LEVELS OF TOTAL MERCURY IN FARMED AND WILD-CAUGHT *Oreochromis niloticus niloticus* (NILE TILAPIA), POND SEDIMENTS AND WATER IN THE MIGORI GOLD MINING BELT, KENYA

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DECLARATION

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

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DEDICATION

I dedicate this work and give special thanks to my family - the K'Ogollah family, and especially to my wife Betty, my sons Timela and Tawali; and to my loving mum *Min* Kola, without whose caring support it would not have been possible. Most importantly, all thanks and glory goes to God, the Almighty for the gift of life and good health.

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LIST OF ABBREVIATIONS

ANOVA Analysis of Variance

BCR	Community Bureau of Reference
CRM	Certified Reference Material
CRmw	Consumption Rate in meals/week
EDIm	Estimated Daily Intake per meal
FAO	Food and Agriculture Organization
Hg	Mercury
I_{Geo}	Geo accumulation index
JECFA	Joint FAO/WHO Expert Committee on Food Additives
KEMFRI	Kenya Marine and Fisheries Research Institute
PTWI	Provisional Tolerable Weekly Intake
RfDo	Reference Doses
THQ	Target Hazard Quotient
T-Hg	Total Mercury
USEPA	the United States Environmental Protection Agency
VGA	Vapour Generation Accessory
ww	Wet weight

ABSTRACT

Mercury is a well-known toxicant with a myriad of ill effects on human health. It occurs naturally in the environment at basal levels. Human activities, such as mercury use in artisanal and small scale gold mining is one of the major sources of environmental mercury pollution. Migori is renowned for artisanal gold mining. Inland fish farming is also practised in this area with Nile tilapia (*Oreochromis niloticus niloticus*) is the main fish reared. Studies have shown that up to 90% of the mercury used during gold panning in Migori is lost to the environment. Consequently, the mine tailings, soils and waters in these areas are heavily contaminated with mercury. Mercury is washed off to the nearby streams and rivers by run-water, thus extending the pollution farther to the water system. Inland fish farms and Lake Victoria draw their waters from these polluted streams and rivers with mercury being deposited in soil sediments, water and eventually taken up by planktons, insects and other lower organisms which form a major part of the diet for the tilapia fish. Mercury gets absorbed into the fish through feeds, skin and gills. In fish, the mercury is partitioned differently across various tissues depending on the partition coefficients of the tissues to the different mercury forms. Tilapia brain, liver and muscle tissues were selected for this study. Tilapia brain and liver have high-fat content hence are likely to concentrate high amounts of methyl- and other organic forms of mercury which are lipophilic. The liver is also the main organ for metabolism and elimination of the mercury from the fish. Tilapia fish muscle is the major part of consumed by man. Thus it is a tissue of interest in this study since its contamination poses a risk to human health. This study assessed the levels of total mercury (T-Hg) in fish pond sediments, water and tissues of farmed and wild-caught Nile tilapia (*Oreochromis niloticus niloticus*) in the Migori gold mining belt. The correlation between the mean T-Hg levels in the tilapia fish tissues and the mean T-Hg in pond water and sediments was evaluated. The potential risk to human health from the consumption of the fish was also

determined. Ten locations in Rongo and Nyatike sub-counties in Migori with known artisanal gold mining and inland fish farming activities were conveniently selected for the study. Five tilapia fish (irrespective of sex) were sampled from each site except Minyenya (where four fish were sampled). Two replicate samples of pond water and sediment were collected from each site except for the lake (soil and sediment not sampled). Each fish sample yielded one sample of brain, liver and muscle tissues. A 0.3 - 0.5g portions of the samples were homogenised and acid-digested to reduce all the mercury forms to mercury metal (this yield is referred to as total mercury (T-Hg) which was analysed using cold vapour atomic absorption spectroscopy and the mean T-Hg levels recorded in µg/g wet weight. All the data generated were organised, aggregated and mean measures established. Microsoft Excel (2016) and Statistical Package for the Social Sciences (SPSS, version 20.0) were used for statistical analysis. Data for mercury analysis was expressed as the mean± standard deviation. One-Way Analysis of Variance (ANOVA) was used to analyse the levels of T-Hg in fish tissues across the sites. Tukey's HSD test was used as a post-hoc test. Pearson's rank correlation and the t-test were used to determine whether there were any relationships between the various parameters in the study. Values of $p \leq 0.05$ were considered significant in all cases. Sediment quality was evaluated using a geo-accumulation index (I_{GEO}) while the estimated daily intake of fish per meal (EDIm), target hazard quotient (THQ), and the maximum allowable fish consumption rate (CRmw) were used as human health risk indices. Concentrations of mean T-Hg in sediments ranged from 0.208±0.000 to 1.113±0.008 µg/g wet weight (n=8, 95% CI); with six of the eight sites sampled being moderately polluted ($1 \leq I_{Geo} < 2$), whereas two sites (Minyenya and Kokaka) being strongly polluted ($3 \leq I_{Geo} < 4$). Mean T-Hg in the water samples ranged from 0.002±0.000 to 0.004±0.001 µg/ml wet weight (n=8, 95% CI) with all the sites having higher values (up to 40 times higher)

for T-Hg than the maximum contaminant level of 0.0001 µg/ml allowable for mean T-Hg in unpolluted surface water set by the Food and Agriculture Organization (FAO). The concentrations of mean T-Hg were highest in the tilapia brain tissues, ranging from 0.128±0.021 to 3.798±1.421 µg/g wet weight (n= 49, 95% CI); with the highest proportion (78%, 38/49 samples) having mean T-Hg levels above (up to eight times higher) the limits of 0.5 µg/g wet weight recommended as safe by WHO for consumption by the general human population. The mean T-Hg in tilapia muscle tissues ranged from 0.179±0.020 to 0.595±0.065 µg/g wet weight (n= 49, 95% CI) with 31% (15/49) of fish muscle tissues tested having the levels above 0.5 µg/g wet weight. Mean T-Hg levels were lowest in tilapia liver tissues, ranging from 0.103±0.118 to 0.588±0.374 µg/g wet weight (n= 49, 95% CI) with only 27% (13/49) of fish liver tissues tested having the levels above 0.5 µg/g wet weight. However, most of the tilapia fish samples (87.8% (43/49) of brain, 69.4% (34/49) of liver and (68.7% 34/49) of muscle tissues respectively had mean T-Hg above the 0.2 µg/g (wet weight) level recommended by WHO for at-risk populations (frequent fish eaters, people with renal and liver diseases, pregnant mothers and developing children). There were positive correlations between mean T-Hg levels in tilapia brain and muscle tissues and the mean T-Hg levels in fish pond sediments ($r=0.528$, $p<0.05$ and $r=0.524$, $p<0.05$ respectively). However, there was no significant correlation between the mean T-Hg content in soil sediments and the mean T-Hg level in fish liver tissues. There was a positive correlation between mean T-Hg levels in tilapia brain tissues and mean T-Hg levels in pond water ($r=0.402$, $p<0.05$) as well as between mean T-Hg levels in tilapia muscle tissues and mean T-Hg levels in pond water ($r=0.616$, $p<0.05$). However, there was no significant correlation between the mean T-Hg content in pond water and the mean T-Hg level in fish liver tissues. The estimated daily intake of fish per meal (EDIm) and target hazard quotient (THQ) for human consumption ranged

from 2.43-15.84 µg/g and 24.3-158.4 µg/g respectively while the maximum allowable fish consumption rate for humans in meals/week (CRmw) ranged from 1-4 whole fish. These findings show that the levels of mean T-Hg in tissues of Nile Tilapia in the Migori gold mining belt are above-recommended limits. Consumption of Nile tilapia, therefore, bears a significant risk of mercury exposure in frequent fish-eaters, pregnant women and children of developmental age in the Migori gold mining belt, but is safe for the general human population.

CHAPTER ONE

INTRODUCTION

1.1 Background Information and Justification

Mercury is a well-known toxicant with a myriad of ill effects on human health. It occurs naturally in the environment at basal levels (UNEP, 2002; 2013abc). Human activities, such as mercury use in artisanal and small scale gold mining is one of the significant sources of environmental mercury pollution (Gibb and O'Leary, 2014). Migori is renowned for small-scale gold mining. Inland fish farming is also practised in this area with Nile tilapia (*Oreochromis niloticus niloticus*) being the main fish reared (Githukia *et al.*, 2014). Studies have shown that up to 90% of the mercury used during gold panning in Migori is lost to the environment (Maroa, 2009). Consequently, the mine tailings, soils and waters in these areas are heavily contaminated with mercury (Mangati, 2005; Odumo *et al.*, 2011, Ngure *et al.*, 2014, Ogendi *et al.*, 2014, Odumo and Carbonell, 2014). The mercury is washed off to the nearby streams and rivers by runoff water, thus extending the pollution farther to the water system in the area. Inland fish farms and Lake Victoria draw their waters from these polluted streams and rivers with mercury being deposited in the soil sediments, water and eventually taken up by planktons, insects and other lower organisms which form a significant part of the diet for the tilapia fish (Boischio and Henschel, 2000; Gibb and O'Leary, 2014). Mercury gets absorbed into the fish through feeds, skin and gills where it is partitioned differently across various tissues depending on the partition coefficients of the tissues to the different mercury forms (Boeining, 2000; Park and Zheng, 2012). Tilapia brain, liver and muscle tissues were selected for this study. Tilapia brain and liver have high-fat content hence are likely to concentrate high amounts of methyl- and other organic forms of mercury, which are lipophilic (Park and Zheng, 2012). The liver is also the primary organ for metabolism and elimination of the mercury from the fish. Tilapia fish muscle is the major part of consumed

by man. Thus it is a tissue of interest in this study since its contamination poses a risk to human health (Boischio and Henschel, 2000).

Over the course of the last two decades, several studies have evaluated the environmental effects of heavy metals in water, sediment and aquatic life in Kenya (Ogola *et al.*, 2002; Campbell *et al.*, 2003; Mangati, 2005; Odumo *et al.*, 2011; Odumo and Carbonell, 2014; Ngure *et al.*, 2014 and Ogendi *et al.*, 2014). Ogola *et al.* (2002) reported that the concentration of mercury (Hg), lead and arsenic in soil and water samples collected from 11 mine sites along the Migori gold mining belt were above acceptable limits. Campbell *et al.* (2003) evaluated mercury content in several forms of fish from three rift valley lakes (Turkana, Naivasha and Baringo). They reported that with the sole exception of two *Hydrocynus forsakhlii* (elongate tiger) fish, the contents of mercury in several other forms of fish were above the WHO recommended levels.

In 2011, Odumo and other workers reported high levels of arsenic (As), copper (Cu), lead (Pb) and zinc (Zn) in 4 sites within the Migori gold mining belt (Odumo *et al.*, 2011). Later on, Odumo and Carbonell (2014) evaluated mercury concentrations in soil, lichens, and mosses within the Migori gold mining belt and reported that they exceeded critical values.

Ngure *et al.* (2014) evaluated the distribution of arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb) in the soil, stream water and whole samples of *Rastrineobola argentea* (silver cyprinid) fish in gold mining areas of the Lake Victoria basin. They reported that the concentration of arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg) in the fish exceeded the joint WHO/FAO (JECFA) maximum allowable concentration. In contrast, Ogendi *et al.* (2014) reported that the levels of cadmium (Cd), copper (Cu) and lead (Pb) in water and common carp (*Cyprinus carpio*) fish from Lake Naivasha in Kenya were significantly lower than the WHO/USEPA guidelines. However, with continued artisanal

gold mining in Migori County over the years, there is increased pollution of the area with mercury thus there was a need to undertake this study.

Artisanal mining in Kenya is a significant contributor to socioeconomic development in areas where it is practised (Ngure *et al.*, 2014). Nonetheless, it is considered a major source of pollution, second only to Agriculture (Ngure *et al.*, 2014). Migori County is home to a vibrant small-scale artisanal mining community where mercury (Hg) is used to extract gold (Au), as shown in Figure 1 below. However, this process generates large volumes of mine waste, tailings, and effluents which contain potentially harmful elements such as arsenic (As), lead (Pb) and mercury (Hg) (Ngure *et al.*, 2014; Gibb and O'Leary, 2014). These elements have the potential to bioaccumulate in aquatic ecosystems and may be subsequently transferred to humans via the food chain (Fallah *et al.*, 2011).

Over the last decade, aquaculture has proliferated in Kenya, mainly due to the Economic Stimulus Programme according to the report by Kenya Marine and Fisheries Research Institute (KEMFRI, 2017). About 160 constituencies, including many within the Migori gold mining belt, have benefited from this programme (Mwamuye *et al.*, 2012). The programme is mostly dependent on fast-growing forms of fish, such as the Nile tilapia (KEMFRI, 2017). However, little is known on the consequences of gold mining using mercury on the contamination levels of mercury in pond sediment, water and farmed as well as captured Nile tilapia in the region. It is against this background that the present study was aimed to assess the levels of total mercury on Nile tilapia reared in fish farms in gold mining areas of Migori County as well as the levels in pond water and sediment, and subsequently assess the potential risk to human health from consumption of the fish.

Figure 1: Use of mercury to extract gold during panning in Migori County.

1.2 Research Objectives

1.2.1 General Objective

The broad objective of this study was to assess the levels of total mercury in pond sediments, water and *Oreochromis niloticus niloticus* (Nile tilapia) tissues from gold mining areas of Migori County, Kenya and subsequently assess the potential risk to human health from consumption of the fish.

1.2.2 Specific Objectives

i. To assess the T-Hg contamination in tissues (brain, liver and muscle) of farmed Nile tilapia fish from the Migori gold mining belt as well as captured Nile tilapia from Lake Victoria in Kenya.

ii. To determine the T-Hg contamination in pond water and sediment collected from the Migori gold mining belt and assess how this contamination relates to pollution in the fish tissues

iii. To assess the risk to human health following dietary exposure to T-Hg in different tissues of Nile tilapia.

1.3. Hypotheses

1.3.1 Null Hypotheses

This study hypothesises that the mean T-Hg levels in the Nile tilapia fish tissues are equal to or below 0.5µg/g and 0.2µg/g wet weights for the general human population and at-risk populations, respectively. These are the maximum T-Hg concentration levels in fish tissues recommended as safe for human consumption by WHO (2016).

Thus the null hypotheses;

H_0; T-Hg $\leq$0.5µg/g wet weight and H_0; T-Hg $\leq$0.2µg/g wet weight for tilapia fish tissues

Additionally, this study hypothesises that the fish pond soil sediments and waters from the area are not polluted with mercury. That is, the mean T-Hg values are below the maximum allowable levels for T-Hg in sediments and water. Sediment contamination levels are evaluated using a geo-accumulation index (I_{GEO}).

Thus, the null hypotheses;

H_0; $I_{Geo} \leq 0$ (unpolluted/class 0) for soil sediments (Müller, 1969)

H_0; T-Hg $\leq$0.0001 µg/g for unpolluted surface water (FAO, 1993)

1.3.1 Alternate Hypotheses

The alternate hypotheses for this study are the converse of all the above stated null hypotheses, suggesting high contamination levels above maximum allowable limits of T-Hg in all the Nile tilapia tissues, pond sediment and water in the area.

Thus, the alternate hypotheses are;

H_a; T-Hg >0.5µg/g wet weight and H_a; T-Hg > 0.2µg/g wet weight for tilapia fish tissues for the general and at-risk human populations respectively (WHO, 2016)

H_a; I_{Geo} > 0 (polluted/class 1- 6) for soil sediments (Müller, 1969)

H_a; T-Hg > 0.0001 µg/g for unpolluted surface water (FAO, 1993)

CHAPTER TWO

LITERATURE REVIEW

2.1 Chemical Forms of Mercury

Mercury exists in several forms. It is a heavy metal and a component element of the earth. In its pure form, it is also called "metallic" or "elemental" mercury. Mercury is hardly ever found in nature in its elemental form but rather often in compounds and inorganic salts (UNEP, 2002). Naturally, Mercury occurs in the environment at deficient concentrations (UNEP, 2002; Göthberg and Greger, 2006) and exists in many forms, designated "forms" (UNEP, 2002). Inorganic mercuric compounds, also known as Hg salts, include mercuric sulphide, mercuric oxide and mercuric chloride. Most mercury salts are in powder or crystalline form (UNEP, 2002; 2013abc).

Compounds formed from a combination of mercury and carbon are called “organic” mercury compounds (organomercurials). The number of organic Hg compounds is potentially significant and includes dimethylmercury, methylmercury, ethylmercury and phenylmercury, the most common being methylmercury (UNEP, 2002; 2013abc). Both phenylmercury and methylmercury exist as salts (such as phenylmercuric acetate and methylmercuric chloride).

In this study, all the mercury forms in the sample analytes were reduced to elemental (metallic) mercury before being swept into the absorption cell for analysis. The resulting mercury yield referred to as total mercury (T-Hg) therefore gives the total concentration of all chemical mercury forms in the samples (USEPA, 1998a; USEPA, 1998b; Perkin-Elmer, 2011).

2.2 Mercury Transformation in the Environment

There are several natural forms of mercury in the environment (Ki-Hyun *et al.*, 2016). The most common natural forms are methylmercury, metallic Hg, mercuric chloride and mercuric

sulphide. Microorganisms and natural processes can convert environmental Hg from one type to another (Driscoll *et al.*, 2013; Zheng *et al*., 2012).

Elemental Hg in the environment can be converted into inorganic Hg forms, making way for deposition of emitted elemental mercury Hg (Driscoll *et al*., 2013; Zheng *et al*., 2012). Methylmercury is the organic form of Hg most frequently generated by microorganisms and natural processes (USEPA, 2001a). Edible mammals living in fresh water, salt water and marine bodies have natural mechanisms to build up (bioaccumulate/biomagnify) methylmercury levels to levels up to a thousand times greater than that of the surrounding water which raises a major health hazard concern (Heath, 1987; Whalin *et al.*, 2007).

It is impossible to break down or degrade Hg into a harmless substance for even in its simplest form, elemental Hg, it still poses a risk of causing harm to both humans and the environment. Mercury, once released from the earth's crust, mineral deposits or fossil fuels into the biosphere, has high mobility, cycling between the surface of the earth and the atmosphere (UNEP, 2002; 2013abc). The primary natural reservoirs for Hg are the soils, water bodies and bottom sediments.

2.3 Significance of Mercury Chemical Forms and Transformation

The different chemical forms Hg exists in plays a critical influence in the toxicity and exposure of Hg to living organisms (Heath, 1987; UNEP, 2002; 2013abc). For instance, the forms affect the physical availability of exposure – Hg that is bound tightly to in-absorbable material cannot be taken up readily (e.g. into the organism's bloodstream). Likewise, it affects the transport inside the organism to the target tissues – for example, the crossing the blood-brain barrier or intestinal mucosa; its toxicity; its accumulation, biomodification, detoxification in tissues and excretion from tissues; its biomagnification as it goes up the trophic levels of the food chain (particularly for methylmercury) (Park and Zheng, 2012).

The chemical forms of Hg also determine its transportation within and between environmental compartments, including the atmosphere and oceans, to mention but a few (Ki-Hyun *et al.*, 2015). For example, how far Hg emitted from the source is transported in the air is determined by its chemical form. Moreover, the controllability of Hg emissions to the atmosphere highly depends on its form. For instance, some control devices (e.g., wet scrubbers) capture inorganic mercuric compounds emissions (such as mercuric chloride) reasonably well, while most emission control devices tend to be slow (Ki-Hyun *et al.*, 2016).

2.4 Sources and Toxicology of Mercury

Different chemical forms of Hg have different levels of toxicity – thus, elemental Hg, organic mercuric compounds and inorganic mercuric compounds exhibit varying symptoms and signs following exposure (Rice *et al.*, 2014). The different forms of Hg also have various sources of exposure.

Exposure to alkylmercury compounds, majorly methylmercury, is mainly dietary, especially via fish and other seafood (Evans *et al.*, 1993; Dorea, 2003, Ki-Hyun *et al.*, 2016). Exposure to elemental Hg vapour is majorly from dental amalgams for the general population, but exposure in the line of work in some cases may exceed this by a great deal. Exposure to inorganic mercurics is mainly dietary (Gibb and O'Leary, 2014). However, a proportion of the population gets exposed to inorganic or elemental Hg from using cosmetics containing mercury, and ritualistic/ cultural use mercury or use in traditional medicine (Ki-Hyun *et al.*, 2016).

Methylmercury is a neurotoxicant (Davidson *et al.*, 1998; Debes *et al.*, 2006; Davidson *et al.* 2011). Dietary exposure of methylmercury to pregnant women can lead to subtle, long-term effects on the developing brain (Myers *et al.*, 2003). It readily crosses the blood-brain barrier and the placental barrier, resulting in adverse effects on the developing brain. The foetus,

new-born and young children are considered at-risk populations (ARPs) since they are more susceptible to mercury exposure due to their developing nervous system. Small increases in methylmercury exposure could also have adversely affected the cardiovascular system (Park and Zheng, 2012; Rice *et al.*, 2014; Gibb and O'Leary, 2014; Ki-Hyun *et al.*, 2016).

Exposure to elemental mercury is mainly through vapour inhalation. Up to 80% of inhaled vapours are absorbed in the lungs (Ki-Hyun *et al.*, 2016). The vapour readily crosses the blood-brain barrier causing toxicity to the nervous system. It, however, does not readily penetrate intestinal membranes. Elemental Hg can undergo oxidation in body tissues to the inorganic divalent form (Park and Zheng, 2012).

Inhalation of mercury vapours has been shown to cause neurological and behavioural disorders in humans with symptoms such as tremors, insomnia, neuromuscular changes, emotional lability, headaches and memory loss (Park and Zheng, 2012). It also causes effects on the thyroid and kidney and death in cases of high exposure. Metallic mercury and inorganic mercury salts classified as carcinogenic compounds, but methylmercury is considered potentially carcinogenic to humans (Rice *et al.*, 2014).

2.5 Uses of Mercury

Despite its potential risks, Hg is still useful in various products and processes worldwide. Elemental mercury is used in the mining of silver and gold, especially in artisanal and small-scale setups (UNEP, 2002; 2013abc) such as in Migori gold mining belt; production of vinyl chloride monomer and chlor-alkali; and in various products such as manometers, electrical switches, thermometers, dental amalgams and fluorescent lamp bulbs. Compounds of mercury are also used in making some pharmaceuticals, paints, batteries, as a preservative in vaccines, pharmaceuticals as well as industrial catalysts and laboratory reagents (UNEP, 2002; 2013abc).

2.6 Mercury Release to the Environment

Globally, the major sources (up to 50%) of mercury released to the environment are through natural processes such as volcanic activity and release from leaching from certain soils (UNEP, 2002; 2013ab). Anthropogenic activities account for the remaining 50% of mercury released to the environment. This release occurs during production, use or after disposal of products and wastes containing mercury. The mercury is released through various industrial sources that result in the release of mercury impurities in input materials such as fuels (UNEP, 2002; WHO, 2016). Examples of such sources include metal smelters, coal plants, and cement factories; these are classified as among the sources with the highest emissions of mercury. The environment is thus contaminated from such discharges, resulting in human exposures. Emission and exposure levels from a given facility are dependent on several factors, including levels of mercury in the inputs (such as fuel or feedstock).

In Migori County, human activities such as the use of mercury during artisanal and small-scale gold mining are the major source of mercury contamination to the environment. Possible deposition of mercury from the air and dust re-mobilised from far off sources of contamination cannot be ruled out since mercury can be carried in the air over long distances of up to 880 kilometres from the contamination sourced (UNEP, 2002). Agricultural inputs and feeds are also possible sources of mercury contamination in the area.

2.7 Routes of Exposure to Mercury

Mercury bioaccumulates and biomagnifies up the food chain, and human beings are majorly exposed to methylmercury through diet, primarily through the consumption of fish and other fish-consuming animals (e.g. marine mammals) (Dorea, 2003, WHO, 2007, 2008; Park and Zheng, 2012; UNEP 2013ab; Gibb and O'Leary, 2014; Rice *et al.*, 2014; Ki-Hyun *et al.*, 2016). The main route of exposure to elemental mercury is through inhalation of air during industrial activities and from dental amalgams (WHO, 2007). Occupational exposures may

occur where mercury or its compounds are produced, used in production processes, or incorporated in final products (WHO, 2008). Such occupational exposures have been reported from mercury mines, thermometer and sphygmomanometer factories, mercury refineries, mercury-based small-scale gold and silver mining, chlor-alkali plants, dental clinics which poorly handle mercury and production of mercury-based chemicals. Additionally, exposures to elemental mercury may also occur following the use of mercury in rituals and cultural practices, mercury-containing skin-lightening cosmetics, mercury-containing traditional medicines, and accidental mercury spillage. Likewise, there are minor exposures to other forms of organic mercury from the use of thimerosal (ethylmercury thiosalicylate), which is usually used as a preservative in some vaccines and other pharmaceuticals (UNEP, 2013abc).

In the Migori gold mining belt, human beings are majorly exposed to methylmercury through diet, primarily through the consumption of contaminated fish (WHO, 20077; Park and Zheng, 2012; UNEP 2013ab; Gibb and O'Leary, 2014; Rice *et al.*, 2014; Ki-Hyun *et al.*, 2016). The main route of exposure to elemental mercury is through inhalation of air during artisanal gold mining using mercury (where the mercury-gold amalgam is heated to vaporize the mercury and leave the gold nuggets) (Ogola *et al.*, 2002; Campbell *et al.*, 2003; Mangati, 2005; Odumo *et al.*, 2011; Odumo and Carbonell, 2014; Ngure *et al.*, 2014 and Ogendi *et al.*, 2014). There is also a risk of mercury exposure through the skin of the mine workers in the region as they stir the mercury-gold amalgam with their naked hands during panning as shown in Figure 1 (page 19).

2.8 Effects of Mercury on Human Health

Every human being is naturally exposed to low mercury levels, but the occurrence and severity of resulting adverse health effects depend on many factors (Kariuki, 2002). These

include the chemical form of mercury; the developmental stage of the person exposed (the foetus being the most susceptible); the duration, dose and route of exposure. Dietary patterns are also key; a fish-eating population is generally at a higher risk of exposure (Gibb and O'Leary, 2014; Rice *et al.*, 2014; Ki-Hyun *et al.*, 2016).

Primarily, mercury and its compounds' toxicity targets the nervous system, the cardiovascular system and the renal system. In overall, developing organ systems such as the developing foetal nervous system are most susceptible to mercury toxicity. Other systems may also be affected; the circulatory, gastrointestinal, respiratory, immune, and reproductive systems (Rice *et al.*, 2014; Ki-Hyun *et al.*, 2016).

Neurotoxicity, especially of the developing nervous system appears to be the most serious toxicological endpoint caused by exposure to elemental mercury and methylmercury (Davidson *et al.*, 1998; Debes *et al.*, 2006; Davidson *et al.* 2011), whereas kidneys' damage is the main result in exposure to inorganic mercury compounds (UNEP, 2002; WHO, 2016).

Several media reports have been made on the toxicity and effects of mercury on the people in the Migori gold mining belt. Media outlets such as Reuters (2018), Citizen online (2018) and the Star Newspaper (2019) have reported cases of mercury toxicity amongst the mine workers. These include health problems, such as weight loss, body weakness, trembling hands, cancer and even death.

2.9 Populations Susceptible to Mercury Toxicity

Susceptible populations can be divided into two, namely, those who are more sensitive to toxic mercury effects and those who are more exposed to higher mercury levels (UNEP, 2002). The foetus, the new-born and children, are susceptible due to the sensitivity of their developing nervous system. On top of *in utero* exposure, neonates can be further be exposed through contamination in breast milk. Thus, it is paramount to enhance awareness of the

potential risks of methylmercury to women who might become pregnant, pregnant women and new mothers. Individuals with liver, kidney, nervous system and lung diseases are also susceptible to mercury toxicity (UNEP, 2002).

The other subpopulation that may be highly susceptible is those exposed to higher levels of methylmercury in the diet. This exposure is due to fish and seafood consumption, especially if consumed in large quantities and regularly (Rice *et al.*, 2014; Ki-Hyun *et al.*, 2016).

Individuals with dental amalgams are generally more exposed to elemental mercury than those without. High exposure levels can also be found in studies with high occupational exposure, and those who use products containing mercury (such as some skin lightening creams and soaps), mercury-containing traditional medications, or use mercury for cultural and religious purposes (WHO, 2007; UNEP, 2002; UNEP 2013a).

In the Migori gold mining belt, there are several at-risk populations (ARPs) to mercury toxicity (Kariuki, 2002). Most notably, pregnant mothers, foetuses and children of developmental age form a major proportion of the ARPs in this area. From the demographic records, pregnant women and children below five years are 4.4% and 19.4% respectively of the general human population in the area (MCG-AWP, 2018). Moreover, the mine workers, who mostly handle the mercury with their naked hands (as shown in Figure 1 - page 19) are also at risk to mercury exposure (Ngure *et al.*, 2014 and Ogendi *et al.*, 2014). Since fish consumption is popular in the area (Githukia *et al.*, 2014), a major part of the community are frequent fish eaters and are thus at increased risk to exposure to mercury in contaminated fish (Rice *et al.*, 2014; Ki-Hyun *et al.*, 2016). Other subpopulations that may be at risk in the area are those with kidney, liver nervous system and lung diseases (UNEP, 2002).

2.10 Mercury Reference Levels

Various countries and international organisations have come up with reference levels for daily or weekly mercury exposure. Based on Available data and research, these levels of exposure are estimated to be without notable risk to human health. Reference levels set methylmercury intake range from 0.7 to 2 μg/kg body weight per week (UNEP, 2002; WHO, 2007; UNEP 2013a). There are also reference levels set to protect against adverse effects of inhaling elemental mercury and ingesting inorganic mercurics.

The Joint Food and Agricultural Organization (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA), established provisional tolerable weekly intakes (PTWIs) at 5 μg/kg body weight (for total mercury) and 1.6 μg/kg body weight (for methylmercury) (UNEP, 2002; UNEP 2013a; WHO, 2016). The PTWI is defined as the amount that can be consumed every week throughout one's lifetime without significant effects on health. Its represents admissible human exposure every week while ensuring protection to the most susceptible subpopulations, to contaminants that cannot be avoided through the consumption of otherwise wholesome and nutritious foods.

Since fish is the main route of exposure to methylmercury in human, Governments usually set legal limits for the maximum allowable mercury or methylmercury levels in marketed fish. For instance, Codex Alimentarius sets guideline levels of 0.5 mg/kg of methylmercury in non-predatory fish and 1 mg/kg in predatory fish. The US FDA has set a limit of 1 mg/kg methylmercury in finfish and shellfish (USEPA, 1997). The European Community states a threshold of 0.5 mg/kg mercury in fish products (with a few exceptions), and Japan allows up to 0.4 mg/kg total mercury (or 0.3 mg/kg methylmercury) in fish in its market (UNEP, 2002; 2013a).

Moreover, some Governments and international organisations provide advice on diet to guide the consumption of certain types and amounts of fish. These guidelines aim to help limit mercury exposures, and both the benefits and risks of consuming the given fish to the particular population is considered. This advice serves to guide on the amounts, types and frequency of fish consumption that is deemed safe or potentially least harmful to the susceptible population (UNEP, 2002; 2013ab)

For this study, the maximum allowable T-Hg concentration levels in the Nile tilapia fish tissues were taken as 0.5µg/g and 0.2µg/g wet weights for the general and at-risk human populations, respectively. These are the maximum T-Hg concentration levels in fish tissues recommended as safe for human consumption by WHO (2016). Additionally, the mean T-Hg levels in fish pond sediments were analysed based on their geochemical accumulation indices, as previously described by Müller (1969) and classified accordingly in classes from class 0 (unpolluted) to class 6 (extremely polluted). The maximum allowable values for mean T-Hg in pond water was taken at 0.0001µg/g for unpolluted surface water as set by FAO (1993).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Ethical Considerations

Approval to undertake the study was approved by the Graduate School of the University of Nairobi (Appendix 1). Ethical approval was obtained from the Biosafety, Animal Care, and Use Committee of the Faculty of Veterinary Medicine, University of Nairobi; REF: FVM BAUEC/2018/148 (Appendix 2). Extreme caution was exercised in handling concentrated mercury reagents and acids. Good laboratory practices (GLPs) were observed (use of gloves, gas masks, overalls and fume chamber) at all times. The working condition of the fume extraction system within the laboratory was ascertained before the commencement of the study through the laboratory's standard operating procedures (SoP) for fume extraction system. The thesis write-up was subjected to a plagiarism screening and the report is as shown in Appendix 4. A manuscript has been developed from the study and submitted for publication (Appendix 5).

3.2 Pre-treatment of equipment and sample bottles

Precautionary steps, as described by Shafer *et al.* (1997), were taken before using the equipment or sample collection bottles. Briefly, all equipment used for sample collection and storage of sediment, water, and fish samples were pre-cleaned using high-purity nitric acid and rinsed with sufficient quantities of reagent water. This cleaning was done to ensure that they were free of trace metals. After cleaning, the bottles were stored in double-bagged zip-lock polyethene bags to ensure that no detectable metal contaminants were present in the sampling equipment.

3.3 Study Area and Sampling Sites

The Migori gold mining belt covers five sub-counties, namely Suna West, Nyatike, Rongo, Kuria West, and Kuria East within Migori County (Figure 2).

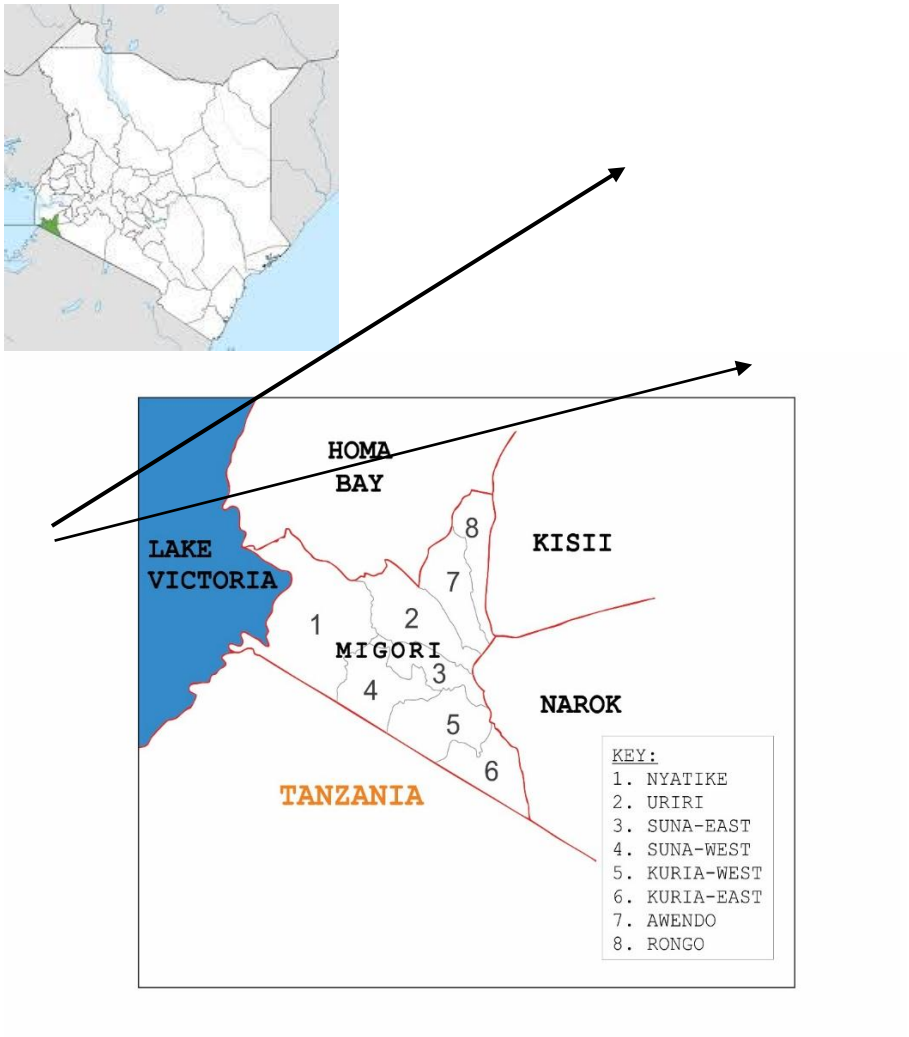


Figure 2: Sub-counties in Migori County, Kenya

The main gold mining sites within the County are situated in Rongo and Nyatike sub-counties: Macalder, Osiri, Mikei, Masara, Kitere, and Namba, as shown in Figure 3.

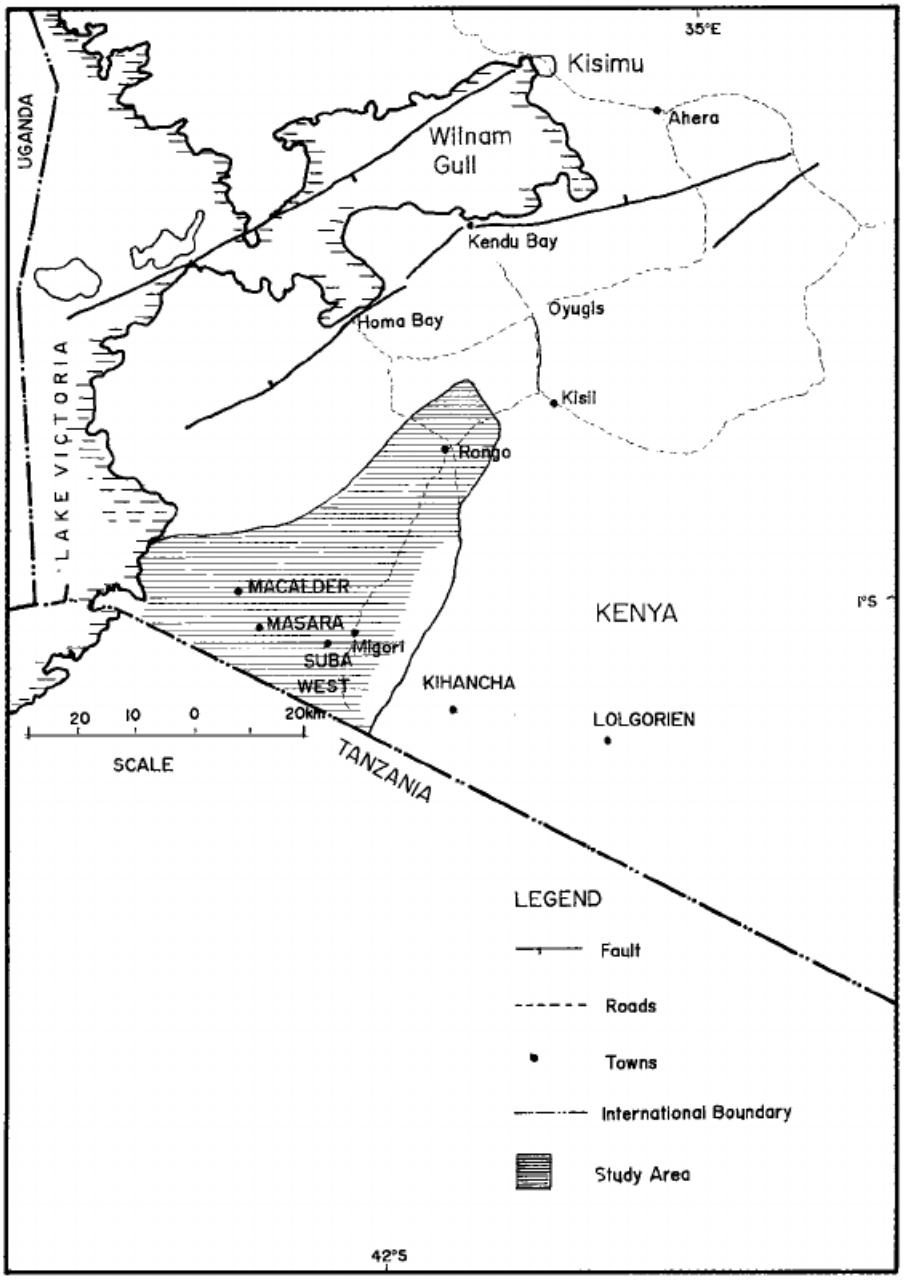


Figure 3: Major gold mining sites within Migori County, Kenya

Adapted from Ogola et al. (2002)

Apart from mining, other economic activities undertaken in the region include livestock farming, maize, tobacco, and sugar cane farming. The main rivers that drain the region are Mara, Kuja and Migori (Odumo and Carbonell, 2014).

Rongo and Nyatike sub-counties were selected for sampling. These regions were chosen because, in addition to being within the gold mining belt, inland fish farming is widely practised in the region (Figure 3 and 4). Ten sites namely Minyenya, Kamagambo North, Masara, Nyabisawa, Ndiwa, Kokaka, Luanda Nyira, Kamagambo South, Siginga beach and Sori beach located within the two sub-counties (Figure 4) and bearing coordinates between 0°6'11.16''0°52'51.6''S and 34°7'8.434°37'55.2''E were purposively selected based on proximity to active gold mining areas.

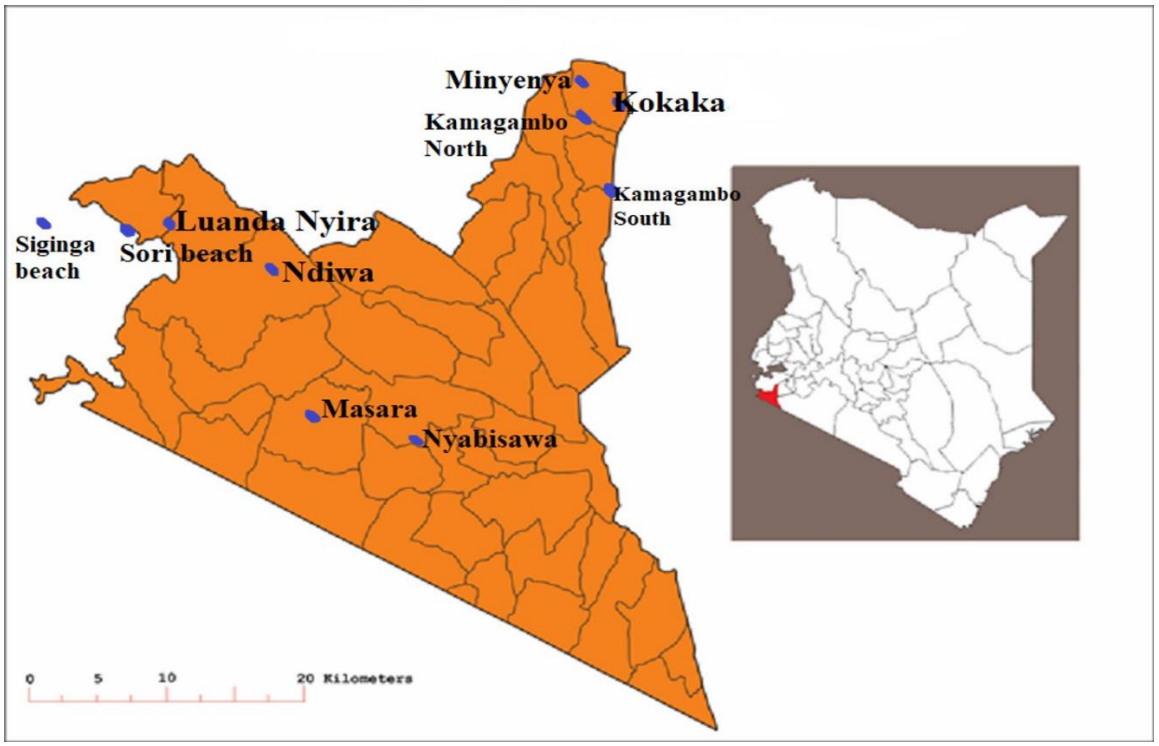


Figure 4: Map of Migori County showing the sampling sites

Eight sites within the gold mining belt (four sites in Rongo and Nyatike sub-counties respectively) and two fish landing sites on Lake Victoria (Siginga beach and Sori beach) were conveniently selected for sampling (Figure 3 above). Samples were collected in November and December 2015. A total of 163 samples (147 fish, eight water and eight sediment samples) were obtained, as shown in Table 1.

Table 1: Total number of sediment, water and Nile tilapia tissue samples from the ten sites in the study area.

Type of sample	Samples from sites in Rongo	Samples from sites in Nyatike	Samples from sites on Lake Victoria	Total number of samples
Sediment	4	4		8
Water	4	4		8
Fish muscle	19	20	10	49
Fish liver	19	20	10	49
Fish brain	19	20	10	49
Total	65	68	30	163

3.4 Sediment Sampling

By use of plastic trowels, near-surface (the top 5 cm) of fish pond sediments weighing approximately 1000g was taken from each of the selected sites as previously described by Campbell *et al.* in 2003. These were then packed in plastic Biological Oxygen Demand (BOD) bottles and labelled (location, sample type and the date of collection). They were then kept on ice and transported to the laboratory and stored at 20° C for 11 months until the time of analysis. Sediment sampling was not done from Lake Victoria due to resource limitations and sampling complexity involved since the lake has a massive area from which to sample. A total of 8 pond soil sediment samples were taken for analysis (Table 1).

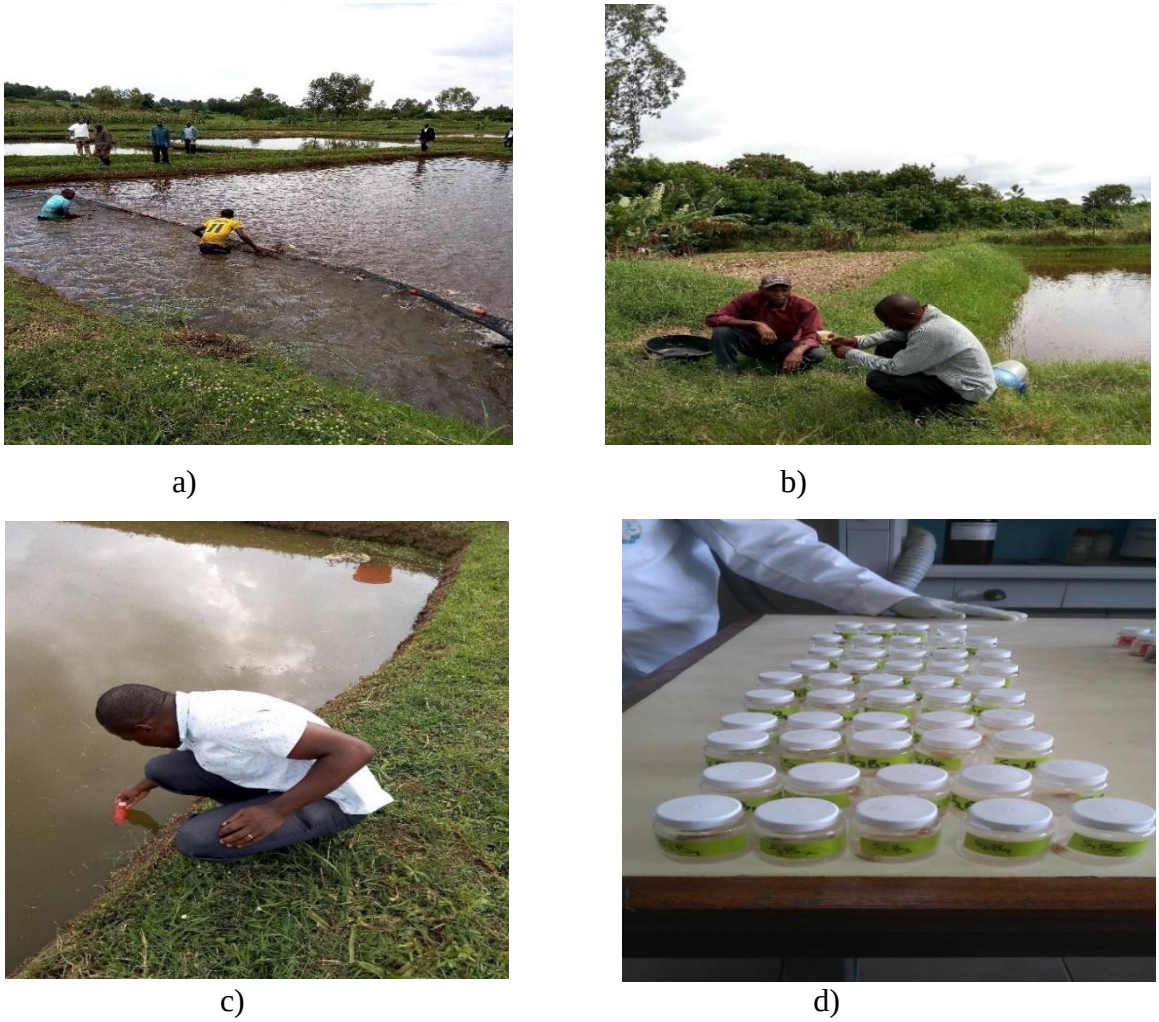
3.5 Water Sampling

A total of 8 fish pond water samples were obtained from the study area; water samples were not obtained from the lake (Table 1) due to the complexity of the sampling protocol involved when taking samples from the lake and resource limitations during the study. Trace metal clean procedures as described by Shafer *et al.* (1997) and Shelton and Capel (1994) were used to collect water samples. Briefly, water samples were collected in 250 ml metal-free

plastic bottles. They were then acidified to a pH below 2 using ultrapure nitric acid (HNO_3) to prevent adsorption of potentially harmful elements onto the interior walls of the storage bottles as well as to minimize microbial activity (Figure 5b). Upon arrival at the laboratory, they were filtered through a 0.45µm pore paper filter (Whatman) and stored in 125 ml metal-free plastic sample bottles and frozen at -20° C until the time of analysis. Mercury in the filtrate, also referred to as "dissolved" mercury was of particular interest in this study since it is more likely to have measurable biological effects on aquatic organisms (Shafer *et al.*, 1997).

3.6 Collection of Fish Samples

By use of gillnets (as shown in Figure 5a), five tilapia fish (regardless of their sex) were taken from each site except the site at Minyenya where four fish were sampled (Table 1).



a) b)

c) d)

Figure 5: shows (a) fish sampling using gill nets, (b) water sampling and filtering, (c) measuring pond water pH and temperature and (d) labelled Nile tilapia brain samples

Guidelines on the humane harvesting of fish as earlier described by Hill (2014) were used to euthanize the collected fish. A two-step process involving electro-narcosis and asphyxiation was used. Fish were initially stunned in an electric field of 2.5V/cm at 1000 Hz to make them insensible to pain. The absence of eye-roll reflex when the fish were moved from side to side was used as a confirmation that insensibility had been achieved. Death was then induced by asphyxiation in the air for 10 seconds and was confirmed by the lack of movement of the

operculum. A 10 cm^2 sample of muscle tissue was taken from each fish using methods earlier described by Campbell *et al.* (2003). Liver and brain tissues were similarly harvested. The collected samples were then transferred to plastic sample bottles which were labelled (Figure 5d above), and packed in self-zipping polyethene bags, frozen and transferred to a -20° C freezer where they were stored awaiting analysis.

Other physicochemical characteristics of the ecosystem where these inland fish ponds were located were also described and noted (Appendix 2). These include the fish pond pH, and temperature (Figure 5c above), the water source for the fish ponds, frequency of water top-up for the fish ponds, type of fish culture practised in the ponds, mean weight, age and length of the sampled tilapia fish. These are some of the parameters that are postulated in this study to directly/indirectly affect the levels of mercury in fish tissues and may form the basis of further research.

3.7 Reagents

All chemicals and reagents used were of analytical grade (Merck, Germany; Sigma-Aldrich, France; Central Drug House, India; Fisher Scientific, UK). Double distilled, de-ionised water was used for preparing working solutions and for all analytical work. Standard stock solutions of mercury were made from a high purity standard stock solution with a concentration of 1000 parts per billion (ppb) and were diluted to the corresponding mercury working standard solutions (i.e.10 ppb, 20 ppb, and 30 ppb). These working solutions were freshly prepared daily by diluting an appropriate aliquot of the stock solution using 1M hydrochloric acid (HCl; Sigma-Aldrich) and diluting the resulting solution to 100 ml with reagent water. Standard reference material for mercury in fish, i.e. the Community Bureau of Reference (BCR) – 463 (European Commission), was analysed to ascertain the accuracy and precision of the experimental procedure. Alkaline solutions of sodium borohydride ($NaBH_4$)

were freshly prepared daily by dissolving 1.0 g of $NaBH_4$ (Merck), and 0.25g of sodium hydroxide (NaOH) pellets (Merck) in 500 ml of distilled water. 3% v/v of HCl (Sigma-Aldrich) was used in the preparation of the carrier gas (Argon C45). Stannous chloride ($SnCl_2$) was freshly prepared by dissolving 62.5 g in 50 ml of 6 M HCl, the solution boiled for about 5 minutes, cooled, and nitrogen bubbled through it to expel any impurities of mercury. For sample digestion, 11 M nitric acid (HNO_3; Merck), 18 M perchloric acid ($HClO_4$; Merck) and HCl (Sigma-Aldrich) were used. Fused alumina anti-bumping granules (Merck) were used to avoid foam formation during sample digestion.

3.8 Equipment and Apparatus

All glassware used in the analysis were soaked overnight in 10 % (v/v) nitric acid (HNO_3), followed by washing with 10% (v/v) hydrochloric acid (HCl). They were then rinsed with double-distilled water and dried before use. Samples were weighed on an analytical balance, and sample digestion was carried out in a steam bath (DK Heating Digester from Velp Scientifica) in the confines of a fume hood. A Varian Model Spectr AA 220Z atomic absorption spectrometer (Figure 6 below) equipped with a mercury hollow cathode lamp was used for the analysis of the total mercury content of samples. Flow injection and cold vapour generation were done via a Varian model vapour generation accessory (VGA) 77. The analytical wavelength and slit widths were 253.7 nm and 0.5 nm, respectively. The Varian model, Spectr AA 220Z software, was used to monitor the output.

Figure 6: The Varian Model Spectr AA 220Z atomic absorption spectrometer fitted with Varian Model vapour generation accessory (VGA) 77 used for the analysis.

3.9 Sample Preparation and Digestion

All samples, certified reference materials, standards, reagent blanks, and spiked samples were processed using methods of the United States Environmental Protection Agency (USEPA) and analytical methods for atomic absorption spectroscopy by Perkin-Elmer (USEPA, 1998a; USEPA, 1998b; Perkin-Elmer, 2011) with minor modifications. Briefly, a top pan analytical balance was calibrated before weighing a batch of samples. Samples were removed from the freezer and allowed to thaw for about an hour. A batch of samples (approximately 20 in number) was digested simultaneously. A 0.3 - 0.5 g aliquots of well-homogenised samples were weighed and placed at the bottom of glass digestion tubes. For samples that were less than 0.5 g (particularly the brain and liver tissues), the whole sample was processed. Nine millilitres of concentrated nitric acid (HNO_3), 3 ml perchloric acid ($HClO_4$) and 1 ml of HCl (to stabilise the pH of the matrix) was slowly added to the glass digestion tubes in a fume

hood. The tubes were allowed to stand at room temperature (in the fume hood) until the initial reaction subsided (about 15 minutes). Spoon scoops of fused alumina anti-bumping granules (Merck) were added to the solutions to prevent them from bumping and spilling over. Glass (Soselex) columns were fixed on top of the glass digestion tubes to prevent spilling over in the event of frothing. The tubes were then placed on top of a steam bath unit (DK Heating Digester; Velp Scientifica) which was programmed to heat gradually to 150 °C over 10 minutes. This heating was maintained for 120 minutes to complete dissolution. The tubes were then removed from the steam bath, and the solutions allowed to cool to room temperature over 30 minutes. The solutions were then carefully transferred into 100 ml flat-bottomed volumetric flasks, the tubes and columns rinsed thoroughly with small amounts of distilled water and the resultant contents transferred into the flat-bottomed volumetric flasks. Six millilitres of saturated potassium persulfate ($K_2S_2O_8$) and 30ml of potassium permanganate ($KMnO_4$) was added to each solution and slightly shaken to mix. The resultant solutions were left to stand for 40 minutes. Additional portions of the $KMnO_4$ solution were gradually added until the resulting purple colour persisted for at least 15 minutes. After thorough mixing, six mL of sodium chloride-hydroxylamine sulphate was added to each solution to reduce the excess permanganate (this was confirmed by the colour change from purple to colourless). Reagent water was then added to the mixtures up to the 100 ml mark, and treated samples were then filtered through grade 541 (diameter 110 µm) filter paper (Whatman). Five millilitres of stannous sulphate was then added to each of the treated samples. After that, each sample bottle was attached immediately to the aeration apparatus (one at a time) of the cold vapour atomic absorption spectrophotometer ready for analysis (USEPA, 1998a; USEPA, 1998b and Perkin-Elmer, 2011).

3.10 Analytical Quality Control

Precautionary steps were taken to rule out any interference that may have arisen in the course of running the analyses. Briefly, blanks were analysed in order to ensure that all the materials (solvents, reagents, glassware, and other sample processing hardware) were free from artefacts or interferences which may have had the potential to compromise the integrity of the analysis. Interference from sulphide was minimised by the use of potassium permanganate ($KMnO_4$). Excess hydroxylamine sulphate reagent (about 25 mL) was used to ensure that free chlorine was absent in the mixture before the mercury was reduced and swept into the cell (USEPA, 1998a; USEPA, 1998b) since chlorine gas absorbs light at 253.7nm, which is close to 253nm - the wavelength at which mercury absorbs light. Also, the dead air space in the BOD bottle was purged before adding stannous sulphate. A preliminary run using reagent water was also used to rule out interference by volatile organic materials which absorb at wavelengths close to the wavelength at which mercury absorbs. Moreover, the accuracy of the procedure was determined by analysing three certified reference materials (CRMs) namely; Tuna fish muscle fapas CRM, BCR 463 from Community Bureau of Reference - European Commission, vegetable puree CRM (EU) and fish CRM (EU). Recovery studies were performed by adding a known amount of standard solution of mercury chloride to spiked samples, which were then taken through the digestion procedure. The concentration of mercury in the resulting solutions was then analysed in order to assess the mercury concentrations in the tilapia tissue samples, factoring in the dilution factors in the sample mixtures (USEPA, 1998a; USEPA, 1998b and Perkin-Elmer, 2011).

3.11 Analysis of Mercury in Collected Samples

The optimum operating temperature of the Cold Vapour Atomic Absorption Spectrometer (CVAAS) instrument was set at 18 °C, and the circulating pump was adjusted to pump at the rate of 1 L/min continuously (Perkin-Elmer, 2011). Maximum absorbance was noted within

30 seconds. The bypass valve was then opened, and aeration continued until absorbance returned to the minimum value. The bypass valve was then closed, the fritted tubing removed from the BOD bottle, and aeration continued. The measurement time was set at 5 seconds, with a pre-reading delay of 45 seconds in between readings. Aliquots of 1.0, 2.0 and 3.0 mL of the mercury working standard (containing 0.1 mg/L or 1000 ppb) of mercury and 1ml of hydrochloric acid (HCl) solution was transferred to a series of 100 mL volumetric flasks and made up to the mark with reagent water. The standards had 10, 20 and 30 ppb of mercury, respectively. A calibration curve was automatically generated from the instrument's software, plotting the absorbance of the standard versus parts per billion (ppb) of mercury (USEPA, 1998a; USEPA, 1998b and Perkin-Elmer, 2011). The absorbance of the samples and standards were determined from the recording device and corresponding mercury concentrations tabulated.

3.12 Evaluation of the Degree of Sediment Contamination

The quantitative geochemical accumulation index (I_{Geo}) was used to evaluate the level of mercury contamination in fish pond sediments collected from different sampling sites. This method follows the formula proposed by Müller (1969) to calculate the degree to which sediment is contaminated by mercury. Thus, $\mathbf{I_{Geo} = \log_2 (C_n / 1.5 \times B_n)}$,

Where $\mathbf{I_{Geo}}$ = the geochemical accumulation index

$\mathbf{C_n}$ = sediment metal concentration

$\mathbf{B_n}$ = geochemical background value of the metal

In this study, the global mercury background value of 0.05 μg/g wet weight, as described by Reimann and de-Caritat (1998) was used. Accordingly, mercury pollution in collected sediments is classified into seven categories (0 - 6) by Müller (1969) as:

- class 0 (unpolluted; $I_{Geo} \leq 0$)

- class 1 (unpolluted to moderately polluted; $0 \leq I_{Geo} \leq 1$)
- class 2 (moderately polluted; $1 \leq I_{Geo} \leq 2$)
- class 3 (moderately to strongly polluted; $2 \leq I_{Geo} \leq 3$),
- class 4 (strongly polluted; $3 \leq I_{Geo} \leq 4$)
- class 5 (strongly to extremely polluted; $4 \leq I_{Geo} \leq 5$)
- class 6 (extremely polluted; $I_{Geo} > 5$)

3.13 Risk‐based consumption limits

Guidelines set by the United States Environmental Protection Agency (USEPA, 1989; 2000) were used to calculate the potential health risk from consumption of Nile Tilapia sampled in the region. An assumption was made that the ingestion dose was equal to the absorbed dose of Hg as has been described previously by Chien *et al.* (2002). Calculations on mercury consumption limits were based on the USEPA reference dose (RfDo). The ratio between exposure and the reference dose indicated by the target hazard quotient (THQ), were calculated on the assumption of an integrated USEPA risk analysis model. The methods described by Copat *et al.* (2013a, 2013b) were used to estimate the daily intake per meal (EDI_m) and the target hazard quotient (THQ) as shown below;

$$\mathbf{EDI_m} = \frac{\mathbf{MS \times C}}{\mathbf{BW}}$$

$$\mathbf{THQ} = \frac{\mathbf{EDI_m}}{\mathbf{Rf\ Do}}$$

Where $\mathbf{EDI_m}$ is the estimated daily intake of mercury per meal size;

MS is the standard weight portion of fish (230 g) for adults (Hosseini *et al.*, 2013);

C refers to the concentration of mercury in mg/kg wet weight (Marrugo-Negrete *et al.* (2008);

BW is the body weight of (taken as 70 kg for an adult human being) (Copat *et al.*, 2013a);

RfDo for T-Hg is 0.1 μg/g/day (USEPA, 2000).

For non-carcinogenic effects, the maximum allowable fish consumption rate in meals/week (CR_{mw}) according to the USEPA (2000) that would not be expected to cause any chronic systemic effects were calculated as below;

$$\mathbf{CR_{mw}} = \frac{\mathbf{49}}{\mathbf{C \times MS}}$$

Where **MS** is the standard weight portion of fish taken as 230 g for adults (Hosseini *et al.*, 2013) **C** is the concentration of mercury in mg/kg wet weight (Marrugo-Negrete *et al.*, 2008).

3.14 Statistical Analysis

Data for mercury analysis was expressed as the mean ± standard deviation. One-Way Analysis of Variance (ANOVA) was used to analyse the levels of mean T-Hg in fish tissues across the sites. Tukey's HSD test was used as a post-hoc test. Pearson's rank correlation was used to determine whether there were any relationships between mercury levels in water and those in fish tissues, levels of mercury in sediment and those in fish tissues, and the levels of mercury in fish tissues and the pH of the pond water. The same test was also used to determine relationships between the levels of mercury in fish tissues and pond water pH, temperature, weight, and age of the fish. The t-test was used to analyse the relationship between the level of mercury and the type of fish culture practised. Contamination levels in the soil and water samples from the different sites were also analysed. Microsoft Excel (2016) and Statistical Package for the Social Sciences (SPSS, version 20.0) were used for statistical analysis. $p \leq 0.05$ was considered significant in all cases.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1 Mean T-Hg levels in the Nile tilapia tissue samples

In brief, mercury contamination was established across all the sampling sites, but the degree of mercury contamination varied from one site to another and from one tissue to another. Table 2 shows the levels of mean T-Hg in Nile tilapia brain, liver and muscle tissues across the sites. All mean T-Hg measures are in µg/g wet weight (ww). The concentrations of mean T-Hg were highest in the tilapia brain, ranging from 0.128±0.021 µg/g ww (n= 5, 95% CI) at Nyabisawa in Nyatike to 3.798±1.421 µg/g ww (n= 4, 95% CI) at Minyenya in Rongo. The mean T-Hg in tilapia muscle ranged from 0.179±0.020 µg/g ww (n= 5, 95% CI) at Kamagambo south in Rongo to 0.595±0.065 µg/g ww (n= 5, 95% CI) at Masara in Nyatike. Mean T-Hg levels were lowest in tilapia liver; ranging from 0.103±0.118 µg/g ww (n= 5, 95% CI) at Kamagambo South in Rongo to 0.588±0.374 µg/g ww (n= 5, 95% CI) at Kokaka in Rongo.

Table 2: Mean T-Hg levels (in µg/g ww) in Nile tilapia tissues across the sites

Site Location	Site name	Brain (n = 5, 95%CI)	Liver (n = 5, 95%CI)	Muscle (n = 5, 95%CI)
Nyatike	Luanda Nyira	2.543±1.095 ^{f}	0.298±0.096 ^{abcd}	0.301±0.069 ^{bc}
Nyatike	Ndiwa	1.994±0.678^{def}	0.176±0.090 ^{ab}	0.374±0.056 ^{c}
L. Victoria	Siginga beach	1.436±0.588^{cde}	0.563±0.338 ^{d}	0.488±0.061 ^{de}
L. Victoria	Sori beach	0.501±0.09 ^{ab}	0.445±0.105 ^{bcd}	0.545±0.159 ^{e}
Nyatike	Masara	0.865±0.281^{abc}	0.483±0.134 ^{cd}	0.595±0.065 ^{e}
Nyatike	Nyabisawa	0.128±0.021 ^{a}	0.226±0.038 ^{abc}	0.385±0.103 ^{cd}
Rongo	Kamagambo South	0.476±0.171^{ab}	0.103±0.118 ^{a}	0.179±0.020 ^{a}
Rongo	Kamagambo North	1.186±0.847^{bcd}	0.447±0.594 ^{bcd}	0.261±0.155 ^{ab}
Rongo	Minyenya*	3.798±1.421$^{* g}$	0.108±0.038$^{* a}$	0.917±0.099$^{* f}$
Rongo	Kokaka	2.161±0.635^{f}	0.588±0.374^{d}	0.349±0.015^{b}

* n = 4

$^{a, b, c, d, e, f, g}$ mean T-Hg levels with different letters are significantly different

4.1.2 Comparison of the mean T-Hg levels in Nile tilapia tissues across the sample sites with the WHO critical values for T-Hg in fish

The WHO (2016) has set the critical values of 0.2 µg/g ww and 0.5 µg/g ww as the maximum allowable levels of T-Hg in fish that are deemed safe for human consumption by the ARPs and the general human population respectively. In this study, the levels of mean T-Hg in all the Nile tilapia tissues (brain, liver, muscle) across the sites were found to be significantly greater than the critical value of 0.2 µg/g ww (n=49, 95%CI) as shown in Table 3. However, only the Nile tilapia brain tissues were found to have mean T-Hg levels which were significantly greater than the critical value of 0.5 µg/g ww (n=49, 95%CI).

Table 3: T-Test of mean T-Hg content in fish tissues and critical values of 0.2 and 0.5 µg/g ww

Fish Tissues	Sample size (n)	Critical Value (µg/g ww)	Mean THg (µg/g ww)	Std. Err	t -values	Sig.
Brain	**49**	**0.2**	**1.353**	**0.150**	**7.687**	**0.000***
		0.5	**1.353**	**0.150**	**5.686**	**0.000***
Liver	**49**	**0.2**	**0.294**	**0.028**	**3.341**	**0.001***
		0.5	**0.294**	**0.028**	**- 7.300**	**1.000**
Muscle	**49**	**0.2**	**0.413**	**0.031**	**6.773**	**0.000***
		0.5	**0.413**	**0.031**	**- 2.787**	**0.996**

*significant values

4.1.3 Comparison of the mean T-Hg levels in Nile tilapia brain samples across the sites

The mean T-Hg levels in the Nile tilapia brain tissues across the sites ranged from 0.128±0.021 µg/g ww (n= 5, 95% CI) at Nyabisawa in Nyatike to 3.798±1.421 µg/g ww (n= 4, 95% CI) at Minyenya in Rongo (Table 2), with the brain tissues showing the highest levels of mean T-Hg compared to the muscle and liver tissues across all the sites.

Table 4 is a summary of the results of the analysis of the mean T-Hg (µg/g ww) in levels in tilapia brain tissues across the sites showing that the mean T-Hg levels in the brain tissues are significantly different from one site to another (sig. = 0.000 at 95%CI).

Table 4: One Way ANOVA of mean T-Hg levels in Nile tilapia brain tissues across the sites

	Sum of Squares	Degrees of freedom	Square	F	Sig.
Between Groups	38.864	9	4.318	12.018	0.000
Within Groups	14.013	39	0.359		
Total	52.877	48			

Further analysis of the variation revealed that there was no significant difference (at 95% CI) in the mean T-Hg levels in the tilapia fish brain from the different sampling locations (Rongo, Nyatike and Lake Victoria) as shown in Table 5.

Table 5: Tukey's HSD results showing homogeneous subsets for mean T-Hg levels in Nile tilapia brain tissues across the sample sites

Site of Data Collection		Subsets' Mean THg (µg/g ww)			
	Location	1	2	3	4
Nyabisawa	Nyatike	0.128			
Kamagambo South	Rongo	0.475			
Sori Beach	Lake Victoria	0.500			
Masara Pond	Nyatike	0.864	0.864		
Siginga Beach	Lake Victoria	1.114	1.114	1.114	
Kamagambo North	Rongo	1.210	1.210	1.210	
Luanda Nyira	Nyatike		2.084	2.084	2.084
Kokaka	Rongo			2.165	2.165
Ndiwa	Nyatike			2.215	2.215
Minyenya	Rongo				3.128
Sig.		0.165	0.076	0.149	0.201

4.1.4 Comparison of the mean T-Hg levels in the Nile tilapia muscle samples across the sites

The mean T-Hg levels in the Nile tilapia muscle tissues across the sites ranged from 0.179±0.020 µg/g ww (n= 5, 95% CI) at Kamagambo south in Rongo to 0.595±0.065 µg/g ww (n= 5, 95% CI) at Masara in Nyatike (Table 2).

Table 6 is a summary of the results of the analysis of the mean T-Hg levels in tilapia muscle tissues across the sites showing that the mean T-Hg levels in the muscle tissues are significantly different from one site to another (sig. = 0.000 at 95%CI).

Table 6: One Way ANOVA of mean T-Hg levels in Nile tilapia muscle tissues across the sample sites

	Sum of Squares	Df	Square	F	Sig.
Between Groups	1.998	9	0.222	27.239	0.000
Within Groups	0.318	39	0.008		
Total	2.316	48			

Further analysis of the variation revealed that there was no significant difference (at 95% CI) in the mean T-Hg levels in the tilapia muscle tissues from the different sampling locations (Rongo, Nyatike and Lake Victoria) as shown in Table 7.

Table 7: Tukey HSD results showing homogeneous subsets for mean T-Hg levels in Nile tilapia muscle tissues across the sample sites

Site of Data Collection		Subsets' Mean THg (µg/g ww)				
	Location	1	2	3	4	5
Kamagambo South	Rongo	0.109				
Kamagambo North	Rongo	0.182	0.182			
Luanda Nyira	Nyatike		0.306			
Kokaka	Rongo		0.349	0.349		
Nyabisawa	Nyatike		0.370	0.370		
Ndiwa	Nyatike		0.373	0.373		
Masara Pond	Nyatike			0.513	0.513	
Siginga Beach	Lake Victoria			0.532	0.532	
Sori Beach	Lake Victoria				0.603	
Minyenya	Rongo					0.880
Sig.		0.957	0.057	0.079	0.859	1

4.1.5 Comparison of the mean T-Hg levels in Nile tilapia liver tissues across the sample sites

The mean T-Hg levels in the Nile tilapia liver tissues across the sites ranged from 0.103±0.118 µg/g ww (n= 5, 95% CI) at Kamagambo South in Rongo to 0.588±0.374 µg/g ww (n= 5, 95% CI) at Kokaka in Rongo (Table 2), with the liver tissues showing the lowest levels of mean T-Hg compared to the muscle and brain tissues across all the sites.

Table 8 is a summary of the results of the analysis of the mean T-Hg levels in tilapia liver tissues across the sites showing that the mean T-Hg levels in the brain tissues are significantly different from one site to another (sig. = 0.000 at 95%CI).

Table 8: One Way ANOVA of mean T-Hg levels in Nile tilapia liver tissues across the sample sites

	Sum of Squares	Df	Square	F	Sig.
Between Groups	0.959	9	0.107	4.568	0.000
Within Groups	0.91	39	0.023		
Total	1.87	48			

Further analysis of the variation revealed that there was no significant difference (at 95% CI) in the mean T-Hg levels in the tilapia liver from the different sampling locations (Rongo, Nyatike and Lake Victoria) as shown in Table 9.

Table 9: Tukey HSD results showing homogeneous subsets for mean T-Hg levels in Nile tilapia liver tissues across the sample sites

Site of Data Collection		Subsets' Mean THg (µg/g ww)		
	Location	**1**	**2**	**3**
Kamagambo South	Rongo	0.0815		
Minyenya	Rongo	0.1128		
Ndiwa	Nyatike	0.1928	0.1928	
Nyabisawa	Nyatike	0.2187	0.2187	0.2187
Kamagambo North	Rongo	0.2278	0.2278	0.2278
Luanda Nyira	Nyatike	0.3041	0.3041	0.3041
Kokaka	Rongo	0.3916	0.3916	0.3916
Siginga Beach	Lake Victoria	0.3959	0.3959	0.3959
Sori Beach	Lake Victoria		0.4505	0.4505
Masara Pond	Nyatike			0.5299
Sig.		0.07	0.236	0.075

4.1.6 Mean T-Hg levels in fish pond sediments samples across the sites

As shown in Table 10, concentrations of mean T-Hg in pond sediments ranged from 0.208±0.000 to 1.113±0.008 µg/g ww (n= 8, 95% CI). Six of the eight sample sites were moderately polluted ($1 \leq I_{Geo} < 2$). Two sites (Minyenya and Kokaka – both from Rongo) were strongly polluted ($3 \leq I_{Geo} < 4$). The geochemical accumulation indices were calculated as explained in section 3.12 of materials and methods (chapter three – page 31) and the sites classified based on the degree of T-Hg contamination. The normal geo-accumulation index of unpolluted soil sediment should be below or equal to 0 (class 0; unpolluted; $I_{Geo} \leq 0$) as proposed by Müller (1969).

Table 10: Mean T-Hg levels in fish pond sediments and geo-accumulation analysis

Site	Location	T-Hg (µg/g) (n=8, 95%CI)	Geo accumulation index (I_{Geo})	Sediment quality
Luanda Nyira	Nyatike	0.208±0.000	1.472	Moderately polluted
Ndiwa	Nyatike	0.211±0.001	1.492	Moderately polluted
Kamagambo South	Rongo	0.240±0.002	1.678	Moderately polluted
Masara	Nyatike	0.249±0.001	1.731	Moderately polluted
Nyabisawa	Nyatike	0.258±0.001	1.782	Moderately polluted
Kamagambo North	Rongo	0.282±0.001	1.911	Moderately polluted
Kokaka	Rongo	1.102±0.013	3.877	**Strongly polluted**
Minyenya	Rongo	1.113±0.008	3.891	**Strongly polluted**

4.1.6 The relationship between mean T-Hg levels in fish pond soil sediments and Nile tilapia fish tissues

The mean T-Hg content in the soil sediments at the various sampling sites were compared with the corresponding levels in tilapia tissues from the same sites, as shown in Table 11. The findings show that an increase in mean T-Hg content in the pond soil sediment coincided with increase in the mean T-Hg content in tilapia brain tissues (r= 0.528, sig. = 0.001, 95% CI) and increased in mean T-Hg content in the tilapia muscle tissues (r= 0.524, sig. =0.001, 95% CI). However, there was no significant correlation between the mean T-Hg levels in pond sediments and tilapia liver tissues (sig. =0.923, 95% CI).

Table 11: Bivariate correlation between mean T-Hg levels in pond sediment and tilapia tissues

		Brain Tissues	Liver Tissues	Muscle Tissues
Mean T-Hg Content in Soil Sediment	Pearson Correlation Coefficient	0.528	0.016	0.524
	Sig. (2tailed)	0.001*	0.923	0.001*
	N	39	39	39

*significant correlation

4.1.7 Mean T-Hg levels in fish pond water samples across the sites

Mean T-Hg in the water samples ranged from 0.002±0.000 to 0.004±0.001 µg/ml (n=8, 95% CI) with all the sites having higher values (up to 40 times higher) for T-Hg than the maximum contaminant level of 0.0001 µg/ml allowable for mean T-Hg in unpolluted surface water set by FAO (1993) as shown in Table 12.

Table 12: Mean T-Hg levels in fish pond water sampled from different sites in the study area

Site	Location	Mean T-Hg (µg/ml)
Minyenya	Rongo	0.002
Kokaka	Rongo	0.002
Masara	Nyatike	0.003
Kamagambo North	Rongo	0.004
Nyabisawa	Nyatike	0.004
Ndiwa	Nyatike	0.004
Luanda Nyira	Nyatike	0.004
Kamagambo South	Rongo	0.004

4.1.8 Comparison of the mean T-Hg levels in pond water samples across the sites with the FAO critical values for T-Hg in unpolluted surface water

The water samples from all the sites were found to have mean T-Hg levels that were significantly greater than the critical value of 0.0001µg/ml (n=8, sig. = 0.000 at 95% CI) set by FAO (1993) for unpolluted surface waters as shown in Table 13.

Table 13: T-Test of mean T-Hg content in water samples and critical values of 0.0001µg/ml

Samples	Sample size (n)	Critical Value (µg/ml)	Mean T-Hg (µg/ml)	Std. Err	t -values	Sig.
Water	8	0.0001	.003375	.0003239	10.111	0.000

4.1.9 The relationship between mean T-Hg levels in water and fish tissues

The relationships between the mean T-Hg content in water and fish samples across the sites were analysed, as shown in Table 14. The results show that an increase in mean T-Hg content in the pond water coincided with decrease in the mean T-Hg content in tilapia brain (r= - 0.402, sig. = 0.011, 95% CI) and muscle tissues (r= - 0.616, sig. =0.000, 95% CI). However, there was no significant correlation between the mean T-Hg levels in pond water and tilapia liver samples (sig. =0.874, 95% CI).

Table 14: Bivariate correlation between mean T-Hg levels in water and tilapia tissues

Levels of T-Hg in		Brain Tissues	Liver Tissues	Muscle Tissues
Water	Pearson Correlation Coefficient (r)	- 0.402	- 0.026	- 0.616
	Sig. (2tailed)	0.011*	0.874	0.000*
	n	39	39	39

*significant correlation

4.1.10 Risk-based consumption limits

Table 15 is a summary of the risk-based consumption limits calculated across the sites, as per the guidelines earlier described in section 3.13 of materials methods (pages 31 and 32), using an integrated USEPA risk analysis model described by Copat *et al.* (2013a, 2013b) and USEPA (1989; 2000). There was no particular pattern of the safety of the Nile tilapia to human consumption across the sites but, from the findings, Nile tilapia reared in Kamagambo South in Rongo was deemed safest.

Table 15: Risk‐ based consumption parameters for Nile tilapia from the sample sites

Sampling site	Location	Nile tilapia Tissue	T-Hg ± SD (µg/g), n= 49	EDIm (µg/g)	THQ	CRmw (Meals per week)
Kamagambo South	Rongo	Brain	0.476±0.171	1.56	15.6	4
		Liver	0.103±0.118	0.34	3.4	
		Muscle	0.179±0.020	0.59	5.9	
Nyabisawa	Nyatike	Brain	0.128±0.021	0.42	4.2	3
		Liver	0.226±0.038	0.74	7.4	
		Muscle	0.385±0.103	1.27	12.7	
Minyenya	Rongo	Brain	3.798±1.421	12.48	124.8	2
		Liver	0.108±0.038	0.35	3.5	
		Muscle	0.917±0.099	3.01	30.1	
Kamagambo North	Rongo	Brain	1.186±0.847	3.90	39	2
		Liver	0.447±0.594	1.47	14.7	
		Muscle	0.261±0.155	0.86	8.6	
Ndiwa	Nyatike	Brain	1.994±0.678	6.55	65.5	2
		Liver	0.176±0.090	0.58	5.8	
		Muscle	0.374±0.056	1.23	12.3	
Luanda Nyira	Nyatike	Brain	2.543±1.095	8.36	83.6	2
		Liver	0.298±0.096	0.98	9.8	
		Muscle	0.301±0.069	0.99	9.9	
Sori beach	Lake Victoria	Brain	0.501±0.09	1.65	16.5	1
		Liver	0.445±0.105	1.46	14.6	
		Muscle	0.545±0.159	1.79	17.9	
Masara	Nyatike	Brain	0.865±0.281	2.84	28.4	1
		Liver	0.483±0.134	1.59	15.9	
		Muscle	0.595±0.065	1.96	19.6	
Siginga beach	Lake Victoria	Brain	1.436±0.588	4.72	47.2	1
		Liver	0.563±0.338	1.85	18.5	
		Muscle	0.488±0.061	1.60	16.0	
Kokaka	Rongo	Brain	2.161±0.635	7.10	71	1
		Liver	0.588±0.374	1.93	19.3	
		Muscle	0.349±0.015	1.15	11.5	

4.1.11 Miscellaneous study findings: the relationships between pond water pH, pond water temperature, tilapia samples' weights and age and the mean T-Hg levels in tilapia samples

The physicochemical and biotic characteristics of the ecosystems of the sites of sample collection were noted and summarised in Appendix 3. These parameters included the pond water pH and temperature at the time of samples' collection, the weight, age and length of the tilapia fish collected, source of water for the fish ponds and frequency of water top-up for the fish ponds and the type of fish-culture practised in the farms. Although it was not part of the objectives of this study, some of these parameters were analysed to gauge their effect on T-Hg in the tilapia tissues sampled. The findings of this analysis are shown in Table 16 and 17.

Table 16: Bivariate correlation between water pH, water temperature, the weight of fish, and fish age with T-Hg content in tilapia fish tissues

		Fish Brain	**Fish Liver**	**Fish Muscle**
Water pH	Pearson Correlation Coefficient	0.48	0.113	0.21
	Sig. (2 tailed)	0.000*	0.44	0.149
	n	49	49	49
Water Temperature (°F)	Pearson Correlation Coefficient	0.404	0.041	0.232
	Sig. (2 tailed)	0.004*	0.778	0.109
	n	49	49	49
Weight of the Fish (g)	Pearson Correlation Coefficient	- 0.623	- 0.033	0.104
	Sig. (2 tailed)	0.000*	0.822	0.476
	n	49	49	49
Fish Age (Months)	Pearson Correlation Coefficient	- 0.154	- 0.196	- 0.221
	Sig. (2 tailed)	0.348	0.231	0.177
	n	39	39	39

*significant correlation

Table 17: Independent two-sample t-test of mean T-Hg in fish tissues and fish-culture practised

Fish Tissue	Group	Observations (n)		t-values	Std. Err	Sig.
Brain	Monoculture	20	1.246	0.588	0.240	0.559
	Polyculture	29	1.426		0.194	
	Diff		0.181			
Liver	Monoculture	20	0.300	2.239	0.030	0.002*
	Polyculture	29	0.490		0.044	
	Diff		0.189			
Muscles	Monoculture	20	0.221	3.248	0.036	0.030*
	Polyculture	29	0.345		0.038	
	Diff		0.123		0.240	

*significant values

Water pH and the mean T-Hg levels in tilapia tissues

A positive correlation was identified ($r=0.48$, sig. $=0.000$, $p<0.05$) implying that as the pH of the water increases, the level of mercury in fish brain tissues also increases (Table 16). However, there was no relationship noted between water pH and the levels of T-Hg in the liver and muscle tissues of the tilapia fish samples.

Water temperature and the mean T-Hg levels in tilapia tissues

There was a positive correlation ($r=0.404$, sig. $=0.004$, $p<0.05$) between the water temperature and the T-Hg level in fish brain tissues (Table 16) which means that high water temperature was associated with higher T-Hg levels in fish brain tissues. There was no relationship noted between the water temperature and the T-Hg levels in tilapia liver and muscle tissues.

Weight of the tilapia fish and the mean T-Hg levels in the tissues

The weight of the fish was found to have a negative correlation ($r = -0.623$; sig. $=0.000$, $p<0.05$) to T-Hg levels in the tilapia brain tissues (Table 16) implying that the larger the fish, the lower the level of mercury in fish brain tissues. No correlation between the weights of the tilapia fish

and the T-Hg levels in the liver and muscle samples was established from the results in this study.

Age of the tilapia fish and the mean T-Hg levels in tissues

The study established that there was no correlation between the age of the fish and the level of mercury in the brain, liver and muscle tissues of fish (Table 16).

Type of fish culture practised and the mean T-Hg levels in tilapia tissues

There was no significant difference in the mean T-Hg levels in tilapia brain tissues regardless of the type of fish culture practised (Table 17), but there was a significant difference in the mean T-Hg levels in fish liver and muscle tissues across the two cultures.

4.2 DISCUSSION

4.2.1 Mean T-Hg levels in the Nile tilapia brain, liver and muscle tissues

Mercury is a global environmental pollutant that poses a significant risk to human and animal health. It is transferred through trophic levels and biomagnification in the food chain (Jinadasa *et al.*, 2013). Fish have been identified as the primary source of mercury in the human diet (Evans *et al.*, 1993; Dorea, 2003). *Oreochromis niloticus niloticus* (Nile Tilapia) is an omnivore with an extensive food web that includes plants, phytoplankton, insects, diatoms, algae, and mosquito larvae (Mwamuye *et al.*, 2012; BBC NEWS, 2007). It has a lifespan of about nine years and is the fish of choice for fish farmers in the Migori gold mining belt (Mwamuye *et al.*, 2012; Githukia *et al.*, 2014). It was therefore ideal for use in monitoring the bioaccumulation of T-Hg in the region. Fish can take up mercury from their environment and store it in relatively high concentrations in their tissues (Giblin and Massaro, 1973; Park and Zheng, 2012; Zheng *et al.*, 2012).

As shown in Table 2, the levels of T-Hg recorded in the Nile tilapia brain tissues were highest in Minyenya – Rongo with the mean T-Hg content of 3.798±1.421µg/g ww (n=4, 95% CI). This value is eight times higher than the WHO/FAO JECFA critical reference guideline value for the general human population of 0.5µg/g ww (WHO, 2007, 2008, 2016) and 19 times higher than the WHO/FAO JECFA critical reference values of 0.2µg/g ww for at-risk populations. The at-risk populations include pregnant women, children under five years, frequent fish eaters and people with lung, kidney and liver diseases (Campbell *et al.*, 2003b). The study findings contradict those of Campbell *et al.* (2003) who had reported that the levels of mercury in several forms of fish captured from African Lakes including Lake Victoria had mercury levels that were within WHO limits. Given that up to 90% of the mercury used in gold mining in the area is lost to the environment (Maroa, 2009), increased contamination of the area with mercury over time, may have led to these higher contamination levels.

Ogola *et al.* (2002) evaluated the impact of gold mining on the environment and human health in the Migori gold mining belt and made several recommendations. Among the recommendations, was the need to accord technical and professional assistance to artisan miners to improve their skills in gold mining and gold processing. The need for the formation of strong mining committees tasked with planning and managing gold mining sites and need for mine owners to collaborate with Government agencies to control pollution were the other recommendations of their study. However, this study's observations of high levels of mercury in fish tissues in the region suggest that these recommendations are yet to be implemented.

In this study, the levels of total mercury in the fish tissues were highest in the brain, followed by liver and lowest in the muscle tissues suggesting that brain tissues of Nile tilapia in the region release mercury at a slower rate than liver and muscle tissues. It may also indicate that the

distribution and accumulation of mercury in tissues of Nile tilapia seems to be biased towards the brain tissue since it is rich in fats and oils and methyl- and other organic forms of mercury which are highly lipophilic partition better in the brain tissues (Park and Zheng, 2012). The role of metabolism on mercury bioaccumulation in the different tissues cannot be ruled out either (Giblin and Massaro, 1973; Park and Zheng, 2012; Zheng *et al.*, 2012). Hypothetically, the metabolic rate in the brain tissues of Nile tilapia within the region was higher than the other tissues, and this may have predisposed fish brain tissues to a higher assimilation efficiency for methylmercury than other tissues. Methylmercury has a strong affinity for sulfhydryl groups in tissues and accumulates to a higher concentration in brain, muscle, and kidney tissues (Grieb *et al.*, 1990).

It is widely accepted that the muscle is the most edible and palatable part of fish. However, among the local community that resides in the region (particularly the Luo), eating the fish brain is an age-old tradition that is widely popular (Githukia *et al.*, 2014). The local folk hypothesise that eating the fish brain improves the intelligence quotient of the consumer significantly. From this study, this may be the most dangerous part of the fish to eat as methylmercury (a significant contributor of total mercury) is not eliminated from fish tissues by any practical cooking method (Grieb *et al.*, 1990).

4.2.2 Levels of mercury in pond soil sediments and geo-accumulation analysis

There were mixed findings, with mercury pollution noted in pond soil sediment samples across all the study sites, as shown in Table 10. In brief, Concentrations of mean T-Hg in sediments ranged from 0.208±0.000 to 1.113±0.008 µg/g ww (n= 3, 95% CI) with six of the eight sample sites being moderately polluted ($1 \leq I_{Geo} < 2$), whereas two sites (Minyenya and Kokaka – both

from Rongo) being strongly polluted ($3 \leq I_{Geo} < 4$). However, the levels reported in this study were significantly lower than those reported by related studies in Africa (Donkor *et al.*, 2006; Asare-Donkor and Adimado, 2016). Notwithstanding, in the context of the Hg threshold of 0.2 µg/g for sediments suggested by Salomons and Förstner (2012) and the geo-accumulation indices realised, on average, all the sample sites in the Migori gold mining belt may be considered to be moderately to highly polluted with mercury.

4.2.3 The relationship between mean T-Hg levels in pond soil sediments and Nile tilapia tissues

There is a consensus that heavy metal uptake by aquatic organisms occurs via water, food and sediment (Kalay *et al.*, 1996; Boischio and Henschel, 2000; Fallah *et al.*, 2011; Park and Zheng, 2012; Gibb and O'Leary, 2014). However, the rate of heavy metal uptake from these sources may be dependent on the ecological needs, metabolic patterns of aquatic organisms and other factors such as salinity, temperature and interacting agents (Health AG, 1987; Roesijadi, 1994; Langston, 2017). As shown in Table 14, this study revealed that the higher the levels of T-Hg in soil sediments, the higher the levels of T-Hg in tilapia fish tissues (notably brain and muscle with r= 0.528, sig. = 0.001, 95% CI and r= 0.524, sig. =0.001, 95% CI respectively). According to Gupta *et al.* (2009), sediments are the most important reservoir of metals and other pollutants in the aquatic environment. The fact that heavy metal contamination in sediment has been shown to affect bioaccumulation of metals in marine organisms may partially explain why tilapia brain and muscle tissues in this study had high levels of mercury (Evans *et al.*, 1993). However, it is unclear why there was no association between the levels of mercury in soil sediments and the levels of mercury in fish liver tissues. More studies are needed to explore this phenomenon further.

4.2.4 Mean T-Hg levels in pond water samples

The study findings (Table 12) showed that all the sites had significant T-Hg pollution in the pond water, with mean T-Hg levels ranging from 0.002±0.000 to 0.004±0.001 µg/ml (n=3, 95% CI). These mean T-Hg levels were higher (in some places up to 40 times higher like in Nyabisawa, Luanda Nyira and Ndiwa in Nyatike and Kamagambo South in Rongo) than the maximum contaminant level of 0.0001 µg/ml allowable for mean T-Hg in unpolluted surface water as set by FAO (1993) as shown in Table 13.

Levels of T-Hg in water are affected by the pH of the water, the presence of suspended solids in the ponds, as well as adsorption and precipitation processes. These factors have been shown to have the potential to remove metals such as mercury from solutions in the form of sulphides under anoxic conditions (Asare-Donkor and Adimado, 2016). Lamborg *et al.*, (2004) have also reported on the tendency of inorganic mercury and methylmercury (components of total mercury) to form complexes with naturally occurring dissolved organic carbon, thereby reducing the amount of mercury available in the water. These may explain the relatively low T-Hg content detected in the pond water samples in comparison to the tilapia tissues and pond sediment samples.

4.2.5 The relationship between mean T-Hg levels in water and Nile tilapia fish tissues

The study results (Table 14) show that an increase in mean T-Hg content in the pond water coincided with decrease in the mean T-Hg content in tilapia brain (r= - 0.402, sig. = 0.011, 95% CI) and muscle tissues (r= - 0.616, sig. =0.000, 95% CI). As per previous studies (Wang and Wong, 2003; Pickhardt *et al.*, 2006), most of the mercury that accumulates in higher trophic level forms originate from consumed food rather than direct aqueous accumulation. However, this does not explain why there was a decrease in the quantity of mercury in fish tissues (brain

and muscle) as the level of mercury increased in the water, a phenomenon that warrants further research. Moreover, there was no significant correlation between the mean T-Hg levels in pond water and tilapia liver samples (sig. =0.874, 95% CI) which needs further investigations too.

4.2.6 Risk-based consumption limits for Nile tilapia from the sample sites

Fish is a rich source of nutrients. However, it is essential to regulate the dietary intake of fish to avoid the possible accumulation of mercury in the human body. Accordingly, the WHO, in conjunction with FAO, has developed guidelines on the maximum mercury intake per week in human (JECFA, 2003). This study's findings on risk-based consumption limits were specific for each Nile tilapia tissue (brain, liver, and muscle) as shown in Table 15. In reality, however, humans usually consume the whole fish. This study's findings indicate that Kamagambo South (with a CRmw of 4) and Nyabisawa (CRmw of 3) are the areas where up to four and three fish respectively can be consumed with a low risk to human health. Conversely, Masara, Kokaka, Siginga beach, and Sori beach (each with a CRmw of 1) are the areas where the threat to human health from consuming fish is the highest.

4.2.7 The relationship between pond water pH, and temperature, fish weight and age, type of fish-culture and the mean T-Hg levels in tilapia samples

Several physicochemical and biotic parameters of the ecosystem of the fish ponds that in this study's opinion is thought to influence the levels of T-Hg in the tilapia fish tissues were noted (Appendix 3) and analysed as shown in Table 16. However, as a precaution, these parameters would need further investigations to ascertain if they do impact on the T-Hg levels in the tilapia fish and the extent to which they do so.

This study noted that as the pond water increased, so did the level of T-Hg in fish brain tissues (but not liver and muscle). Previous studies indicate that once fish assimilate mercury, it is

distributed via the blood and stored in various tissues (Boudou and Ribeyre, 1983). Thus, in the process of excretion, there is a transfer of mercury between 'donor' and 'receiver' organs, thereby implying that fish tissues are bound to have varying concentrations of mercury. Based on this, it can be hypothesised that alterations in the pH of fish pond water within the Migori gold mining belt may have favoured bioaccumulation and distribution of mercury to the brain tissue as a receiver organ relative to liver and muscle tissues. Additionally, alterations in pH may also have influenced the release of mercury from brain tissues of Nile tilapia relative to liver and muscle tissues. The lack of a relationship between pH and mercury levels in liver and muscle tissues of Nile tilapia seems to suggest that the rate of bioaccumulation, distribution, and release from these tissues may not be dependent on changes in the pH of water in fish ponds within the Migori gold mining belt.

The study also found that higher water temperatures were associated with higher levels of mercury in fish brain tissues (but not liver and muscle tissues). Mechanistically, rising temperature in water may lead to an increase in the feeding rates of fish in response to higher metabolic demand (Dijkstra *et al.*, 2013). Such an increase in food consumption could result in greater methylmercury uptake and accumulation (Dijkstra *et al.*, 2013). Thus, hypothetically, there may be a temperature dependent distribution and release of mercury from tissues of Nile tilapia.

Further study findings showed that the larger the fish, the lower the levels of T-Hg in fish brain tissues. Controversy abounds over the relationship between the weight of fish and the levels of mercury in fish tissues. Some studies have reported positive correlations between the two variables (Snyder *et al.*, 1998), while others have reported negative correlations (Sedláèková *et*

al., 2015). These conflicting findings, hypothetically, may be dependent on the type and species of fish.

The study suggests that the type of culture practised in the Migori gold mining belt has some effect on the bioaccumulation and distribution of mercury in tissues of the Nile tilapia (particularly in liver and muscle tissues). The polyculture system appears to be associated with higher levels of mercury in fish tissues. It may be that polyculture practised in fish ponds within the Migori gold mining belt may expose Nile tilapia to unique physiological stressors that increase the rate of metabolism in the fish tissues. Consequently, the rate of consumption of food among these fish rises, resulting in a more pronounced uptake of mercury from food as well as a diminished elimination from the tissues. There is a need for studies to investigate these findings further.

4.3 LIMITATIONS OF THE STUDY

Several factors may limit the interpretation of this study's results. First, although the study showed widespread contamination of the Migori gold mining belt with mercury, there was no clear pattern of mercury pollution. The statistical approach adopted may have ignored spatial correlations and drainage patterns between sampling points and thus may have missed relevant information. Secondly, the diet has been reported by other studies to be one of the major pathways for the overall accumulation of mercury. In this study, the levels of mercury in feed and how potentially this would have translated to bioaccumulation in fish tissues were not investigated. Furthermore, this study used a single species of fish as a bio-indicator of pollution. Multi-species' comparisons covering different feeding habitats of fish and a wide range of age categories may provide data that may facilitate stakeholders to distinguish recent exposure from the long-term load. Finally, this study did not explore the seasonal variation of mercury

concentrations and what effect this might have had on bioaccumulation of mercury in the tilapia fish.

4.4 CONCLUSIONS

The study findings show that there is widespread mercury pollution across all the inland study sites. For instance, the mean T-Hg levels in sediments ranged from 0.208±0.000 to 1.113±0.008 µg/g ww (n=8, 95% CI); with six of the eight sites sampled being moderately polluted ($1 \leq I_{Geo} < 2$), whereas two sites (Minyenya and Kokaka) being strongly polluted ($3 \leq I_{Geo} < 4$). Likewise, the pond waters had high mercury pollution with the mean T-Hg in the water samples ranging from 0.002±0.000 to 0.004±0.001 µg/ml (n=8, 95% CI). All the sites had higher mean T-Hg levels (up to 40 times higher) than the maximum allowable limit for T-Hg of 0.0001 µg/ml in unpolluted surface water set by FAO (1993).

There was also the presence of mercury contamination in the Nile tilapia tissues. Concentrations of mean T-Hg were highest in the tilapia brain tissues with values ranging from 0.128±0.021 to 3.798±1.421 µg/g ww (n= 49, 95% CI); with the highest proportion (78%, 38/49 samples) having mean T-Hg levels above (up to eight times higher) the limits of 0.5 µg/g ww recommended as safe by WHO (2016) for consumption by the general human population. The mean T-Hg in tilapia muscle tissues ranged from 0.179±0.020 to 0.595±0.065 µg/g ww (n= 49, 95% CI) with 31% (15/49) of fish muscle tissues tested having the levels above the critical value of 0.5 µg/g ww. Mean T-Hg levels were lowest in tilapia liver tissues with values ranging from 0.103±0.118 to 0.588±0.374 µg/g wet weight (n= 49, 95% CI) with only 27% (13/49) of fish liver tissues tested having the levels above the critical value of 0.5 µg/g ww. However, most of the tilapia fish samples (87.8% (43/49) of brain, 69.4% (34/49) of liver and (68.7% 34/49) of muscle tissues respectively had mean T-Hg above the 0.2 µg/g (wet weight) level recommended

by WHO for at-risk populations (frequent fish eaters, people with renal and liver diseases, pregnant mothers and developing children).

There were positive correlations between the mean T-Hg levels in tilapia brain and muscle tissues and the mean T-Hg levels in fish pond sediments ($r=0.528$, $p<0.05$ and $r=0.524$, $p<0.05$ respectively). However, there was no significant correlation noted between the mean T-Hg content in soil sediments and the mean T-Hg level in fish liver tissues. There were negative correlations between mean T-Hg levels in tilapia brain tissues and mean T-Hg levels in pond water ($r= -0.402$, $p<0.05$) as well as between mean T-Hg levels in tilapia muscle tissues and mean T-Hg levels in pond water ($r= -0.616$, $p<0.05$). However, there was no significant correlation noted between the mean T-Hg content in pond water and the mean T-Hg level in fish liver tissues.

The estimated daily intake of T-Hg in fish per meal (EDIm) and target hazard quotient (THQ) for human consumption ranged from 2.43-15.84 µg/g and 24.3-158.4, respectively. The maximum allowable fish consumption rate for humans in meals/week (CRmw) ranged from 1- 4 whole fish with Kamagambo South (with a CRmw of 4) and Nyabisawa (CRmw of 3) being the areas where up to 4 and 3 fish respectively can be consumed with a low risk to human health. Conversely, Masara, Kokaka, Siginga beach, and Sori beach (each with a CRmw of 1) are the areas where the threat to human health from consuming fish is the highest.

In summary, this study's findings show that the levels of mean T-Hg in tissues of Nile Tilapia in the Migori gold mining belt are above-recommended limits for mean T-Hg levels allowable in fish for human consumption to the at-risk human populations (frequent fish-eaters, pregnant women and children of developmental age). However, the fish is safe for consumption by the

general human population as long as they don't exceed the recommended consumption rate of 4 tilapia meals per week. Thus, there is a need for advisories from the Government on human consumption of the tilapia fish reared in the Migori gold mining belt.

4.5 RECOMMENDATIONS

From the findings in this study, there is a need for food advisories from the Government on human consumption of the tilapia fish reared in the Migori gold mining belt. Additionally, there is a need to enforce safe mining practices to minimize environmental mercury pollution in the area as proposed by Ogola *et al.* (2002), Mangati (2005), Ngure *et al.* (2014), Odumo *et al.* (2011) and Odumo and Carbonell (2014) as well as enforce the ban on the use of mercury in gold mining as per the Kenyan mining act (2016). The community members in the gold belt in the area of study also need to be followed up for assessment of possible mercury intoxication which may present in myriad ill-health effects and subsequently they should be given appropriate medical attention.

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APPENDICES

Appendix 1: **Approval of study proposal by the Graduate School of the University of Nairobi**

UNIVERSITY OF NAIROBI
BOARD OF POSTGRADUATE STUDIES
P.O. Box 30197, 00100
NAIROBI, KENYA
Telephone: 3318262 Ext. 28267
Fax Number: 243626
Telegrams: "Varsity of Nairobi
E-mail: bps@uonbi.ac.ke
YOUR REF:
OUR REF: J56/74432/2014

27th January 2016

Dr. Owuor Samwel Kola
c/o Chairman,
Department of Public Health,
Pharmacology & Toxicology
Faculty of Veterinary Medicine
CAVS

Dear Dr. Owuor,

REF: RESEARCH PROPOSAL AND SUPERVISORS

This is to inform you that the Director, acting on behalf of the Board of Postgraduate Studies has approved your Master of Science research proposal titled:"**Levels of mercury in tilapia fish, water and sediment from gold mining regions of Migori County, Kenya".**

She has also approved, **Dr. Laiitia W. Kanja, Prof. James M. Mbaria** and **Dr. Dr. Joyce G. Maina** as supervisors of your thesis.

You should therefore begin consulting them and ensure that you submit your thesis for examination on or before 26th July 2016. The Guidelines on Postgraduate Supervision can be accessed on our website (www.bps.uonbi.ac.ke) while the Research Notebook is available at the University Bookstore.

Yours sincerely,

SUSAN DARYA (MS)
FOR: DIRECTOR, BOARD OF POSTGRADUATE STUDIES

Cc: Dean, Faculty of Veterinary Medicine
Chairman, Department of Public Health, Pharmacology & Toxicology
Dr. L.W. Kanja, (Supervisor) – Department of PHPT
Prof. J.M. Mbaria, (Supervisor) – Department of PHPT
Dr. J.G. Maina, (Supervisor) – Department of Animal Production
SD/bwg

Appendix 2: **Approval of study proposal by the Biosafety, Animal Use and Ethics Committee**

UNIVERSITY OF NAIROBI
FACULTY OF VETERINARY MEDICINE
DEPARTMENT OF VETERINARY ANATOMY AND PHYSIOLOGY

P.O. Box 30197,
00100 Nairobi,

Tel: 4449004/4442014/ 6
Ext. 2300
Direct Line. 4448648

Dr Samuel Kola Owuor
c/o Dept of PHP&T

REF:FVM BAUEC/2018/148

Dear Dr Owuor

07/02/2018

RE: Approval of Proposal by Biosafety, Animal use and Ethics committee

Levels of Mercury in Tilapia fish, water and sediment from gold mining areas of Migori County, Kenya

By Samuel Kola Owuor (Reg. No:J56/74432/2014)

We refer to the above proposal that you submitted to our committee for review and approval. We have now reviewed the proposal and have noted that you will be sampling fish in different areas for determination of mercury levels. Furthermore, we note that the principal investigator will ensure safe use and disposal of the toxic chemicals for analysis and fish carcasses.

We hereby approve your work as per the proposal you submitted.

Rodi O. Ojoo BVM M.Sc Ph.D
Chairman,
Biosafety, Animal Use and Ethics Committee,
Faculty of Veterinary Medicine

Appendix 3: **Physico-chemical and biotic characteristics of the ecosystem of the sites of sample collection**

Site	Water pH	Water temp (°F)	Pond type	Water source	Water top-up frequency	Type of fish culture	Estimated age (months) of the fish	weight of fish (g)	length of fish (cm)
Minyenya	7.8	86.2	Earthen	Mining wastewater	Occasionally	Polyculture	7	80 ± 8.164	14.75 ± 2.5
Kamagambo N	7.2	85.3	Earthen	Spring	Weekly	Polyculture	7	128 ± 33.466	18.2 ± 1.483
Masara	7.8	83.1	Earthen	Stream/underground	Occasionally	Polyculture	5	84 ± 8.944	14.2 ± 1.095
Nyabisawa	7.0	82.0	Earthen	Spring	Weekly	Monoculture	8	364 ± 32.863	26.2 ± 0.447
Ndiwa	8.2	80.2	Liner	Spring	Weekly	Monoculture	5	112 ± 4.472	16.6 ± 0.894
Kokaka	7.4	83.4	Earthen	Underground	Monthly	Monoculture	9	92 ± 10.954	15 ± 1.225
Luanda Nyira	8.6	86.1	Earthen	Underground	Never	Polyculture	6	114 ± 16.733	16.8 ± 0.837
Kamagambo S	7.3	80.9	Earthen	Spring	Occasionally	Monoculture	8	332 ± 83.187	24.4 ± 2.30
Siginga	7.3	82.5	Lake	Lake	N/A	Polyculture	Unknown	344 ± 55.498	25 ± 1.225
Sori	7.6	82.2	Lake	Lake	N/A	Polyculture	Unknown	364 ± 69.857	25.2 ± 2.280

Appendix 4: **Thesis Plagiarism Report**

LEVELS OF MERCURY IN TILAPIA FISH, WATER AND SEDIMENT FROM GOLD MINING AREAS OF MIGORI COUNTY, KENYA

CHAIRMAN
Signed DEPT OF PUBLIC HEALTH, 10/7/2019.
PHARMACOLOGY & TOXICOLOGY
Supervisor

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