63.38 for Pb - SQHI), there was a negative association between health assessment index with Cadmium in the sediment (r = -9.06), the same was observed with Cadmium (r = -4.43), Zinc (r = -10.07) and copper (r = -1.90) respectively in the fish liver. Condition factor revealed a negative association with heavy metal both in the sediment and fish liver. There was a no association between heavy metals in sediment and fish liver with hepatosomatic index

based on the Pearson product moment correlation values recorded, while Splenosomatic index revealed a moderate association with Zinc and Lead in sediment and with chromium in the fish liver. Table 4.8 shows the correlation results

Table 4. 8: Effects of heavy metals in Lake Kanyaboli sediment and fish liver on histology-based biomarkers as indicated by Pearson product moment with percentage correlation r during the study period

		SQHI	HAI	CF	HSI	SSI
BIOMARKERS	Semi-quantitative index					
	Health Assessment Index	34.25				
	Condition factor	11.05	-7.80			
	Hepatosomatic index	15.19	13.34	-4.65		
	Splenosomatic index	15.12	17.28	-12.47	13.08	
HEAVY METALS IN SEDIMENT	Cadmium (Cd)	58.41	-9.06	-53.52	13.95	-16.32
	Chromium (Cr)	73.09	26.83	-72.66	-0.08	7.52
	Zinc (Zn)	54.26	57.90	-40.81	-3.69	45.09
	Copper (Cu)	67.91	13.07	-66.25	7.67	0.38
	Lead (Pb)	25.53	45.31	-18.77	-14.19	38.03
HEAVY METALS IN LIVER	Cadmium (Cd)	60.41	-4.43	-21.64	13.38	-12.23
	Chromium (Cr)	49.09	52.20	-37.97	1.50	47.48
	Zinc (Zn)	47.40	-10.07	-38.69	23.44	-11.51
	Copper (Cu)	61.96	-1.90	-56.48	12.65	-10.88
	Lead (Pb)	63.38	35.02	-48.39	3.32	21.26

KEY: SQHI- Semi-Quantitative Histological Index, HAI- Health Assessment Index, CF- Condition Factor, HSI- Hepatosomatic Index, SSI- Splenosomatic Index

CHAPTER FIVE

DISCUSSION

5.1 Heavy metal concentration in sediments

The sediments are the biotic matrix of the environment that was investigated. Sediments serve as reservoirs for heavy metal residues and play a significant role in their remobilization and distribution in aquatic systems through interaction between water and sediment under favourable conditions (Őztürk *et al.,* 2009; Varol, 2011). Since heavy metals in the aquatic systems are usually predominantly associated with sediment, their concentrations in sediment are more sensitive and accurate indicators of recent and current pollution.

The concentration of the heavy metals in sediment was significantly higher ($p < 0.05$) in station 1 and 2 than in the reference station, station 3. In addition, the results indicate that Zn had the highest concentration followed by Pb and copper. The high Pb and Zn contents found in sediments samples from station 2 could be due to the inflow of contaminated sediments from the catchment. Station 2 is a key source of sediment deposition into the lake as it originates from the upper catchment areas, and is a major inlet into the lake. These results agrees with the observation by Mutia *et al*. (2012) who found higher concentration of Pb and Zn at the mouth of River Malewa which drains into the Lake Naivasha.

The discharge outlet from rice farms (Kadenge) showed significant Cd and Cu metal loads in station 1; this could be attributed to the quality of the agricultural waste water from the rice farms, which is rich in fertilizers and other agrochemicals, although these

metals were below lowest effect level in sediment (Appendix D). The results also indicate that Cd and Cr concentration in sediments were low which can be explained by their possible occurrence in dissolved form, still being suspended in water and not settled at the bottom due to turbulence or that most Cd and Cr are taken up by aquatic plants.

The Gangu beach (Station 3) had the lowest levels of the heavy metals in sediments samples. This could be attributed to lack of discharge canals and other inflows. However, the impacts of anthropogenic activities are shown in relatively higher concentration of Pb. Soil erosion within Gangu area coupled with the petroleum-based fuels from the motorcycles being washed in Gangu beach could be the source of Pb levels observed in the study. The mouth of River Yala had the highest concentration of heavy metals in sediment showing that they emanate from natural processes through erosion and anthropogenic activities up stream.

5.2 Heavy metal concentration in *C. gariepinus* liver

Results from the study show that the concentration of Cd, Cr, Zn, Cu and Pb in the fish liver are quite variables but the general trend is that the fish sampled from station 2 which is upstream, had a higher concentration of heavy metals as compared to the downstream site in Lake Kanyaboli.

The concentration of heavy metals in fish sampled in station 2 was significantly higher ($p < 0.05$) than that of the fish caught in station 3 this is due to the higher concentration of these metals in the sediment at station 2. The results reported a high concentration of Zn followed by Pb and Cu in the liver of *C. gariepinus* as compared to the reference station. The higher levels of heavy metal concentration in *C. gariepinus* in station 2 could be

attributed to leaching from fertilizers, urban runoff, extensive use of pesticides sprays which contain Cu compounds and municipal sewage.

Similar levels of Cr, Zn, Cu and Pb to that found in the present study were measured by Nnaji *et al*. (2007) in *Oreochromis niloticus* and *Synodontis schall* from the Galma River in Nigeria, which has high loading of agrochemicals. According to Skelton (2001), the *Synodontis* spp are bottom feeders, occupying the same niche as *C. gariepinus*. In Kenya, Oyoo-Okoth *et al*. (2010), measured levels of Cd, Cr, Cu and Pb from Lake Victoria affected by industrial effluents, mining and agricultural activities. The levels measured in *Rastrineobola argentea,* were more or less similar to the *C. gariepinus* of this study, but *R. argentea* is herbivorous, thus lower on the food chain than *C. gariepinus*. It can be expected that *C. gariepinus* from Lake Victoria could have higher levels, based on the study by Oyoo-Okoth *et al.* (2010), due to bio-magnification, as *R. argentea* are prey to *C .gariepinus. C. gariepinus* is omnivorous and preys on small fish of other species. This could have led to high levels of heavy metal in *C. gariepinus* compared to *R. argentea* which feed on zooplankton and macroinverterbrates.

Comparing the mean concentration of these heavy metals in *C. gariepinus* obtained from the study area with permissible standards limits by European Union (2006) and suggested by Wegner and Bomen (2003), these results indicate that the levels of heavy metals are within the permissible limits safe for human consumption. Although these results do not indicate a clear manifestation of toxic effects, the possibility of harmful effects cannot be ruled out after a long period of consumption of fish caught in the study area. Accumulation of metal toxicants from the aqueous environment by fish depend upon availability and persistence of the contaminants in water and food thus, the less available

it is the less it will be accumulated. Heavy metals have a tendency to accumulate in various aquatic organisms especially fish which may in turn enter into human metabolism through consumption of fish causing serious health hazards (Kamaruzzan *et al*., 2011).

The concentration of heavy metals in lake sediment has a positive linear relationship to the heavy metals found in fish liver (Fig. 4.1) except for ZnS - ZnF which showed a negative linear relationship. As the levels of heavy metals increased in the lake sediments, so did the levels in the fish liver. The negative relationship observed between zinc concentration in sediments and zinc concentration in fish liver could be explained by their possible existence in suspension form and hence have not settled at the lake sediments. Fish found in heavy metal contaminated water bodies, accumulate higher amounts of metals than those found in uncontaminated water bodies because they absorb these metals from gills, skin oral consumption of water, food and non-food particles (Nzeve *et al*., 2014). This explains the heavy metal toxicity of the water and the accumulation of the heavy metals in the sediments. Fish absorbs heavy metals from the sediment, polluted water and food and thus leads to contamination of the food chain. Heavy metals are not easily biodegradable and consequently can be accumulated in humans when they each contaminated fish over long period of time. This situation causes varying degrees of illness based on acute and chronic exposure (Demirezen and Ahmet, 2006; Mathenge, 2013). The introduction of these elements into the food chain may affect human health (Coutate, 1992).

The result presented predicts a possible risk associated with consumption of fish contaminated with heavy metals since there is a positive correlation between the concentration of heavy metals in sediment and in fish liver. The fish might look

apparently healthy despite accumulating heavy metals to concentration which substantially exceed maximum values considered safe for human consumption (Pheiffer *et al*., 2014). It is therefore becoming a matter of concern to environmentalist since the presence of pollutants, particularly; toxic metals apparently accumulate in the sediments. Heavy metal in the liver of *C. gariepinus* was significantly associated with heavy metal concentration in lake sediment indicating that fish liver is an effective indicator of fish exposure to heavy metals.

5.3 Health Assessment Index

The HAI results suggest that fish from Station 1, are in a significant poor condition ($p < 0.05$) compared to fish from Station 3. The conclusion of a decreasing environmental health status towards the most impacted station 1 was exemplified by several factors such as liver with fatty changes, discoloration and the enlargement of the spleen of some fish. The coefficient of variation was higher for fish from the station 3 which is less impacted station than those from more impacted stations. One possible interpretation of the finding is that fish living in more impacted environments are also all exposed equally to poor environmental quality and, therefore, the variability in physiological condition in fish from a pollutant- exposed population may tend to be less than for fish in unstressed environment.

There were significant differences in HAI of fish sampled from all the sampling stations. The highest mean HAI value for *C.gariepinus* were recorded at the severely polluted station whereas the lowest mean HAI value was recorded at the least polluted station. Enrichment of the lake by organic and inorganic wastes could be the main contributing factor as station 1 faces the effect of agricultural activities while Station 2 experience the

influx of pollutants from the highlands of Rift Valley which is the source of River Yala. This explains the high HAI values in these particular stations.

The health assessment index has been previously implemented in several studies (Adams *et al*., 1993; Barnmon *et al.,* 2010). Results from a study by Adams *et al.,* (1993) showed HAI values ranging from 17-79 from a reservoir that receives pollutants from numerous sources. Marchand *et al.* (2008) found high HAI values in *C. gariepinus* and *O. mossambicus* found in a polluted systems and a low HAI value from a reference station. Similar results were reported by McHugh *et al.* (2013) who recorded a high HAI values in Pongolapoort Dam which is polluted by agrochemicals and low values in Okavango Delta which is considered to be relatively pristine natural ecosystem without any effects of agricultural activities. The HAI provided similar conclusions relative to health status as other confirmed by the histopathological alterations and proved to be a reliable approach for assessing the general health status of fish population. These results confirms that fish exposed to pollutants, such as in station 1, have a higher HAI score indicating more alterations and poorer fish health.

5.4 Biometric Indices

The biometric indices determined in the current study included CF, HSI and SSI which are useful in assessing the health status of fish and toxic effects. According to Van der Oost *et al*. (2003), initial screening to identify exposure and effects of pollutants can be achieved, using basic, simple and cost-effective measures of condition. Such measures may be used to assess the ability of animals to tolerant toxic challenges or other environmental stress (Mdegela *et al.,* 2010).

5.4.1 Condition factor (CF)

Condition factor (CF) has been used extensively in fish health and population assessments and the calculation used for this study, namely Fulton's Condition factor described by Carlander (1969) can be indicative of the overall condition and nutritional status of an individual (Schmitt and Dethloff, 2000). A value of 1 is indicative of a very good health status.

In the current study, the mean condition factor was significantly greater in fish in station 3 than in fish in station 1 and 2. The values recorded for *C. gariepinus* in station 3 was close to 1, with values below 1 recorded at station 1 and station 2. This may suggest differences in the condition of fish from upper and lower reaches of Lake Kanyaboli with fish at station 2 and 1 in the upper reaches being exposed to pollutants, including heavy metals. The decrease in CF under the effect of pollutants might be as a result of loss in appetite or excessive use of energy reserves to compensate requirements. The high CF value observed at station 3 could be explained by the simulation of detoxification mechanisms which prevented metabolic reactions to be affected by pollutants.

These results represents similar results obtained in studies by Gaber (2013) in Lake Nasser and Keyombe *et al*. (2015) in Lake Naivasha, Kenya who reported low CF values in these lakes due to chemical pollution. Furhan *et al*. (2013) while studying the health status of *Clarias gariepinus* in Lake Kallar Kahar (polluted with heavy metals) reported a CF value below 1 in stations impacted by heavy metals. Thus, being able to monitor fish well-being is extremely useful in making management recommendations concerning fish population. Because the condition factor integrates different levels of sub- organismal

processes, it is therefore able to signify the overall health and nutritional status of an individual fish.

5.4.2 Hepatosomatic index

A measurement of liver weight in fish, in relation to histopathological alterations, has been reported in several studies as a useful indicator of exposure to chemical pollutants, as well as fish health (Yang and Bauman, 2006). Hepatosomatic index is one of the liver measurement that can increase or decrease depending on the toxicity of effluents. Exposure of fish to sub lethal concentration of pollutants generally leads to increased HSI (Barse *et al*., 2006; Abdel- Hameid, 2007; Carrola *et al*., 2009), as a result of induction or activation of biotransformation oxidase enzymes and thus develops increased ability to metabolize xenobiotics. On the other hand, pollutants with high toxicity usually lead to reduced HSI, as a result of hepatocellular injury associated with cell death (Shailaja and D' silva 2003; Ma *et al*., 2005).

In the current study, HSI was significantly greater in fish from station 1 and station 2 than fish sampled from station 3, which suggest an exposure of fish to effluents with sub-lethal concentration of pollutants. These results are in agreement with the microscopic findings in fish from these stations. The histopathological analysis showed a higher incidence of nuclear hypertrophy as well as vacuolation which are associated with increased cell size in station 1 and 2 whereas these alterations were lacking in fish sample from station 3. Similar results have been reported by Gaber, (2013) and Mabika and Barson., (2013) while studying the health *C.gariepinus* in Lake Nasser and Lake Kariba respectively. These results also correlate with the findings by Van Dyk *et al.* (2012) who

showed that HSIs of *C.gariepinus* from various aquatic ecosystems range from 0.5 to 0.9%.

Enlargement of livers by hyperplasia or hypertrophy could be reflected in an increased liver weight to body weight (Hinton and Lauren, 1990). Therefore hepatosomatic index has been used to indicate the contaminant exposure of fish. Non-contamination factor, such as sex, seasonal changes, nutritional status, and infection of parasites might cause variation in HSI values (Everaarts *et al*., 1993).

5.4.3 Spleno-somatic Index

In the current study low SSI values were obtained in all sampled stations for *C. gariepinus*. There were significant differences between stations. Botha, (2010) obtained similar findings with values ranging from 0.20 to 0.43 which compares well with this study. Low SSI values have also been recorded in different fish species (including carp, catfish and bass) from Colarado River Systems subjected to pollutants (Hinck *et al.*, 2007). In all these cases, the deterioration of SSI has been attributed to pollution especially from the impact of heavy metals. Decrease in SSI may result from necrosis and perturbation in a cell processing which impairs an individual fish health.

The spleen size is considered a useful diagnostic factor because it is a haemotopoietic organ (Anderson, 1990) and dysfunction could have effects at the whole organism's level (Bornman *et al.*, 2010). The SSI has not been as thoroughly investigated as the HSI, but certain endogenous and exogenous factors such as gender, age, size, gonadal development, growth rate and seasonal changes are known to affect it (Schmitt and Dethloff, 2000).

Enlargement of the spleen has been considered to be indicative of abnormal pathological condition such as disease and immune systems problems (Goede and Barton, 1990). Contracted or smaller spleen in fish has also been associated with exposure to organic contaminants including PCBs and PAHs (Pulsford *et al.*, 1995; Payne *et al.*, 1978).

5.5 Histological Assessment

The histological alterations identified in the liver organ of fish sampled from station 1 and 2 were similar, both in nature and severity of occurrence, compared to the same organ of the fish from station 3 except for vascular degeneration which was observed in station 2. In addition, the liver showed intra vascular haemolysis, vascular degeneration, vacuolation, necrosis and hypertrophy. These changes may be attributed to direct toxic effects of pollutants on hepatocytes, since the liver is the site of detoxification of all types of toxins and chemicals (Deore and Wagh, 2012). The liver is also one of the organ most affected by contaminants in the water (Comorgo *et al.*, 2007) and vacuolization of hepatocytes might indicate an imbalance between the rate of synthesis of substances in the parenchymal cells and their rate of release into the circulation system (Mohamed, 2009). The vascular dilation and intra vascular haemolysis may also be responsible for the cellular degeneration and necrosis in the liver (Mohamed, 2009).

The toxicity effects of heavy metals and other pollutants on liver of fish have been studied by many workers. Velcheva *et al.* (2010) reported necrotic and hyperemic changes in the parenchyma of liver due to heavy metal pollution. Mohammed *et al.* (2013) reported the histopathological changes in the liver of *Clarias gariepinus* fish, collected from El-Rahawy drain, to include loss of cellular architecture of liver, vacuolar degeneration, pycnotic nuclei and focal areas of necrosis of hepatocytes. In this study,

leucocytes infiltration and hyaline degeneration were also detected in the hepatic tissue of fish while dilation of the central vein accompanied by blood congestion was detected due to heavy metal pollution. Further, Olojo *et al.* (2005) observed degeneration of hepatocytes and focal necrosis in the liver of *Clarias gariepinus* exposed to lead. Exposure of *Oreochromis niloticus* to copper sulphate was found to induce fatty degeneration, intense haemolysis and massive necrosis in liver parenchyma.

Mean liver index values for both sample group fall within class I (station 3) and class 2 (Station 1 and 2) representing slight to moderate histological alterations present. The results represent similar liver index values obtained by Marchand *et al*. (2008) and Botha (2010) for *C. gariepinus* from polluted aquatic systems in South Africa. These findings suggest that the organ is still in functional state. Although the results showed that fish from both study stations are similarly affected in terms of histology, the causative agents of these alterations remain unclear. It is known that histological alterations are not toxicant specific (Pheiffer *et al.,* 2014), and that identifying a single causative agent is complicated, especially in wild fish populations, as such fish are exposed to a combination of biological, physical and chemical variables (McHugh *et al.,* 2013). Nevertheless, histological changes have been associated with certain pollutant exposure, among these are DDT and its degradation products, petroleum compounds, naphthalene and phenol; other miscellaneous compounds; acid or alkaline pH; nitrogenous compounds NH_3^- and heavy metals.

As only the heavy metals in sediment sample from Lake Kanyaboli were determined as part of specific study, and showed a positive association with the histological alterations, the presence of other chemicals in the Lake Kanyaboli system that could have resulted in

the histological responses identified could not be conclusively established. Fish that display histological alteration are evidently unhealthy, they show structural abnormalities that may lead to the suppression or inhibition of physiological functions.

Multidimensional scaling analysis revealed a positive association between semi-quantitative histological index and health assessment index a result which was confirmed by the Pearson product moment correlation analysis. The reason for lack of association between semi-quantitative and the condition factor, Hepatosomatic index and splenosomatic index and the negative association condition factor and health assessment index, condition factor and hepatosomatic index and condition factor and splenosomatic index is unclear. One possibility is that the sizes of the organs (liver, spleen) are independent of the length and weight of the fish. Another possibility is that the environmental pollutants were harmful to the liver and spleen and led to the abnormality in the organ sizes. The latter hypothesis is supported by the positive association found between the semi-quantitative histological index and sediment and liver concentration of heavy metals (Fig. 4.3).

No significant associations were observed between hepatosomatic index and concentration of any selected heavy metals (Cd, Cr, Zn, Cu, and Pb). One possible reason for lack of association in the current study is that variations in exposure levels were not great enough to be determinative factor for hepatosomatic index and, thus allowing variation in other factors nutrition and disease to obscure any relationship. This line of reasoning is consistent with the highest HSI and SSI being associated with the highest heavy metal concentrations (Table 4.4) and the study by Vignier *et al*. (2004) in which

the HSI significantly increased in fish exposed to high concentration of heavy metals but not fish exposed to low concentration of heavy metals compared to the control group.

Interaction of various contaminants present in the study stations might be another reason for the absence of association between pollutants and the HSI. Since increases and decreases in HSI have been identified in fish following exposure to various toxicants, effects in different direction might offset each other once fish were exposed to a mixture of contaminants.

In summary, no clear change could be seen between the impacted and less impacted sites in HSI values of *C.gariepinus*. However, semi-quantitative histological index values appeared to be positively associated with concentration of heavy metals in sediments and fish liver, respectively. This suggest that exposure to elevated concentration of heavy metals in Lake Kanyaboli may cause increase in the liver histological alterations. Interestingly in this study, there was a negative association between the condition factor and the concentration of heavy metals in sediment and fish liver. This suggest that as the concentration of heavy metals reduces in the lake sediment and the fish liver, the condition of the fish gets better. Alternatively, as the concentration of the heavy metals in the sediment and fish liver increases the condition of the fish deteriorates.

Relationship between HSI or SSI and pollution were lacking within the study lake system. While the impacts of heavy metals on HSI and SSI might be apparent when contamination levels are very high, organo-somatic indices in general seem to be affected by too many non- contaminant variables to be useful in delineating mildly from moderately contaminated locations.

CHAPTER SIX

CONCLUSION & RECOMMENDATIONS

6.1 CONCLUSION

In this project, the biomarker approach based on histology-based fish health assessment index was studied to evaluate their usefulness in assessing pollutant exposure and effects in Lake Kanyaboli. Results from the study showed the following

1. Elemental analysis of the sediments and fish liver showed them to be enriched with heavy metals, polluting the Lake Kanyaboli, Cd, Cr, Cu, Zn and Pb were elements contributing most to the sediments pollution. The concentration of these heavy metals in sediments and fish liver differed significantly between the sampled sites hence rejection of the first null hypothesis.
2. This study demonstrated that HAI derived from the fish sampled have the potential use in assessing the fish health and therefore the environmental quality. This is due to the difference observed for the HAI score value for the three sites and hence the rejection of the null hypotheses that pollutant load has no significant effect on health of *Clarias gariepinus* in Lake Kanyaboli. This is a step forward towards HAI development as a bioassessment and monitoring tool for the Lake Kanyaboli especially so for the concentration of Zinc and Lead in sediments.
3. The hypotheses that condition factor, the organ weight (liver and spleen to the body weight ration) (biometric indices) of fish will not change which indicate no change in organ function in Lake Kanyaboli was rejected. *C. gariepinus* condition

factor, HSI and SSI showed some significant differences between some sites hence depicting fish were in poor health.

4. Although the alterations observed in this study were mild in terms of severity, they were nevertheless present. The presence of necrotic hepatocytes in some fish indicates pollution has affected the livers of these fish to the point where liver cells have started to die. In the specimen where necrosis was observed, they were only observed in focal areas which are why liver index values are at class 2 (tissue with moderate alteration). The multidimensional scaling analysis showed a positive association between the concentration of Pb, Zn and Cu in fish liver as well as the concentration of Cu, Cr, Zn, and Pb in sediments and the SQHI consequently, the Pearson product moment analysis confirmed the existence of the association between heavy metal concentration in sediments and fish liver and the SQHI hence the rejection of the fourth null hypotheses. This is a step forward towards the development of quantitative histological index that serves as an early warning system for bioassessment and monitoring for the Lake Kanyaboli.

6.2 RECOMMENDATIONS

Following the results of this study, the recommendations below are advanced:

1. Since there are several expected impacts from reclamation of Yala swamp for agricultural development activities and the intensification of agriculture in the upper catchment of Lake Kanyaboli, continuous assessment and monitoring of pollutants especially heavy metals in sediments as well as in fish liver in Lake Kanyaboli is recommended.

2. As a starting point towards bioassessment and monitoring of Lake Kanyaboli towards mitigation process which would result to improvement of its ecological system, the use of Health Assessment Index (HAI) is recommended especially so for zinc and lead concentrations in sediments.
3. The biometric indices for *Clarias gariepinus* were not within the accepted range thus suggesting that fish at the impacted stations showed poorer health status than from the reference station, although this trend was not expressed in every individual fish. The use of biometric indices as initial screening biomarkers to indicate exposure and effects of micro-pollutant is recommended.
4. Histological alterations were identified in the fish liver. The mean liver index for all study sites fall within class 2 with the exception of the liver index from control site which was in class 1. The results suggest that the liver histopathology of the *Clarias gariepinus* fish could be useful biomarker for bioassessment and monitoring of aquatic pollution in Lake Kanyaboli. Despite this, a more comprehensive chemical analysis of water and sediments samples including the measurements of endocrine disrupting chemicals is recommended for future studies to further investigate possible causative agents regarding liver alterations identified.

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APPENDICES

Appendix I: Health Assessment Index Data Sheet

Health Assessment Index (HAI) Data Sheet									
Site:									
Sampling No:									
Date:									
Specimen:									
Fish No.	**Gender**	**Eye**	**Skin**	**Fin**	**Opercula**	**Gills**	**Liver**	**Spleen**	**Parasites**
		Normal Exopthal Heamo Blind Missing Other	Normal Aberation Moderate Severe	Normal Erosion Severe	Normal Shortening Severe	Normal Frayed Clubbed Discolour Pale Other	Normal Slight disc Fatty Nodules/ cysts Focal disc Discolouration Other	Normal Granular Nodular Enlarged Other	No Few Moderate Severe
		Normal Exopthal Heamo Blind Missing Other	Normal Aberation Moderate Severe	Normal Erosion Severe	Normal Shortening Severe				

		Normal Exopthal Heamo Blind Missing Other	Normal Aberation Moderate Severe	Normal Erosion Severe	Normal Shortening Severe				
Notes:----------									

Appendix II: Histological Assessment Data Sheet

Liver						
n			Specimen no:			
RP	Functional unit	Alterations		a	w	ax w
CD	Blood vessels	Haemorrhage/hyperaemia/aneurysm	e.g. induce congestion	0	1	0
		Intercellular oedema		0	1	0
			RP INDEX	0		
RC	Liver tissues	Structural alterations	e.g. cord disarray & cell structure	0	1	0
	Hepatocytes	Plasma alterations	e.g. Granular degeneration/ intra cellular deposits	0	1	0
			Fatty degeneration (e.g. fatty change)	0	1	0
			Glycogen vacuoles	0	1	0
			Vacuolation	0	1	0
		Inter cellular deposits		0	1	0
		Increase in MMC		0	1	0
		Nuclear alterations	Pleomorphism/Chromatin clearing	0	2	0
			Pyknosis	0	2	0
		Atrophy		0	2	0
		Necrosis		0	3	0
	Bile ducts	Structural alterations		0	1	0
		Plasma alteration	Granular degeneration/ intra cellular deposits	0	1	0
			Vacuolation	0	1	0
		Intercellular deposits		0	1	0
		Nuclear alteration		0	2	0
		Atrophy		0	2	0
		Necrosis		0	3	0
			RP INDEX	0		
PC	Liver tissue	Hypertrophy		0	1	0
		Hyperplasia		0	2	0
		Wall proliferation	e.g. blood vessels	0	1	0
	Interstitial tissue	Hypertrophy		0	1	0

		Hyperplasia	e.g. cirrhosis	0	2	0
	Bile ducts	Hypertrophy		0	1	0
		Hyperplasia		0	2	0
		Wall proliferation		0	3	0
			RP INDEX			0
1		Exudate		0	1	0
		Activation of RES		0	1	0
		Infiltration	e.g. leucocytes	0	2	0
			e.g. granulocytes	0	2	0
			RP INDEX	0		
T		Benign		0	2	0
		Malignant		0	3	0
			RP INDEX	0		

Appendix III: Quality guidelines for metals in fish for human consumption as suggested by EU, 2006 and Wegner and Boman (2003)

Metals	Cd	Cr	Zn	Cu	Pb
Mg/g	5E-04		40-100	10-100	0.5-10
Site 1 (Kadenge)	0.22		13.40	2.88	5.40
Site 2 (Yala River mouth)	0.16		8.50	4.16	14.40
Site 3 (Gangu)	nd		7.12	2.12	1.63

Cd- Cadmium, Cr- Chromium, Zn- Zinc, Cu- Copper, Pb- Lead. nd- not detected

Appendix IV: Selected sediment quality guidelines (Source ANZECC, 2000)

Elements	Threshold value	Site 1	Site 2	Site 3
	Mg/g	Mg/g	Mg/g	Mg/g
Cd	10	0.77	0.60	0.49
Cr	26	7.10	7.48	4.26
Zn	123	81.23	87.09	79.61
Cu	16	20.43	18.61	14.03
Pb	35	14.38	155.56	19.83

Cd- Cadmium, Cr- Chromium, Zn- Zinc, Cu- Copper, and Pb- Lead
ANZECC- Australian and New Zealand Environment and Conservation Council