Analysis of Fish Lipids and Heavy Metal Contents in Selected Fish Species from Lake Naivasha and the Kenyan Coast and Fish Eating Habits of the Inhabitants

Caroline Wanjiru Chege

A Thesis Submitted in partial fulfillment for the Degree of Master of Science in Chemistry in the Jomo Kenyatta University of Agriculture and Technology

2011

DECLARATION

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

Signature: ..... Date: .....

Caroline Wanjiru Chege

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

1. Signature: ..... Date: .....

Prof. Joseph M. Keriko

JKUAT, Kenya

2. Signature: ..... Date: .....

Prof. Eric C. Mwachiro

Pwani University College, Kenya

3. Signature: ..... Date: .....

Dr. Mercy N. Githua

JKUAT, Kenya

DEDICATION

This work is dedicated to my loving parents Mr. John Chege and Mrs. Margaret Chege, for all the reasons you have taught me in life and for giving me the great gift of education.

ACKNOWLEDGEMENTS

First of all, I thank the almighty God, by whose grace I have successfully achieved what I strived for.

I gratefully acknowledge the guidance given by my supervisors, Prof. Joseph M. Keriko, Prof. Eric C. Mwachiro and Dr. Mercy N. Githua throughout this work. Their limitless effort, guidance, zeal, deep interest in the problem, supervision, positive criticism, helpful and interesting discussions, for making me see the positive side of the world and continued inspiration throughout the work, without which this study would not have been possible.

I wish to thank the Jomo Kenyatta University of Agriculture and Technology through the Research, Production and Extension (RPE) division for finances under the Lipid research project (Vote Ref. 601-197).

I would like to express my sincere gratitude to Prof. Thiong'o, Prof. Tsanuo and Dr. Oyaro for being generous with their time, knowledge and valuable discussions concerning the research work. Great appreciation goes to Dr. Salim for all administrative and technical assistance. Thanks to Prof. Gachanja, Mr. Ngumba and Mr. Mining for introducing me to the practical lab work in Gas Chromatography-Mass Spectrometry and columm packing respectively. Mr. Magu for his kind assistance in all computer related issues. May God bless the works of their hands.

I appreciate the support of the chief technician, Chemistry Department Mr. Mawili and the assisting technicians.

I also wish to thank my fellow classmates: Ms. Murago, Mr. Matei, Ms. Muiruri and Mr. Wahome who always remained positive to give any assistance and for providing a friendly and comfortable atmosphere to work in.

All my friends especially Ms. Kahoro and all my lab colleagues: Ms. Kaaria, Ms. Mulatya, Mr. Shileka and Ms. Mulimi for their continuous encouragement, advice, motivation and social life.

Finally, my family - The Chege's for their continuous motivation, inspiration, moral and financial support throughout my study period and for always understanding when I did not always show up when they expected me to.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

ALA	α-Linolenic acid
CFO	Crude Fish oil
CHD	Coronary Heart Disease
CVD	Cardivascular Disease
DAG	Diacylglycerides
DAGE	Diacylglyceryl esters
DHA	Docosahexaenoic acid
EFA	Essential Fatty Acids
EPA	Eicosapentaenoic acid
FAAS	Flame Atomic Absorption Spectroscopy
FFA	Free Fatty Acids
FID	Flame Ionization Detector
GC-MS	Gas Chromatography- Mass Spectrometry
GE	Glyceryl esters
GLC	Gas Liquid Chromatography
HDL	High Density Lipo-protein
LDL	Low Density Lipo-protein
MI	Myocardial Infarction
PC	Phosphatidylcholine
PCBs	Polychlorinated biphenyls
PE	Phosphatidylethanolamine
PHFO	Partially Hydrogenated Fish Oil
PL	Phospholipids

PUFA	Polyunsaturated fatty acids
Rf	Relative fraction
SCD	Sudden cardiac death
SE	Steryl esters
ST	Sterols
TAG	Triacylglycerides
TAGE	Triacylglyceryl esters
TLC	Thin layer chromatography
UV	Ultra-Violet
VLDL	Very Low Density Lipo-protein
WE	Wax Ester
WHO	World Health Organization

ABSTRACT

The consumption of aquatic products especially fish is very beneficial to the human body since they provide essential nutrients that are unavailable in plant natural products and terrestrial animals. The purpose of this research work was to determine and document the level of lipid contents and classes in the fish species commonly found in Lake Naivasha and the Kenyan coast, determine heavy metal concentration in fish muscle and water and to establish the fish eating habits of Juja, Naivasha and Mombasa communities.

GC-MS analysis was performed to determine qualitatively the fatty acid composition in fish oils. Heavy metal (Cu, Cd, Pb, Ni and Zn) concentrations in the fish muscle tissue were determined using Flame Atomic Absorption Spectrometer equipment. Fish eating habits of Juja, Naivasha and Mombasa inhabitants was established by conducting interviews using the questionnaire method.

Triacylglyceride was the highest proportion of the neutral lipids in all the tissues examined. Polar lipids including the phosphatidylcholine and phosphatidylethanolamine was the major lipid class in the muscle, pyloric ceacum, liver and orbital tissues of the specimen studied.

The study revealed that selected L. Naivasha fish has more omega-6 series of the polyunsaturated fatty acids while the Kenyan coast fish has more omega-3 series. The prominent omega-3 being C22:6 while the C18:2 are for the omega-6 series.

Lead and nickel metal concentration were significantly higher than other heavy metals, however there was no significant differences between them. Cadmium seemed to be in low concentration in every fish species examined, while zinc levels

throughout the study were in high amounts compared to other heavy metals studied. Nickel was significantly lower than zinc levels but significantly higher than other metals such as cadmium, lead and copper. In addition, the study was extended to determine these elements in the waters of L. Naivasha and the Kenyan coast. Heavy metals under study in the edible tissue of the fish specimen were in the safety permissible levels for human use.

Respondents are becoming aware of the importance marine products have to growth and development of human body and are willing to consume more fish if made readily available and at subsidized prices.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

The term "fish" is most precisely used to describe any non-tetrapod chordate, that is, an animal with a backbone, has gills throughout life, and has limbs, in the shape of fins (Stone, 1996). Unlike groupings such as birds or mammals, fish are not a single clade but a paraphyletic collection of taxa, including hagfishes, lampreys, sharks and rays, ray-finned fishes, coelacanths, and lungfishes (Helfman *et al.,* 1997).

Fish constitute an important source of protein for mankind throughout the world, and fish consumption has increased in importance among health-conscious people because it provides a healthy, low cholesterol source of protein and other nutrients (Knuth *et al.*, 2003). Fish provide omega-3 (ω-3) fatty acids that reduce cholesterol levels and the incidence of heart disease, stroke, and preterm delivery (Daviglus *et al.,* 2002).

1.2 Role of Aquatic Products in Human Nutrition

Aquatic products especially fish may serve a more important role in human nutrition and health than previously realized. Unfortunately, food consumption habits of Kenyans highly depend on their habitat i.e. their geographical set-up. For instance, in the areas where the Kikuyu, Embu, Meru and the Akamba tribes live, there are at present few fish eaters. The only people who are used to eating fish are those who live near the rivers, lakes and the ocean. The Kikuyu, who live in the vicinity of the Sagana Fish Farm, also eat fish and there are many hundreds of members of the other tribes who have been

employed or educated elsewhere and have become accustomed to eating fish (Huss, 1995).

Consumption of fish in many developed and developing communities around the world has increased greatly in recent years. This is because of the awareness created by the usefulness they provide to the general healthcare of humans. Lipids are the building blocks of the fats or fatty substances found in animals and plants. They are microscopic layered spheres of oil which, in animals are composed mainly of fats and oils, waxes, phospholipids, steroids (like cholesterol), and some other related compounds. All lipids are hydrophobic (Shirai *et al.*, 2002).

1.3 Classification of Fish Oils

Fish oils like other oils and fats are classified into their basic class lipid fractions such as triacylglycerol (triglycerides), diacylglycerol (diglycerides), monoacylglycerol (monoglycerides), phospholipids, steryl esters, sterols and free fatty acids. The quantity of the total lipid may differ between various tissues and organs and also between different species. For instance, the belly flap is a notoriously fat section of many fish bodies e.g. belly flap of mackerel contain 29% lipid while dark muscles have 18.3% and light muscles contain 7.6% (Ackman and Eaton, 1979).

The distribution of fats of the cellular type in muscle is such that in lean fish, dark muscle has about twice the lipid as compared to the white muscle in Skipjack tuna. In male mackerel the skin fat contained 40% of the fat in the whole fish (Lohne, 1976).

Most of the fish contain saturated fatty acids that have carbon length that generally ranges from C_{12} (lauric acid) to C_{24} (lignoceric acid). Small amounts of C_{10} and C_{12} monoenoic acids have been found in some fish oils (Stansby, 1967). The fatty acid pattern of ocean fish appears to be different as compared to that of freshwater fish. Gas Chromatography – Mass Spectrometry analysis of fish oil of ocean fish showed that it contained 58 fatty acids ranging from C_{11} to C_{21} carbon atoms. Among these acids, 35 were unsaturated compounds with 1 - 6 double bonds. On the contrary, freshwater fish contained 16 saturated fatty acid with C_{11} to C_{19} carbon atoms and as many as 14 unsaturated acids with 1 to 5 double bonds. Moreover, the ocean fish oil contained considerable amount of unsaturated fatty acids such as $C_{21:4}$ and $C_{21:5}$, whereas all the fatty acids of the freshwater fish oil showed chain length of 20 carbon atoms (Manal, 2009).

Fatty acids except $C_{20:1}$ and $C_{22:1}$ which are of exogenous origin, are a basic composition for the fish oils from temperate and northern latitudes, with similar totals for saturated ($C_{14:0}$; $C_{15:0}$), monounsaturated ($C_{16:1}$, $C_{18:1}$) and polyunsaturated (primarily ω-3) fatty acids. Thus, any of these oils are potential raw materials for urea complexing of acids or esters to give concentrations enriched in eicosapentaenoic (EPA) and docosahexaenoic (DHA) fatty acids (Ackman *et. al.*, 1988).

1.4 Contaminants in Fish

Levels of contaminants in fish are of considerable interest because of potential effects on the fish themselves or the organisms that consume them, including top-level receptors including humans. Contaminant levels, particularly methylmercury and polychlorinated biphenyls (PCBs), are sufficiently high in some fish to cause adverse human health effects in people consuming large quantities (Hightower and Moore, 2003).

Methylmercury is reported to counteract the cardioprotective effects (Guallar *et al.*, 2002) and to damage developing fetuses and young children. Maternal exposures can threaten the fetus because chemicals can be transferred across the placenta to the developing fetus (Gulson *et al.*, 1997). Several groups have reported a positive relationship between mercury and/or PCB levels in fish. For example, fish consumption by pregnant women and deficits in neuro-behavioral development in children (Jacobson and Jacobson, 1996; Lonky *et al.*, 1996).

Metals can be taken up by fish from water, food, sediments, and suspended particulate material (Hardersen and Wratten, 1998). However, the presence of a given metal at high concentrations in water or sediments does not involve direct toxicological risk to fish, especially in the absence of significant bio-accumulation. It is known that bio-accumulation is to a large extent mediated by abiotic and biotic factors that influence metal uptake (Rajotte *et al.*, 2003).

Some trace metals such as zinc and copper are important in small quantities for biological processes in plants and animals, and occur naturally in soil, water and the

atmosphere. However, when they are discharged in large quantities from sewage, industrial or agricultural run-off, they ultimately find their way into water bodies including the ocean and constitute an increasing hazard to humans through the food chain. Alongside increasing urbanization and industrialization in the Kenya, there is increasing awareness of the need to control waste discharges into the environment. In order to properly formulate pollution control policies, it is undoubtedly necessary to ascertain the actual state and trend of pollution (Onyari, 1981). A few studies have been carried out to investigate the distribution of heavy metals in some lakes in Kenya (Wandiga and Onyari, 1987).

Most people obtain their fish from fish markets and supermarkets (Burger *et al.*, 2004), making it important to know the levels of contaminants in these fish. Consumers cannot make informed decisions about what species of fish to eat if they do not know how contaminants vary among fishes.

CHAPTER TWO

LITERATURE REVIEW

2.1 General Overview

Omega-3 (ω-3) marine lipids play a vital role in preventing coronary heart diseases and this has led to a change in food consumption habits Worldwide. Coronary heart diseases are known to cause more cases of death and premature disability than does any other disease in many economically developed countries of the World (WHO, 1982). It has been reported that dietary intervention with marine lipids on patients with cardiovascular risk has been stimulated by epidemiological observation showing that population groups consuming large amounts of seal and fish experienced low mortality from coronary heart disease (Dyerberg *et al*., 1978).

Clinical tests suggest that dietary fish lipids offer protection/treatment against coronary heart diseases, cancer, diabetes, high blood pressure, gout and other diseases. Two significant components in all sea foods, and not present in other foods, are the omega-3 fatty acids such as, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The three main effects on body function and maintenance associated with fatty acids are that they accumulate in the: eye, brain, testes, placenta and they lower blood lipid concentration specifically the triglycerides (Catherine, 2006).

It is necessary to build up local demand by vigorous campaigns, pointing to the nutritional benefits of fish and to follow the campaign up by making available supplies

of fish, if necessary, initially at subsidized prices. The Fisheries Department places a lot of importance on the conduct of such a campaign in Nairobi and in the areas immediately to the North and to the South-East, where three million non-fish eaters live.

Epidemiological studies within populations have reported that men who ate at least some fish weekly had a lower CHD mortality rate than that of men who ate none (Stone, 1996). Fish consumption favorably affects CHD mortality, especially non sudden death from MI, has been reported in a 30-year follow-up of the Chicago Western Electric Study (Daviglus *et al.,* 1997). Men who consumed 35 g or more of fish daily compared with those who consumed none had a relative low risk of death from CHD of 0.62 and a relative low risk of non sudden death from MI of 0.33 (Morris *et al.*, 1995).

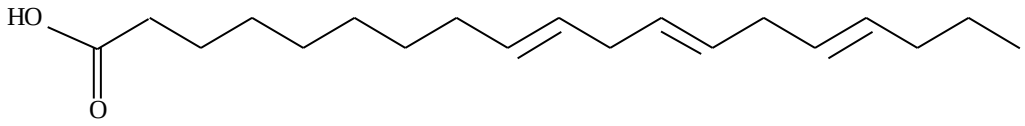
In an ecological study, fish consumption was associated with a reduced risk from all-cause, ischemic heart disease and stroke mortality across 36 countries (Zhang *et al.,* 1999). In the US Physicians' Health Study showed that men who consumed fish at least once weekly had a relative risk of sudden death of 0.48 versus men who consumed fish less than once per month (Siscovick *et al.,* 1995). Information is now available on the effects of fish and omega-3 fatty acids and risk of CHD in women (Mizushima *et al.,* 1997; Lee, 2006). A study conducted with women in the Nurses' Health Study reported an inverse association between fish intake, omega-3 fatty acids and CHD death (Hu *et al.,* 2002). Compared with women who rarely ate fish (less than once per month), the risk for CHD death was 21%, 29%, 31%, and 34% lower for fish consumption 1 to 3 times per month, once per week, 2 to 4 times per week, and less than 5 times per week, respectively. Comparing the extreme quintiles of fish intake, the reduction in risk for

CHD deaths seemed to be stronger for CHD death than for non-fatal MI (Hu *et al.*, 2002). Fish consumption has been shown to be related to reduced sudden cardiac death. In a population-based, nested, case-control study, a strong negative relationship was reported between fish intake and risk for sudden death that is, 5.5 g of omega-3 fatty acids per month, equivalent to two fatty fish meals per week, was associated with a 50% reduced risk of primary cardiac arrest (Albert *et al.*, 1998).

2.2 Omega-3 (ω-3) fatty acids

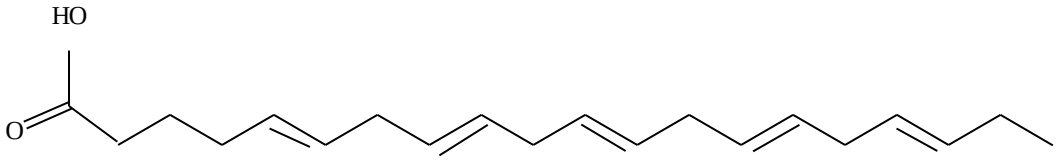
Omega-3 (ω-3) fatty acids are a family of polyunsaturated fatty acids (PUFA) which have in common a carbon-carbon double bond in the ω-3 position. Important omega-3 fatty acids in nutrition include:

(i) Octadeca-9, 12, 15-trienoic acid (α-linolenic acid) (ALA) [18:3n-3] **(1)**



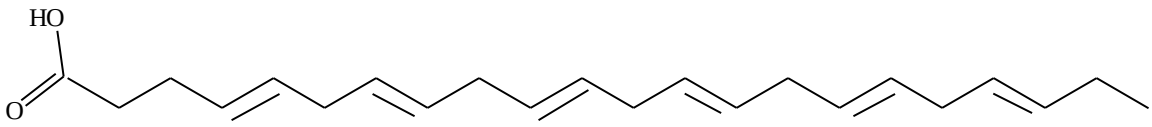
(1)

(ii) Eicosa-5, 8, 11, 14, 17-pentaenoic acid (EPA) [20:5n-3] **(2)**



(2)

(iii) Docosa-4, 7, 10, 13, 16, 19-hexaenoic acid (DHA) [22:6n-3] **(3)**



(3)

The human body cannot synthesize omega-3 fatty acids *de novo*, but can synthesize all the other necessary omega-3 fatty acids from the simpler omega-3 fatty acid such as α-linolenic acid. Therefore, α-linolenic acid is an essential nutrient which must be obtained from food, and the other omega-3 fatty acids which can be either synthesized from it within the body or obtained from food are sometimes also referred to as essential nutrients.

The term omega-3 (a.k.a. "n-3", "ω-3") signifies that the first double bond exists at the third carbon-carbon bond from the terminal methyl end (ω) of the carbon chain. Omega-3 fatty acids which are important in human nutrition are: α-linolenic acid (18:3 n-3, ALA), eicosapentaenoic acid (20:5 n-3, EPA), and docosahexaenoic acid (22:6 n-3, DHA). These three polyunsaturates have 3, 5 or 6 double bonds in a carbon chain of 18, 20 and 22 carbon atoms, respectively. All double bonds are in the *cis*-configuration that is the two hydrogen atoms are on the same side of the double bond.

2.2.1 Health benefits of Omega-3 fatty acids

Consumption of fish in many developed and developing nations around the World has increased greatly. This is as a result of the awareness created by their usefulness to the general healthcare of mankind. DHA is an essential component of the nervous system and an important nutrient in the diet. Only DHA and EPA ω-3 fatty acids reach significant percentages of the brain total fatty acids. DHA is the only ω -3 fatty acid essential in the nervous system. There are three ways in which the brain or retina may accumulate and retain DHA:

a) The brain may take up $C_{18:3n-3}$ or longer chain n-3 intermediate and brain enzymes may then form the DHA.

b) DHA may be present in the diet and taken up into the brain and other organs.

c) DHA may be formed in the liver from $C_{18:3n-3}$ or other long chain intermediates and taken up in the brain (Simopoulos, 1999).

DHA supplements in the diet are necessary because dietary DHA-rich fish oils influences brain development, learning, memory and visual function (Salem *et al.,* 1986; Suzuki *et al.,* 1998a) increasing the uncorrected visual acuity in young people with myopia (Suzuki *et al.,* 1998b), and even improvement of intelligence and visual acuity in the elderly (Suzuki *et al.,* 2001).

Epidemiological, clinical and nutritional studies on animals and humans have shown that fish oils contain high amount of n-3 polyunsaturated fatty acids (PUFA) such as EPA

and DHA that may be responsible for preventing atherosclerosis, cardiovascular diseases, aging and certain forms of cancer (Neuringer *et al.*, 1988).

Administration of purified EPA improves the thickness of carotid arteries along with improving blood flow in patients with unhealthy blood sugar levels (Keli *et al.*, 1994).

Women who ate fish once a week during their first trimester had 3.6 times less risk of low birth weight and pre-mature birth than those who ate no fish (Olsen and Secher, 2002). Omega-3 fatty acids are known to have membrane-enhancing capabilities in brain cells. Omega-3 fatty acids comprise approximately eight percent of the average human brain (Meisner and Burns, 1997). A benefit of omega-3s is helping the brain to repair damage by promoting neuronal growth. In a six-month study involving people with schizophrenia and Huntington's disease who were treated with EPA or a placebo, the placebo group had clearly lost cerebral tissue, while the patients given the supplements had a significant increase of grey and white matter (Puri, 2006).

2.3 Common fishes in Kenya

Table 1 shows some common marine fish species found along the Kenyan coast.

Table 1 Common marine fishes found at the Kenyan coast

COMMON NAME	LOCAL NAME (Swahili)
Demersal	
Rabbit fish	Tafi
Scavenger fish	Changu /Tangu
Red snapper	Shogo
Parrot fish	Pono
Surgeon fish	Kangaja
Unicorn	Puju
Grunter	Pamamba
Pouter	Chaa
Black skin	Fute
Goat fish	Mkundaji
Streaker	Murongo/ Mshikashagwi
Rock cod	Tewa
Cat fish	Fume
Pelagics	
Cavalla Jacks	Kolekole
Mullets	Mkizi
Little Mackerel	Una
Barracuda	Mzio
Milk fish	Mwatiko/Mborode
King fish	Nguru/Baurega
Queen fish	Pando
Sail Fish	Sulisuli
Marlin	Nduaro/ Sulisuli mviringo
Dolphin	Fulusi
Bonito	Sharui
Tuna	Jodari
Wahoo	Nguru Msumari
Crustaceans	
Prawns	Kamba
Lobsters	Kamba Mawe
Crabs	Kaa
Others	
Sharks/Rays	Papa/Kipungu
Sardines	Simu
Octopus	Pweza
Squids	Ngisi
Oysters	Mashaza
Crocodile	Ngwena/Mamba

Some freshwater fish species found in the inland waters of some Kenyan lakes and rivers are shown in Table 2.

Table 2 Some freshwater fish species and where they are found

		Where Found				
		L. Victoria	L. Turkana	L. Baringo	L. Naivasha	Tana R. Dams
Scientific Name	**Common Name**					
Alestes		+	+			
Bagrus		+	+			
Barbus		+	+	+		
Macropterus Salmoides	Black bass				+	
Clarias	Cat fish	+	+	+		+
Rastreonobola argentea		+				
Labeo		+	+			
Haplochromis		+				
Lates niloticus		+	+			
Momyrus kannume		+				
Protopterus		+		+		
Schilbe		+	+			
Synodontis		+	+			
Tilapia niloticus		+	+	+		
Tilapia others			+	+	+	+
	Trout					
	Cray fish				+	
	Carps				+	+
	Eels					
	Sardines					
Citharinus			+			
Hydrocynus			+			
Distichodu niloticus			+	+		

2.4 Heavy metal contamination in fish muscle and water

2.4.1 Heavy metal contamination in fish muscle

Accumulation of metals is species-dependent and therefore feeding habits and life style can be strongly related to the sediment exposure (Chen and Chen, 1999). On the other hand, availability of metals can be influenced by inorganic and organic factors that control metal speciation and thereby bioaccumulation (Sekhar *et al.*, 2003).

The uptake of sediment-associated contaminants by fish may occur by respiratory and dietary routes, whereas the dermal route is usually a minimal contributor of exposure, due to the often effective barrier provided by the external epithelium.

The metal accumulation in different fish organs depends on their physiological role, behavior, and feeding habits, as well as regulatory ability (Clearwater, 2002). Other factors, such as sex and size, may also influence metal accumulation (Canli and Atli, 2003). The concentrations of essential metals, such as Cu and Zn in organisms, tend to be highly regulated compared to nonessential. Fish can use different strategies of metal homeostasis to achieve a steady-state balance. The mechanisms of reducing metal accumulation and toxicity include uptake inhibition, increased elimination and detoxification, and storage.

Heath, 1987 indicated that fish development can be affected by the presence of heavy metals in water and especially the early life stages such as hatching time, larval development and juvenile growth as they are more sensitive than the mature stages.

Although both essential and non-essential metals may retard fish development, some metals like mercury seems to be more effective than the others.

Friedmann *et. al*. (1996) showed that even low levels of dietary mercury inhibited growth of juvenile *Stizostedion vitreum*. Weis and Weis, 1989 also indicated that both essential and non-essential metals could alter embryonic development of fish embryos causing retardation of normal development, disability of organs or mortality.

2.4.2 Sources of heavy metal pollution in the Kenyan waters

Studies conducted in the Kilindini and Makupa Creeks (Mombasa, Kenya) revealed elevated levels of heavy metals (copper, cadmium, iron and zinc), although these levels were considered to be substantially lower than those recorded in other polluted coastal areas (Kamau, 2001). Other studies in Kenya (Makupa and Tudor Creeks) revealed that overall lead and cadmium concentrations in the water column were low. A few incidents of elevated levels in sediment and some fish species were recorded, but levels of lead and cadmium in most of the fish species analysed were generally within acceptable limits (FAO/WHO, 1986).

Extensive mangrove forests in Mombasa and Maputo have been destroyed by oil spills (Munga, 1993). Spillage from the British tanker *Cavalier* caused considerable damage and destruction of mangrove forests in Mombasa in 1972. Since then, this coastline has been subjected to five other severe spills. Such spillage has resulted in mangrove dieback, especially in Mida Creek where the effects of oil spills were still evident 10 years after the last oil spill incident (Abuodha and Kairo, 2001). The main effects of

oiling on mangrove ecosystems are complete smothering of estuarine vegetation and organisms (Abuodha and Kairo, 2001). Seagrass habitats are similarly affected and studies in Kenya have indicated cases of complete smothering of these benthic plants, as well as their associated organisms (Abuodha and Kairo, 2001). To exacerbate the problem, dispersants which are commonly used to clean up oil spills contain toxic solvents which penetrate the protective waxy cuticles of seagrass blades. This affects the biological functioning of cellular membranes and chloroplasts, thereby causing plant loss and as well as harmful effects in other benthic biota (Abuodha and Kairo, 2001).

In Kenya, the increase in industrial activity in Mombasa was largely a result of an expansion in the food processing, metal and textile industries. Although industries generally discharge their waste directly into Kilindini harbour and Port Reitz, the more hazardous wastes are disposed of at the Kibarani dumpsite, leachate from which enters the creek system. Monitoring data for the concentrations of metals and organic contaminants associated with pollution from industrial activity are limited study for waters and sediments around Mombasa. No substantial increases in heavy metal concentrations attributable to anthropogenic activities have been reported, with the exception of localised Pb, Zn, and Cu contamination in the vicinities of Makupa Creek and Mombasa harbour. However, it is important to note that the hydrodynamic setting of Mombasa, in which the average residence time of contaminated water was shown to be less than one week (Rees *et. al.,* 1996), may result in the rapid attenuation of contaminant concentrations. The study reported elevated concentrations of lead, zinc and copper in waters and suspended particulate matter along the reef front between Nyali

and Mtwapa. This could not be explained by an anthropogenic influence and may be the source is ships which flush their tanks in near-shore waters (FAO, 1999).

In Kenya, domestic sewage and storm water run-off in Mombasa were reported to account for 18% (4588 tonnes per year) and 37% (12802 tonnes per year) of the total BOD and suspended solid loads respectively. Currently no sewage treatment facilities are operational in Mombasa (Mwaguni and Munga, 1997) resulting in the release of untreated domestic sewage and microbial contamination of waters in Kilindini, Port Reitz and Tudor Creek.

Analyses of water samples from wells and bore holes indicate that microbial contamination (total and faecal coliform) of groundwater has occurred in the Mombasa district. Only 3 of the 23 wells sampled passed drinking water standards, while none of the 11 borehole water samples was classified as potable (Mwaguni and Munga, 1997). Although municipal solid waste is dumped at the Kibarani dumpsite (Makupa Creek), only 53% of the 103,000 tonnes of annual solid waste production in Mombasa is collected. The dumping of domestic waste on mangrove shores has been reported around Makupa Creek and Lamu (Linden and Lundin, 1997).

2.5 Statement of the Problem

Lack of awareness of the importance of aquatic foods, levels and composition of fish lipids hence the need for data on these. Cases of death due to coronary heart disease, cancer, diabetes, high blood pressure and other diseases associated with the high intake of food that contain high levels of cholesterol are on the rise in Kenya today. This is because majority of the population depend on foods that contain high levels of cholesterol from terrestrial animals as a source of protein in their diet thus, resulting in all these complications. A shift to consumption of sea foods/marine products can reverse the trend. For instance, the human body requirement for the long chain polyunsaturated fatty acids (PUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which are important for growth and development are not adequately provided by vegetables or meat from terrestrial sources. The indirect provision through the carbon elongation followed by the C-C- desaturation process of C-18 (18:3*n*-3) by the body enzymatic system is usually not adequate.

Most people obtain their fish products from supermarkets, making it important to know the levels of contaminants in these fish as consumers cannot make informed decisions about what species of fish to eat if they do not know the variation of the contaminants among fish species.

2.6 Justification

Kenya has a long section of the coastal strip, several inland lakes and rivers that provide the country with the necessary sources of aquatic products for a healthy nation. Unfortunately, not many people are aware of the numerous benefits associated with consumption of marine natural products.

The alarming increase of deaths associated with the consumption of foods containing high cholesterol levels has led to the need to investigate and understand the underlying problem. To move from such feeding habits therefore, awareness that regards the benefits associated with consumption of sea food as opposed to relying only on protein from terrestrial animals is necessary. It is important to create awareness of the benefits of eating fish. In 1985, the fisheries department of Kenya conducted a "eat more fish" campaign and created awareness to the then non-eating fish communities of Kenya. Such campaigns should be encouraged armed with information from research studies like this one. The country will therefore, be able to reduce the cases of death from over-dependence on meat with high level of cholesterol. This will go a long way in saving the country a lot of foreign exchange earnings from coronary heart disease drug imports.

2.7 Null hypothesis

Lipid contents, lipid classes, fatty acid composition and heavy metal levels do not vary among the selected fish species from L. Naivasha and the Kenyan coast.

2.8 Objectives

2.8.1 Main Objective

The main objective of this study was to determine the total lipid content, the lipid classes, fatty acids composition of selected fish species found in Lake Naivasha and the Kenyan coast and heavy metal contamination in selected fish muscles and water.

2.8.2 Specific Objectives

i. To measure the average total lipid content and lipid classes in selected fishes from Lake Naivasha and the Kenyan coast

ii. To determine the fatty acid composition of selected fish species in Lake Naivasha and the Kenyan coast.

iii. To measure Cd, Cu, Ni, Pb, Zn of the selected fish species and the waters of Lake Naivasha and the Kenyan coast.

iv. To establish the fish eating habits of the Juja, Mombasa and Naivasha communities.

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study sites

3.1.1 Lake Naivasha

Lake Naivasha is a small fresh water lake situated at an altitude of 1890 m above sea level, its size is about 150 Km^2 and with an average depth of 6 m. Lake Naivasha is fed by River Malewa and River Gilgil and is surrounded by agricultural and industrial establishments. The lake has become increasingly hyper-eutrophic in recent times and local fishermen have reported an increase in both the size and incidence of algal blooms. There are virtually few native species in the system such as the Tilapia (*Oreochromis leucostictus*) and the introduced aquatic species especially the Largemouth bass (*Micropterus salmoides*) (locally called black bass), Common carp (*Cyprinus carpio*), Mirror carp (*Cyprinus specularis*), and Crayfish (*Procambarus clarkii*) support the local fisheries.

Plate 1 shows a map of L. Naivasha and its environs. L. Naivasha area is also renowned for wildlife and natural resources, especially after the establishment of some national parks and game reserves such as Hell's gate, Kigio wildlife conservancy, L. Naivasha national park which is home to wildlife, including mammals (hippos, zebras, giraffes, waterbucks, buffaloes, antellopes, gazelles) and more than 350 birds species, very important for conservation and tourism (www.tourism.go.ke, Accessed on 27-06-11).

Plate 1 A map of L. Naivasha (Source: www.webkenya.com)

3.1.1.1 Fish species of Lake Naivasha

3.1.1.1.1 Common Carp (*Cyprinus carpio*)

The Common carp or European carp (*Cyprinus carpio*) is a widespread freshwater fish and belongs to the carp family, Cyprinidae. Common carp is native to Asia and Eastern Europe and so far has been introduced into different environments Worldwide. It can grow to a maximum length of 1.5 m, a maximum weight of over 37.3 Kg, and an age of at least 65 years. The common carp prefer large bodies of slow or standing water and soft vegetative sediments. It naturally lives in a temperate climate in fresh or brackish water (Koehn, 2004).

Plate 2 Common carp (*Cyprinus carpio*)

.

3.1.1.1.2 Mirror Carp (*Cyprinus specularis*)

Mirror Carp (*Cyprinus specularis*) is commonly found in the United Kingdom and Europe. The difference between mirror carp and common carp is both genetic and visual but biologically they are similar. The mirror carp was the first mutation of common carp. Common carp have an even, regular scale pattern, whereas mirror carp have irregular and patchy scaling, making the fish unique and possible to identify by sight. This lack of scales is widely believed to have been bred in by monks in order to make the fish easier to prepare for the table (Talwar and Jhingran, 1991).

Plate 3 Mirror carp (*Cyprinus specularis*)

3.1.1.1.3 Largemouth Bass (*Micropterus salmoides*)

The largemouth bass (*Micropterus salmoides*) is also known by the following names; Widemouth bass, Bigmouth, Black bass, Bucketmouth, Florida bass, Florida largemouth, Green bass, Green trout, Oswego bass, and Southern largemouth. It is marked by a series of dark, sometimes black, blotches forming a jagged horizontal stripe along each flank. The largemouth is the largest of the black basses, reaching a maximum recorded overall length of 97 cm and a maximum recorded weight of 11.3 Kg (Welcomme, 1988).

Plate 4 Largemouth bass (*Micropterus salmoides*)

3.1.1.1.4 Tilapia (*Oreochromis leucostictus*)

Tilapia is the common name for nearly a hundred species of cichlid fishes from the tilapiine cichlid tribe. Tilapias inhabit a variety of fresh and, less commonly, brackish water habitats from shallow streams and ponds through to rivers, lakes, and estuaries. Most tilapias are omnivorous with a preference for soft aquatic vegetation and detritus. They have historically been of major importance in artisanal fishing in Africa and the Levant, and are of increasing importance in aquaculture around the World. Where tilapias have been deliberately or accidentally introduced, they have frequently become problematic invasive species (Trewavas, 1983).

Plate 5 Tilapia (*Oreochromis leucostictus*)

3.1.2 The Kenyan Coast

Kenya's marine fish landings are almost all from the 12,000 artisanal fishermen operating 4,000 small boats with gillnets, hooks and lines, shark nets, beach seines and traps within the inshore areas. Factors of marketing, communication and administration have influenced siting of landing beaches most of which have been provided with handling facilities by the Fisheries Department. These are the points for collecting fish landing data. The major fishing areas are the Kiunga coastline and Lamu islands in the North; Tana River mouth, Ngwana Bay and Malindi area including the offshore North Kenya Bank and Shimoni, Vanga, Funzi Island and coral reef areas in the Southern border. In addition to the types of boats, gears and the monsoon weather pattern, social and economic factors affect fish landings. Religious fastings, holidays, festivities and diversion of fishing boats into more lucrative tourist transportation and mangrove pole cutting and transport are also some factors that affect fish landings (Mwashote, 2003).

Wasini Island lies off the Southern Indian Ocean coast of Kenya next to Shimoni. The Island is sparsely populated, undeveloped and there are no cars or roads. The island is next to the Kisite - Mpunguti Marine National Park which is a site of early Swahili civilization, its attractions includes exposed coral reefs. The coral island is occupied by the Fuba people who number about 1500. Shimoni is a port in south eastern Kenya. It is known for its Swahili ruins and slave caves (Mwashote, 2003).

Plate 6. A map of Shimoni and Wasini Island
(Source: www.webkenya.com)

3.1.2.1 Common fish species of the Kenyan coast

3.1.2.1.1 Red Snapper (*Lutjanus campechanus*)

The Red snapper (*Lutjanus campechanus*), is a reef fish that commonly inhabits waters from 10 to 60 m, but can be caught as deep as 100 m or more on occasion. It stays relatively close to the bottom, and inhabit rocky bottom, ledges, ridges, and artificial reefs, including offshore oil rigs and shipwrecks (Robins *et. al*., 1991).

Red snapper will eat almost anything, but prefer small fish and crustaceans. They can grow to a maximum length of 50 cm, a maximum weight of over 18 Kg. A red snapper attains sexual maturity at age 2 - 5 years and an adult snapper can live for more than 50 years. The vibrant red color of these fish comes from high levels of carotenoid pigments, largely astaxanthin, coming from shrimp in their natural diet (Robins *et. al*., 1991).

Plate 7. Red snapper (*Lutjanus campechanus*)

3.1.2.1.2 White Snapper (*Macolor niger*)

The White snapper (*Macolor niger*), is a snapper of the family Lutjanidae found across the Indo-Pacific oceans. The White snapper can reach a maximum length of 75 cm. Its fins and eyes are black and their bodies vary in color from light grey to black depending on the age. Juveniles are lighter in color and adults can be almost completely black. It feeds on crustaceans and small fishes and inhabit reef walls and outer lagoons. White snapper are a prized food fish and are caught commercially, as well as recreationally (Robins *et. al*., 1991).

Plate 8. White snapper (*Macolor niger*)

3.1.3.1.3 Rabbit fish (*Siganus ludridus*)

Rabbit fish (*Siganus ludridus*) are perciform fishes in the family Siganidae. It is found in shallow lagoons in the Indo-Pacific and Eastern Mediterranean. Rabbit fishes grow to about 40 cm and have small, rabbit-like mouths and large dark eyes. An unusual feature among rabbit fish is their pelvic fins, which are formed from two spines, with 3 soft rays between them that are equipped with well-developed venom glands. They are herbivorous, feeding on benthic algae in the wild. It is fished for food, and the more colorful species are often kept in aquaria (Woodland, 1990).

Plate 9. Rabbit fish (*Siganus luridus*)

3.2 Fish sampling in L. Naivasha

Four fish species that are commonly available in L. Naivasha were purchased directly from fishermen at the Central fish landing. For one species, 10 specimens were purchased. These four species included; the common carp (*C. carpio*), mirror carp (*C. specularis*), largemouth bass (*M. salmoides*) and tilapia (*O. leucostictus*). Sampling in L. Naivasha was done twice in the months of October, 2007 and May, 2008 to represent the wet and dry seasons and also because there are regulations that are set by the fisheries department such that no fishing takes place for three months (June - August) in a year to allow maturation and breeding of fishes. The specimens were transported to the JKUAT laboratory in cool-boxes filled with ice-cubes.

3.3 Fish sampling in the Kenyan coast

The first sampling at the Kenyan coast was done in September, 2007 while the second sampling was done in April, 2008. Three common fish species that are locally available were chosen for this study. They included; the Rabbit fish (*S. Luridus*), Red snapper (*L. campechanus*) and White snapper (*M. niger*). The fish species were purchased directly from the fishermen at the Wasini Island and the Shimoni market. The two sites were chosen because they are the major fishing areas at the south coast.

3.4 Waters sample collection from L. Naivasha and the Kenya coast

Water samples were collected from five stations on L. Naivasha and five stations at the Kenya coast for heavy metal analysis. In selecting these stations the following criteria were considered: point of effluent discharge into the lake, point of confluence between the lake and R. Malewa and R. Gilgil and nearness of sampling points to the flower farms. The stretch between Shimoni mainland and Wasini island which is approximately 1 km was divided into five so as to obtain the stations at the Kenya coast. At each station four water samples were collected using 1 litre clean acid - washed polyethylene bottles by gently lowering the bottles into the subsurface of the water and filling them completely beneath the surface. Approximately 2 ml of concentrated HNO_3 was added to each of the samples as a preservative and it lowers the pH of the water sample thus metals do not dissociate. The bottles were stored in a cool-box and transported to the laboratory.

3.5 Pre-Treatment of fish samples

On arrival in the laboratory, the fish specimen were rinsed with tap water, labeled and put in polyethylene bags then frozen at -4°C to prevent autolytic post-mortem changes after rigor mortis.

During analysis, the fish samples were thawed and their physical parameters like width, weight and length of each fish specimen was recorded. Specific organs such as the muscles, liver and pyloric ceacum for the freshwater fishes were removed separately and weighed. For the marine fishes, the muscle and the orbital tissues were used for lipid

analysis as we were not able to obtain fishes that had not been dissected. Physical parameters are indicated on Table 3.

3.6 Lipid extraction and the analysis of the lipid classes

Each individual part that is the pyloric ceacum, liver and the muscle tissue of each fish species was separated and weighed and then minced. The lipid in the tissue was solvent extracted with chloroform/methanol (2:1). After dispersion, the whole mixture was agitated for 15 - 20 min. in an orbital shaker at room temperature.

The homogenate was filtered to recover the liquid phase. The interface was rinsed one or two times with methanol/chloroform (1:1). After centrifugation and siphoning of the upper phase, the lower chloroform phase containing lipids was filtered off and the water phase was removed from the extract by passing it through a filter paper containing anhydrous sodium sulphate (Na_2SO_4 Sigma-Aldrich, Germany). The individual lipids were recovered after evaporating the solvent under vacuum in a rotary evaporator (Folch *et al.*, 1957). The total lipid (TL) extracted was weighed and transferred to a vial and any remaining solvent was left to evaporate in the fume chamber. The dry extract was covered with a parafilm and was stored in the fridge. This procedure was repeated for the liver, the pyloric ceacum and the muscle tissues.

3.7 Column Fractionation of Total Lipids (TL)

The crude total lipids (TL) were separated into classes on silica-gel packed columns (Merck and Co., Kieselgel 60, 70-230 mesh ASTM), and a quantitative analysis of the lipid constituent was performed using gravimetric analysis of fractions collected from column chromatography. The first eluate (dichloromethane : *n*-hexane, 2:3, v/v) was collected as a mixture of steryl esters (SE), wax esters (WE), and glyceryl esters (GE) [fraction 1]. This was followed with 100% dichloromethane eluting a mixture of glyceryl esters (GE) and triacylglycerides (TAG), [fraction 2]. Dichloromethane : diethyl ether (35:1, v/v) eluting a mixture of triacylglyceryl esters (TAGE) and diacylglyceryl esters (DAGE), [fraction 3]; dichloromethane : diethyl ether (9:1, v/v) eluting the diacylglyceryl esters (DAGE) and sterols (ST), [fraction 4]; dichloromethane : methanol (9:1, v/v) eluting the free fatty acids (FFA), [fraction 5]; dichloromethane : methanol (1:1, v/v) eluting phosphatidylethanolamine (PE), [fraction 6] while dichloromethane : methanol (1:5, v/v) eluting other lipids such as inositol, sphingolipids, [fraction 7] and finally dichloromethane : methanol (1:20, v/v) eluting the phosphatidylcholine (PC), [fraction 8] respectively, (Saito and Murata, 1998).

After fractionation, the individual lipids in each class were identified using standards by comparison of the relative fraction (Rf) values using thin-layer chromatography (TLC pre-coated aluminium sheets, Merck and Co., Kieselgel 60 F_{254}, thickness of 0.25 mm) and sprayed with 12-molybdo (IV) phosphoric acid *n*-hydrate (phosphomolybdic acid, BDH Chemicals Ltd., Poole, England) dissolved in water (Dittmer and Lester, 1964;

Rouser *et al.*, 1966). All lipid fractions were dried under the vacuum rotary evaporator and stored in a freezer.

3.8 Preparation of methyl esters for GC-MS analysis

Individual lipid components of triacylglycerols (TAG) fraction was converted into fatty acid methyl esters through direct transesterification by refluxing each component using methanol (10 ml) containing 1% concentrated hydrochloric acid for a period of three hours. Each resulting crude product was poured into saturated brine (200 ml) containing 10 ml of saturated sodium hydrogen carbonate ($NaHCO_3$) in a separating funnel after allowing it to cool down to room temperature. The upper organic layer was then extracted with *n*- hexane (50 ml), purified by passing it through a short column of silica gel after dehydrating it overnight using anhydrous sodium sulfate ($NaSO_4$). Elution was carried out using dichloromethane:*n*-hexane (2:1) solvent system (AOAC, 1994).

3.9 Fish and water sample preparation for heavy metal analysis

For the analysis of cadmium (Cd), copper (Cu), nickel (Ni), lead (Pb) and zinc (Zn) in the respective samples, the digestion protocol described by AOAC (1970, 1975) was followed. 1.0 g of each of the oven dried fish samples was accurately weighed and transferred into a 250 ml conical flask. A little distilled-deionized water was added to moisten the sample then 5 ml of concentrated HNO_3 was added and warmed for 30 min to remove the brown fumes. 1 ml of concentrated $HClO_4$ was then added followed by 5 ml of concentrated H_2SO_4 to complete the digestion.

The digestion was done on a hot plate in the fume cupboard for 90 min. at 130 °C with the flask covered with a watch glass. The sample was then cooled, diluted with 5 ml of 6% HNO_3 and filtered through Whatman filter paper No. 42 into 50 ml volumetric flasks and made up to the mark with distilled-deionized water.

For water samples, 50 ml of each sample was placed in a beaker and evaporated to about 20 ml to concentrate the metal ions. They were then acid-digested following the same procedure as that of the fish samples.

3.10 Preparation of the blank solutions

The calibration blank was prepared by acidifying distilled-deionised water to the same concentrations of the acids found in the standards and samples. A sufficient quantity was prepared to flush the system between standards and samples. The calibration blank was also used for all initial and continuing calibration blank determinations.

The method blank contained all of the reagents in the same volumes as used in the processing of the samples. The method blank was carried through the complete procedure and contained the same acid concentration in the final solution as the sample solution used for analysis.

The calibration blank was used in establishing the analytical curve and the method blank was used to identify possible contamination resulting from either the reagents (acids) or the equipment used during sample processing including filtration.

3.11 Preparation of the standard stock solutions

3.11.1 Cd 1000 mg ml^{-1}

0.250 g of $Cd(NO_3)_2 \cdot 4H_2O$ (Purity : 99 %) was weighed and transferred into a beaker. The metal was dissolved in 5 ml of HNO_3:distilled water (1:1, v/v). The solution was transferred into a 250 ml volumetric flask and diluted to the mark with distilled de-ionized water. The solution was put in a polyethylene bottle and stored in a fridge.

3.11.2 Cu 1000 mg ml^{-1}

0.985 g of $CuSO_4.5H_2O$ (Purity: 98 %) was weighed and transferred into a 250 ml volumetric flask. Distilled de-ionized water was added to dissolve the salt. 5 ml of HNO_3:distilled water (1:1, v/v) was added and diluted to the mark with distilled de-ionized water. The solution was put in a polyethylene bottle and stored in a fridge.

3.11.3 Ni 1000 mg ml^{-1}

1.120 g of $NiSO_4.6H_2O$ (Purity: 99 %) was weighed and transferred into a 250 ml volumetric flask. Distilled de-ionized water was added to dissolve the salt. 5 ml of HNO_3:distilled water (1:1, v/v) was added and diluted to the mark with distilled de-ionized water. The solution was put in a polyethylene bottle and stored in a fridge.

3.11.4 Pb 1000 mg ml^{-1}

0.400 g of $Pb(NO_3)_2$ (Purity: 99 %) was weighed and transferred into a 250 ml volumetric flask. Distilled de-ionized water was added to dissolve the salt. 5 ml of

HNO_3:distilled water (1:1, v/v) was added and diluted to the mark with distilled de-ionized water. The solution was put in a polyethylene bottle and stored in a fridge.

3.11.5 Zn 1000 mg ml^{-1}

0.250 g of zinc granules (Purity: 99 %) was weighed and transferred into a beaker. The metal was dissolved in 5ml HNO_3:distilled water (1:1, v/v). The solution was transferred to a 250 ml volumetric flask and diluted to the mark with distilled de-ionized water. The solution was put in a polyethylene bottle and stored in a fridge.

3.12 Determination of fish feeding habits of Juja, Naivasha and Mombasa communities

Questionnaires (Appendix 1) were distributed and fish consumption habits of Juja residents including the JKUAT community, Mombasa and Naivasha communities were determined. A sample of a hundred residents from each sampling site was picked at random. The information collected was processed to show the fish consumption habits among these communities.

3.13 Sample analysis

3.13.1 GC-MS analysis of fatty acid methyl esters

The fatty acid methyl esters thus prepared (1 µl) was injected into the GC column. GC-MS analysis was performed using GC Voyager-800 series equipped with Trio-01 MS detector operating in electron ionization (EI) mode. Separations were achieved with a fused silica capillary column Omega-wax (30 m x 0.25 mm I.D, 0.25 µm film). The carrier gas was helium (flow 1 ml/min) with a split injection of 50:1. The temperature

profiles were as follows: initial temperature, 140 °C for 4 min; ramped at 10°C/min; final temperature, 240 °C and held for 10 min (final time, 50 min.); injector temperature, 250 °C; and detector temperature, 280 °C. EI-MS data was obtained under the following conditions. The transfer line temperature was set at 280°C and the mass range was 50 – 450 amu. Solvent cut was set at 8 min. Full scan mode. The mass spectrometer was calibrated every day before use with perfluorotributylamine (PFTBA) as a calibration standard.

3.13.2 FAAS analysis of selected fish species muscles and water from L. Naivasha and the Kenya coast

To ensure that the VGP 210 flame atomic absorption spectrometer remained calibrated during the experimentation, standards were analyzed after every 10 runs. The appropriate salts of the metals of interest were used to prepare aqueous stock solutions of the metal by dissolution in distilled water and dilute Nitric acid and subsequent dilution with distilled water to 1000 ppm. Five working standards were prepared in triplicate for each metal by serial dilution of the stock solutions. These and blank solutions were aspirated into the equipment. A calibration curve of absorbance versus concentration was prepared for each metal and used for determination of metal concentration in the samples.

3.13.2.1 Method validation (Spiking Method)

The digestion method and the FAAS analysis were validated by preparation of a multi element standard solution which was prepared from commercial available standards. A sample from a given sampling point was digested, run on FAAS and metal contents in this unspiked sample determined from the calibration curve. An aliquot of the multi element standard solution was drawn with graduated pipette and used to spike a sample from the same sampling point. This was followed by the digestion of the spiked sample, aspiration into FAAS and determination of metal contents from the calibration curve. The amount of spiked metal recovered after the digestion of the spiked samples was used to calculate percentage recovery. Spiking, digestion and analysis were done in triplicate. The spiking experiment gave the following percentage recoveries: Zn, 101.56 ± 4.03%: Cu, 97.62 ± 2.92% ; Cd, 92.25 ± 3.28%; Ni, 94.48 ± 3.37% and Pb, 93.69 ± 1.95%. This shows that the digestion method used and the FAAS analysis are reliable.

3.14. Data analysis

Data entry management and preliminary summaries were done on MS Excel spreadsheet. Data from repeated experiments for both total lipid content and lipid classes in various tissues of selected fish species from L. Naivasha and the Kenyan coast were subjected to ANOVA for each treatment and means were separated using Tukey's test (SAS/IML software; Version 9.1; SAS Institute 1999). Mean separation of heavy metal levels in fish muscle and water samples from L. Naivasha and the Kenyan coast was accomplished using Tukey's test.

Probability value of $p < 0.05$ was used for the entire tests to show statistical significance of mean values for the parameters analyzed.

CHAPTER FOUR

RESULTS AND DISCUSSION

Physical parameters of selected fish species from L. Naivasha and the Kenyan coast were recorded as shown in Table 3.

Table 3. Physical parameters of selected fish species from L. Naivasha and the Kenyan coast. Mean ± SD (n=6)

Study site	Fish species	Weight (g)	Length (cm)	Width (cm)
L. Naivasha	**Common carp** (*C. carpio*)	618.27 ± 40.02	36.93 ± 2.42	5.37 ± 1.05
	Mirror carp *(C. specularis)*	549.14 ± 22.12	34.90 ± 1.74	4.70 ± 0.17
	Largemouth bass (*M. salmoides*)	589.02 ± 4.52	32.50 ± 0.58	4.85 ± 0.55
	Tilapia (*O. leucostictus*)	180.78 ± 8.84	21.22 ± 0.22	2.62 ± 0.35
Kenyan coast	**White snapper** (*M. niger*)	351.13 ± 48.86	28.08 ± 1.54	4.47 ± 1.29
	Red snapper (*L. campechanus*)	331.41 ± 29.58	28.38 ± 2.14	4.18 ± 1.32
	Rabbit fish (*S. luridus*)	234 ± 27.79	25.6 ± 1.02	4.53 ± 1.28

4.2 Lipid contents in various tissues of different fish species

4.2.1 Total lipid content in the muscle tissue of selected freshwater fish species

The total lipid content in the freshwater fish species ranged between 1.40 ± 0.31% and 4.77 ± 0.66% in the muscle of the wet tissues (Table 4). Kamal, *et al*., (2007) found out that the muscle lipid contents among seven freshwater fishes from the River Mouri, Khuina, Bangladesh ranged between 3.45 ± 0.92% in *Heteropneustes fossilis* to 7.90 ± 1.91% in *Clarias batrachus*. In a similar study, lipid contents were found in *Clarias batrachus* (7.90 ± 1.91%), *Anabas testudineus* (7.79 ± 2.73%), *Mystus vittatus* (7.53 ± 1.10%) and *Nandus nandus* (7.34 ± 1.49%). Hossain *et al*., (1999) reported the lipid contents of some selected muscle fishes from Mymensingh District, Bangladesh ranging from 1.87 to 9.55%. The results obtained in the present study were within the ranges reported by Hossain *et al*., (1999). Rahman *et al*., (1994) reported a range of 2.18 to 9.38% in the total lipids content of some Bangladeshi zeol fish. The results of this study compare well with these results. Fish with lipid contents in the muscles below 5% are considered lean (Stansby, 1982; Ackman, 1989) and hence all the fish species under this study indicate lean fish specimen. The average total lipid content in the four selected freshwater fish species was significantly different at the $p < 0.5$ except for *M. salmoides* and *C. specularis*.

Table 4. Average percentage total lipid content present in the muscle tissue of selected fish species of L. Naivasha. Mean ± SD (n=6)

TYPE OF FISH	Muscle
Common carp (*Cyprinus carpio*)	1.40 ± 0.31c
Largemouth bass (*Micropterus salmoides*)	2.25 ± 0.08b
Mirror carp (*Cyprinus specularis*)	2.56 ± 0.05b
Tilapia (*Oreochromis Leucostictus*)	4.77 ± 0.66a

4.2.2 Lipid content in the liver tissue of different freshwater fish species

The total lipid content in these species ranged between 11.57 ± 0.25% and 28.33 ± 2.22% in the liver of the wet tissues as shown in Table 5. The results of the present study were significantly low compared with the results obtained by Saito *et al*., (2002) in that the total lipid contents based on the fresh liver weight of five species; *Coryphaenoides yaquinae, Coryphaenoides armatus, Coryphaenoides acrolepis, Coryphaenoides pectralis and Coryphaenoides longifilis*) were 45.5 – 69.1%.

Table 5. Average percentage total lipid content present in the liver tissue of selected fish species of L. Naivasha. Mean ± SD (n=6)

TYPE OF FISH	Liver
Common carp (*Cyprinus carpio*)	28.33 ± 2.22a
Largemouth bass (*Micropterus salmoides*)	11.57 ± 0.25c
Mirror carp (*Cyprinus specularis*)	23.14 ± 1.05b
Tilapia (*Oreochromis leucostictus*)	14.59 ± 0.43c

4.2.3 Lipid content in the pyloric ceacum tissue of different freshwater fish species

The total lipid content in these species ranged between 16.99 ± 0.26% and 28.25 ± 0.00% in the pyloric ceacum of the wet tissue as shown in Table 6. The results obtained in the present study are too high compared to what was obtained by Saito *et al*., (2002) in the total lipid contents based on the pyloric ceacum of five *Coryphaenoides* species which ranged between 1.8% and 3.0%. However, in another study by Saito *et al.*, (1997), the *Euthynnus pelamis* contained 38 ± 4% and 37 ± 4% total lipids in the pyloric ceacum tissue in sub tropical and temperate regions, respectively. These values compare well with the values obtained in the present study. Due to the small size of the *O. leucostictus*, it was very difficult to separate the pyloric ceacum from other tissues. Therefore, its average total lipid content was not measured.

Table 6. Average percentage total lipid content present in the pyloric ceacum tissue of selected fish species of L. Naivasha. Mean ± SD (n=6)

TYPE OF FISH	Pyloric ceacum
Common carp (*Cyprinus carpio*)	17.50 ± 0.79b
Largemouth bass (*Micropterus salmoides*)	28.25 ± 0.56a
Mirror carp (*Cyprinus specularis*)	16.99 ± 0.26b

4.2.4 Lipid content in the muscle tissue of different marine fish species

The total lipid content in the marine fish species ranged between 0.71 ± 0.06% and 0.77 ± 0.02% in the muscle of the wet tissues (Table 7). The figures for marine species compares well with Saito *et al*., (2002) findings in that the total lipid contents in the muscle of five marine species; *Coryphaenoides yaquinae, Coryphaenoides armatus,*

Coryphaenoides acrolepis, Coryphaenoides pectralis and Coryphaenoides longifilis) ranged between 0.1 and 0.7% although from temperate regions.

Table 7. Average percentage total lipid content present in the muscle tissue of selected fish species of Kenyan coast. Mean ± SD (n=6)

TYPE OF FISH	Muscle
Rabbit fish (*Siganus luridus*)	0.77 ± 0.02a
Red Snapper (*Lutjanus campechanus*)	0.71 ± 0.06a
White snapper (*Macolor niger*)	0.73 ± 0.02a

4.2.5 Lipid content in the orbital tissue of different marine fish species

The total lipid content in these species ranged between 14.13 ± 6.68% and 24.67 ± 0.00% in the orbital of the wet tissues as shown in Table 8. Saito *et al*., (1997) reported the total lipids in *Euthynnus pelamis* fish orbital ranging between 13.1 ± 1.14 g/kg and 15.3 ± 1.18 g/kg in sub-tropical and temperate regions respectively. Their figures were low compared to the values obtained in the present study.

Table 8. Average percentage total lipid content present in the orbital tissue of selected fish species of the Kenyan coast. Mean ± SD (n=6)

TYPE OF FISH	Orbital
Rabbit fish (*Siganus luridus*)	24.667 ± 0.00a
Red Snapper (*Lutjanus campechanus*)	14.133 ± 6.68a
White snapper (*Macolor niger*)	17.630 ± 0.02a

4.3 Lipid classes in various tissues of different fish species

4.3.1 Lipid classes present in the muscle tissue of selected L. Naivasha fish species

Four species of fish were sampled from L. Naivasha to establish the amount of lipid class composition in their muscles (Table 9). There were siginificant differences (ANOVA, $p < 0.05$) in the percentage lipid classes in the muscles of *O. leucostictus*, *M. salmoides*, *C. specularis* and *C. carpio*. *O. leucostictus* had the highest percentage of all lipid classes while *C. specularis* had the lowest (ANOVA, $p < 0.05$).

O. leucostictus muscle tissue had significantly high amount of wax ester content (2.04 ± 0.90) compared to other fishes. *C. specularis* had the lowest wax ester composition in the muscle. This could be attributed to the different feed the two species consume. *O. leucostictus* is known to feed mainly on green algae while the C. *specularis* feeds on small fish. Cranwell *et. al.,* 1988, reported small quantities of wax ester for a few species of freshwater algae.

The triacylglycerides content of the four fish species muscles was significantly different from each other ($p < 0.05$). *M. salmoides* recorded the highest percentage of triacylglycerides (11.16 ± 1.14) while *C. specularis* recorded the lowest percentage of triacylglycerides (7.48 ± 2.96). In general, the depot subcutaneous fats in fishes are mostly composed of triacylglycerides, which are neutral lipids consisting mainly of saturated and monoenoic fatty acids. When fishes are exposed to migration, they actively use saturated and monoenoic fatty acids as energy source and hardly use the

polar lipids which mostly comprise the phospholipids as a result, they are conservatively stored in the tissues and various cell membranes.

Whilst undergoing normal swimming, rapid outbursts when escaping predators or when catching their prey, during spawning and the development of gonads, these fishes over a short period of time feed little, conditions that are similar to that of the long migration distances. This suggests that these fishes do not simply incorporate the total lipids of their feed fish in the same ratios, but selectively accumulate the polar lipids and actively use the saturated and monoenoic fatty acids. The main reasons why these fish species selectively use the saturated and monoenoic fatty acids is because they are dispensable for these fishes and are an effective high calorie energy source as they are saturated with hydrogen (Saito et. al., 1999).

Table 9. Average percent lipid classes present in the muscle tissue of selected L. Naivasha fish species. Mean ± SD (n=6)

Fish species	Lipid classes							
	WE[1] (%)	**TAG (%)**	**DAGE(%)**	**ST (%)**	**FFA(%)**	**PE (%)**	**Other lipids (%)**	**PC (%)**
C. carpio	$1.250\pm0.30_{bc}^{C2}$	$8.138\pm1.36_{bc}^{A}$	$6.097\pm1.49_{a}^{C}$	$0.870\pm0.03_{b}^{C}$	$1.279\pm0.12_{a}^{C}$	$21.257\pm1.61_{a}^{B}$	$5.904\pm0.41_{a}^{C}$	$22.651\pm1.18_{a}^{B}$
M. salmoides	$1.815\pm0.13_{b3}^{CD}$	$11.158\pm1.14_{a}^{A}$	$4.023\pm0.44_{a}^{C}$	$0.570\pm0.17_{b}^{D}$	$1.254\pm0.26_{a}^{CD}$	$16.073\pm1.69_{a}^{B}$	$2.699\pm0.09_{b}^{CD}$	$17.776\pm1.13_{a}^{B}$
C.specularis	$1.041\pm0.10_{c}^{D}$	$7.476\pm1.96_{c}^{A}$	$4.418\pm0.75_{a}^{D}$	$1.670\pm0.11_{a}^{D}$	$2.680\pm0.09_{a}^{D}$	$14.179\pm2.01_{a}^{C}$	$3.515\pm0.82_{b}^{D}$	$25.694\pm1.13_{a}^{B}$
O. leucostictus	$2.040\pm0.90_{a}^{E}$	$10.212\pm0.43_{b}^{A}$	$3.362\pm0.41_{a}^{DE}$	$1.351\pm0.44_{ab}^{E}$	$1.358\pm0.32_{a}^{E}$	$15.000\pm0.96_{a}^{C}$	$5.297\pm0.55_{a}^{D}$	$20.497\pm1.10_{a}^{B}$

[1]WE = Wax esters, TAG = Triacylglceride, DAGE = Diacylglceride, ST = Sterol, FFA = Free fatty acids, PE = Phosphatidylethanolamine,
PC = Phosphatidylcholine other lipids = inositol, sphingolipids

[2] Different means with different capital letters within a row are significantly different at the $P < 0.05$ level (Tukey's Least Significant Differences).

[3]Different means with different small letters within a column are significantly different at the $P < 0.05$ level (Tukey's Least Significant Differences).

4.3.2 Lipid classes present in the pyloric ceacum tissue selected L. Naivasha fish species

Phosphatidylcholine and phosphatidylethanolamine in *C. specularis* pyloric ceacum were significantly high at ANOVA $p<0.05$ compared with the other average lipid classes examined as shown in Table 10. However, wax esters and diacylglcerides were significantly lower compared with other lipids classes present. Phosphatidylethanolamine and phosphatidylcholine lipid classes in *C. carpio* were in large proportion compared with other lipids classes that is inositol and sphingolipids but they were not statistically different from each other. Wax esters were the lowest in the pyloric ceacum of *C. carpio* fish but they were not statistically different from diacylglcerides.

Phosphatidylcholine lipid class was significantly the highest lipid class compared with other lipids in the pyloric ceacum of *M. salmoides*. Sterols, diacylglcerides and wax esters were not significantly different from each other although they were least in percent lipid class in the pyloric ceacum of *M. salmoides*.

There were no siginificant differences (ANOVA, $p > 0.05$) in diacylglcerides, sterols, free fatty acids, phosphatidylethanolamine, phosphatidylcholine and other lipid classes' composition in the pyloric ceacum of *C. specularis*, *C. carpio* and *M. salmoides* as shown in Table 10.

Generally, the phospholipids were in higher percent content compared to the other lipid classes. phosphatidylethanolamine and phosphatidylcholine mostly contain mono and

polyunsaturated fatty acids that are seldomly used by the fishes during their daily activities and therefore, tend to accumulate in the tissues.

4.3.3 Lipid classes present in the liver tissue of selected L. Naivasha fish species

The average percentages of lipid classes in the liver tissue of four freshwater fish species were found to be significantly differently (ANOVA, $p < 0.05$) as recorded in Table 11. The amount of triacylglycerides in the liver tissue of *C. carpio* and *M. salmoides* were not statistically different from each other.

Generally, the amounts of triacylglycerides and phospholipids were present in high proportions as compared to the wax esters, diacylglycerides and sterols. The percentage content of phosphatidylethanolamine and phosphatidylcholine were higher in the liver and the pyloric ceacum tissues as compared to the muscle of the selected L. Naivasha fish species. This is probably due to the phospholipids are always present in the cell membranes and tends to accumulate in the various tissues in different amounts. The liver tissue provides enzymes that are responsible of breaking down the lipids leading to their accumulation in the various body organs. Thus, the high levels of free fatty acids (FFA) in the liver and the pyloric caecum tissues may have been as a result of such breakdown (Bligh and Scott, 1996).

Table 10. Percentage lipid classes present in the pyloric ceacum tissue of selected L. Naivasha fish species. Mean ± SD (n=6).

Fish species	Lipid classes							
	WE[1] (%)	**TAG (%)**	**DAGE (%)**	**ST (%)**	**FFA (%)**	**PE (%)**	**Other lipids (%)**	**PC (%)**
C. carpio	$0.843\pm0.11_{b}^{D2}$	$14.504\pm0.91_{b}^{B}$	$1.515\pm0.08_{a}^{D}$	$1.865\pm0.13_{a}^{CD}$	$12.262\pm0.57_{a}^{B}$	$32.016\pm3.25_{a}^{A}$	$6.615\pm0.81_{a}^{C}$	$30.380\pm3.07_{a}^{A}$
M. salmoides	$1.271\pm0.09_{a3}^{F}$	$20.133\pm1.73_{a}^{C}$	$1.816\pm0.36_{a}^{F}$	$2.054\pm0.30_{a}^{F}$	$13.473\pm0.96_{a}^{D}$	$25.101\pm1.95_{a}^{B}$	$6.384\pm1.00_{a}^{E}$	$29.767\pm1.15_{a}^{A}$
C. specularis	$0.720\pm0.07_{b}^{D}$	$15.887\pm0.96_{ab}^{B}$	$1.507\pm0.14_{a}^{D}$	$1.890\pm0.10_{a}^{CD}$	$12.756\pm0.85_{a}^{B}$	$30.997\pm4.82_{a}^{A}$	$7.116\pm0.71_{a}^{C}$	$32.584\pm1.02_{a}^{A}$

[1]WE = Wax esters, TAG = Triacylglceride, DAGE = Diacylglceride, ST = Sterol, FFA = Free fatty acids, PE = Phosphatidylethanolamine,
PC = Phosphatidylcholine other lipids = inositol, sphingolipids
[2] Different means with different capital letters within a row are significantly different at the $P < 0.05$ level (Tukey's Least Significant Differences).
[3]Different means with different small letters within a column are significantly different at the $P < 0.05$ level (Tukey's Least Significant Differences).

Table 11. Percent lipid classes present in the liver tissue of selected L. Naivasha fish species. Mean ± SD (n=6).

Fish species	Lipid class							
	WE[1](%)	**TAG (%)**	**DAGE (%)**	**ST (%)**	**FFA (%)**	**PE (%)**	**Other (%)**	**PC (%)**
C. carpio	$1.415\pm0.33_{a}^{D}$[2]	$25.681\pm1.39_{a}^{A}$	$1.081\pm0.19_{b}^{D}$	$2.958\pm0.36_{a}^{D}$	$11.279\pm0.87_{b}^{C}$	$22.153\pm1.53_{bc}^{B}$	$8.869\pm0.92_{ab}^{C}$	$26.562\pm0.84_{b}^{A}$
M. salmoides	$1.714\pm0.76_{a}^{D}$[3]	$25.679\pm1.21_{a}^{A}$	$2.340\pm0.17_{a}^{D}$	$2.210\pm0.13_{ab}^{D}$	$12.723\pm0.63_{b}^{C}$	$23.707\pm0.92_{b}^{A}$	$10.658\pm0.32_{a}^{C}$	$20.969\pm0.98_{c}^{B}$
C. specularis	$1.708\pm0.20_{a}^{E}$	$12.662\pm2.56_{b}^{C}$	$2.441\pm0.38_{a}^{E}$	$2.700\pm0.30_{a}^{E}$	$13.978\pm1.29_{b}^{C}$	$18.916\pm1.22_{c}^{B}$	$7.073\pm1.64_{b}^{D}$	$32.041\pm0.53_{a}^{A}$
O. leucostictus	$1.408\pm0.05_{a}^{E}$	$16.707\pm0.29_{b}^{C}$	$1.254\pm0.27_{b}^{E}$	$2.125\pm0.06_{b}^{E}$	$16.919\pm0.15_{a}^{C}$	$27.969\pm0.24_{a}^{A}$	$11.275\pm0.22_{a}^{D}$	$22.346\pm0.74_{c}^{B}$

[1]WE = Wax esters, TAG = Triacylglceride, DAGE = Diacylglceride, ST = Sterol, FFA = Free fatty acids, PE = Phosphatidylethanolamine,
PC = Phosphatidylcholine other lipids = inositol, sphingolipids

[2] Different means with different capital letters within a row are significantly different at the $P < 0.05$ level (Tukey's Least Significant Differences).

[3]Different means with different small letters within a column are significantly different at the $P < 0.05$ level (Tukey's Least Significant Differences).

4.3.4 Lipid classes present in the muscle tissue of selected Kenyan coast fish species

Table 12 shows the average percentage of various lipid classes in the muscles of three fish species sampled from the Kenyan coast. *M. niger* had the highest content of wax esters (3.13 ± 0.13) while *S. luridus* recorded the least amount. Phleger *et al.*, (1997) reported that wax ester lipid class composition of fish from Antarctica waters - *Electrona antarctica* ranged between 86.2 and 90.5% of the total lipids. The figures were significantly high compared to the results obtained in the present study. This could be as a result of differences in environmental condition, cold weather especially during winter. Deep sea fishes tend to store their long term energy reserves as wax esters unlike the triaclyglycerides that are short term energy reserve molecules. Wax esters have lower density than triaclyglcerides hence are more buoyant which help mid water fishes to maintain their position in the water column.

Table 12. Percentage lipid classes in the muscle tissue of selected Kenyan coast fish species. Mean ± SD (n=6).

Fish species	Lipid class							
	WE[1] (%)	**TAG (%)**	**DAGE (%)**	**ST (%)**	**FFA (%)**	**PE (%)**	**Other lipids (%)**	**PC (%)**
S. luridus	$1.772\pm0.04_{c}^{D2}$	$20.987\pm0.38_{a}^{B}$	$0.087\pm0.00_{c}^{E}$	$1.325\pm0.07_{a}^{DE}$	$11.582\pm0.34_{a}^{C}$	$20.085\pm0.34_{c}^{B}$	$11.322\pm0.27_{a}^{C}$	$32.840\pm1.01_{a}^{A}$
L. campechanus	$2.623\pm0.04_{b3}^{F}$	$17.815\pm0.01_{b}^{C}$	$1.551\pm0.02_{b}^{G}$	$1.304\pm0.02_{a}^{G}$	$7.592\pm0.16_{c}^{E}$	$24.469\pm0.05_{a}^{B}$	$9.363\pm0.27_{b}^{D}$	$35.283\pm0.26_{a}^{A}$
M. niger	$3.131\pm0.13_{a}^{EF}$	$15.092\pm0.81_{c}^{C}$	$1.695\pm0.62_{a}^{F}$	$6.192\pm3.44_{a}^{DE}$	$10.096\pm0.27_{b}^{D}$	$23.096\pm0.48_{b}^{B}$	$6.829\pm0.41_{c}^{DE}$	$33.868\pm1.47_{a}^{A}$

[1]WE = Wax esters, TAG = Triacylglceride, DAGE = Diacylglceride, ST = Sterol, FFA = Free fatty acids, PE = Phosphatidylethanolamine, PC = Phosphatidylcholine other lipids = inositol, sphingolipids

[2] Different means with different capital letters within a row are significantly different at the $P < 0.05$ level (Tukey's Least Significant Differences).

[3]Different means with different small letters within a column are significantly different at the $P < 0.05$ level (Tukey's Least Significant Differences).

There were significant differences recorded in the amount of diacylglyceride in the fish muscles. *M. niger* had the highest amount of DAGE (1.70 ± 0.62) while *S. luridus* recorded the least amount (1.09 ± 0.20). *L. campechanus* had (1.55 ± 0.02%) of DAGE in its muscle tissue. Triacylglyceride was the highest lipid class in the neutral lipids. The lipid composition of the TAG ranged between 15.10 and 21.00% with the highest average percent in the *S. luridus*.

The selected marine fishes had higher levels of TAG composition than the selected L. Naivasha fishes from the results obtained in this study. This could be attributed to the differences in environmental conditions and dietary factors. Most freshwater fishes feed on the green algae while marine fishes mostly feed on phytoplankton and zooplankton. Season of catch could also influence the amount of TAG in the muscle tissue of fish.

4.3.5 Lipid classes present in the orbital tissue of different marine fish species

Lipid class composition study carried out in the orbital section of the three marine fish revealed significant differences (ANOVA, $p < 0.05$). However, there were no significant differences in the composition of sterols, free fatty acids, phosphatidylethanolamine and phosphatidylcholine in the orbital tissue of the three species (Table 13). The orbital section of *S. luridus* had the highest amounts of wax esters (WE) (9.15 ± 0.40) compared with *L. campechanus* (1.69 ± 0.30) and *M. niger* (2.12 ± 0.03) that were not statistically (ANOVA, $p>0.05$) different from each other. The orbital section of *S. luridus* had the highest amount of triacylglycerides. However,

the amount of triacylglycerides (TAG) in *S. luridus* (12.30 ± 0.10) did not differ statistically with *M. niger* (12.29 ± 0.27) at ANOVA, $p>0.05$.

Table 13. Percentage lipid classes present in the orbital tissue of selected Kenyan coast fish species. Mean ± SD (n=6).

Fish species	Lipid class							
	WE[1] (%)	TAG (%)	DAGE (%)	ST (%)	FFA (%)	PE (%)	Other lipids (%)	PC (%)
S. luridus	$9.154\pm0.40_a^{D2}$	$12.295\pm0.10_a^{C}$	$2.471\pm0.17_b^{E}$	$2.029\pm0.06_a^{E}$	$15.680\pm0.33_a^{B}$	$23.067\pm0.73_a^{A}$	$12.180\pm0.55_b^{C}$	$23.123\pm0.30_a^{A}$
L. campechanus	$1.674\pm0.30_{b3}^{D}$	$10.299\pm0.04_b^{C}$	$3.236\pm0.02_a^{D}$	$2.176\pm0.12_a^{D}$	$18.010\pm3.05_a^{B}$	$26.967\pm2.90_a^{A}$	$14.001\pm0.57_{ab}^{BC}$	$23.637\pm0.57_a^{A}$
M. niger	$2.117\pm0.03_b^{D}$	$12.291\pm0.27_a^{C}$	$3.233\pm0.03_a^{D}$	$2.095\pm0.02_a^{D}$	$14.598\pm0.09_a^{B}$	$23.766\pm0.82_a^{A}$	$16.466\pm0.63_a^{B}$	$25.434\pm1.67_a^{A}$

[1]WE = Wax esters, TAG = Triacylglceride, DAGE = Diacylglceride, ST = Sterol, FFA = Free fatty acids, PE = Phosphatidylethanolamine,
PC = Phosphatidylcholine other lipids = inositol, sphingolipids

[2] Different means with different capital letters within a row are significantly different at the $P < 0.05$ level (Tukey's Least Significant Differences).

[3]Different means with different small letters within a column are significantly different at the $P < 0.05$ level (Tukey's Least Significant Differences).

4.4 Peak identification of fatty acid methyl esters

Fatty acid methyl esters were identified using marine lipid methyl esters certified reference standards (PUFA-1, Marine source Cat. No. 47033 and PUFA-3, Menhaden oil Cat. No. 47085-U, Supelco UK) and comparison of the mass spectral data otained by GC-MS with that of the NIST Library. The fatty acids were identified by comparison of the retention times with those of standard purified fatty acids (Shirai, *et al.*, 2001).

4.4.1 Qualitative analysis of fatty acid composition in the TAG deposit lipids

The results of the GC separation of the fatty acid methyl esters derived from the TAG fraction in fish species from L. Naivasha are shown in figures 9 - 15 whereas figures 16 - 21 represent fatty acid methyl esters from marine fishes (Appendices).

There is an inter and intra species variability in the composition of fatty acids of fish lipids and of the specific polyunsaturated fatty acids in particular. This may be attributed to the existence of a large number of external and internal factors. The external factors include; environment, culturing method, and tropic effects. The internal factors include fish species, feeding regime and digestion, life cycle stage, quantitative and qualitative characteristics of lipids- triacyglycerols, phospholipids and their topographical origin- dorsal and ventral part of muscle tissue (Buchtova *et al*., 2004).

M. salmoides, C. carpio, C. specularis and *O. leucostictus* are commercially important to freshwater fisheries in L. Naivasha while *L. campechanus, M. niger* and *S. ludridus* are just but a few commercially important marine fishes along the Kenya coast. They

constitute a major part of landing, aquaculture and sources of animal protein. The fatty acids compositions of the selected L. Naivasha fishes (Table 14) vary greatly from the selected marine fishes (Table 15).

The major saturated fatty acids and their relative retention times included: methyl myristate (Rt, 25.572), methyl pentadecanoate (Rt, 28.106), methyl palmitate (Rt, 30.406), methyl margarate (Rt, 31.895) and methyl stearate (Rt, 34.174) while the major unsaturated fatty acids included: methyl palmitoleate (Rt, 31.275), methyl oleate (Rt, 34.640), methyl linoleate (Rt, 35.657), methyl linolenate (Rt, 41.625), methyl arachidate (Rt, 44.810), methyl eicosenoate (Rt, 49.144) and methyl docosahexaenoate (Rt, 49.594).

Table 14. Fatty acid compositions of selected L. Naivasha fish species (+ = present, - = absent).

Fatty Acid	Fish species										
	M. salmoides			***C. carpio***			***C. specularis***			***O. leucostictus***	
	Muscle	Pyloric ceacum	Liver	Muscle	Pyloric ceacum	Liver	Muscle	Pyloric ceacum	Liver	Muscle	Liver
Myristic (C14:0)	+	+	-	-	+	-	+	+	-	+	-
Pentadecanoic (C15:0)	+	+	+	-	+	+	-	+	+	+	+
Palmitic (C16:0)	+	+	-	+	+	-	+	+	+	+	+
Palmitoleic (C16:1)	+	+	-	-	+	-	-	-	-	+	+
Stearic (C18:0)	-	-	+	+	-	-	-	+	-	-	-
Oleic (C18:1)	+	-	+	+	-	+	-	-	-	-	-
Linoleic (C18:2)	+	+	+	-	+	+	-	+	+	-	+
Linolenic (C18:3)	+	-	+	-	+	-	+	-	-	-	+
Margaric (C19:0)	-	-	-	-	+	-	-	-	-	+	-
Arachidonic (C20:4)	-	-	+	-	+	+	+	-	-	-	+
Eicosapentaenoic (C20:5)	-	-	-	-	-	-	-	-	-	-	-
Docosahexaenoic (C22:6)	-	-	-	-	-	-	-	-	-	-	-

These varieties, as well as the quality of fatty acids observed may be due to differences in sub species, diet, spawning cycle, season and environment. The degree of unsaturation of fish oils vary with seasons. It rises as the water temperature falls and vice versa (IFFO BULLETIN No.18, 2000). The knowledge of fatty acids composition of fishery species has fundamental importance in the application of different technological processes in fish preservation, processing and product development.

All freshwater fishes except *C. carpio* seem to have saturated fatty acid in the muscle and the pyloric ceacum (Table 14). The major saturated fatty acids identified in these species were C14:0 and C16:0. However, substantial presence of C18:2n-6 and C18:1 (Table 14) was also noted. C18:1 and C18:2n-6 presence may encourage lower melting point. C18:2n-6 and C18:3n-3 content among other factors (viscosity, colour, iodine value and peroxide value) determine oil quality for industrial purposes. Saturated fatty acids and cholesterol are major dietary contributors to coronary heart disease, due to their oxidation in the presence of light and molecular oxygen through a free radical reaction (Diplock, 1993).

The branched chain fatty acids contents in the freshwater species studied were generally absent in the muscle tissue especially in all fishes except *C. specularis*. Branched chain fatty acid has an important advantage. Branched chain fatty acids influence lower melting point, lower cholesterol levels, provide energy, influence some ribosomal functions which are necessary for peptide elongation and form an integral part of

biomembranes while those esterified with cholesterol stimulate protein synthesis (Hradec *et al*, 1974).

Table 15. Fatty acid composition of selected Kenyan coast fish species (+ = present, = absent).

Fatty Acid	**Fish species**					
	L. campechanus		***M. niger***		***S. luridus***	
	Muscle	Orbital	Muscle	Orbital	Muscle	Orbital
Myristic (C14:0)	+	+	+	+	+	+
Pentadecanoic (C15:0)	-	+	-	-	+	-
Palmitic (C16:0)	+	+	+	+	+	+
Palmitoleic (C16:1)	-	+	-	-	-	-
Stearic (C18:0)	-	-	-	-	-	-
Oleic (C18:1)	-	-	-	+	-	-
Linoleic (C18:2)	-	+	+	+	-	-
Linolenic (C18:3)	+	-	-	+	+	-
Margaric (C19:0)	+	-	+	-	-	-
Arachidonic (C20:4)	-	-	-	-	-	+
Eicosapentaenoic (C20:5)	+	-	+	+	+	-
Docosahexaenoic (C22:6)	+	-	+	-	+	+

The fatty acid composition of marine fish species (Table 15) have almost similar pattern. All marine fishes are higher in saturated fatty acids, particularly C14:0 and C16:0, higher in polyunsaturated fatty acids (C20:5 and C22:6), lower in monounsaturated fatty acids (C16:1 and C18:1), similar in fatty acids composition.

4.4.2 Comparison of fatty acids composition of freshwater and marine fishes

The results obtained in the present study revealed that freshwater fish has more omega-6 series of the polyunsaturated fatty acids while the marine has more omega-3 series (Tables 14 and 15). The prominent omega-3 being C22:6 while the C18:2 are for the omega-6 series. This may suggest that the dietary essential fatty acids requirements for marine fish for omega-3 polyunsaturated fatty acids may be higher than that of freshwater fish. It could also suggest that marine species are suppliers of C18:3n-3; the initial building blocks for all omega-3 fatty acids in the human body. The marine species are better in C22:6n-3. The freshwater species are in C18:2n-6 as well as in C20:4n-6 contents. The latter fatty acid is a major constituent of membrane lipids (phospholipids) and is the principal precursor by enzymatic action of hormone-like compounds known as eicosanoids including the prostaglandins (prostanoids, isoprostanes, and isofurans). The eicosanoids produced from C20:4n-6 cause the strongest inflammatory response in humans. Inflammation is one of the body defense mechanisms that reduce the spread of infection.

4.4.3 Mass spectrum of some polyunsaturated fatty acids

The mass spectra of methyl eicosaenoate (C20:5) is shown in Fig. 1. The molecular ion (M^+) at m/z 316 is present in extremely low abudance. The diagnostic peak at m/z 273 (M^+-43) and 242 (M^+-74) are prominent in the spectra. The position of the double bond could not be deduced from the mass spectrum of any unsaturated compound because the isomeric compound having double bond at different positions gives identical spectrum. One common feature to most of the polyunsaturated fatty acids is the observance of C_3H_7 (m/z 43), C_4H_7 (m/z 55), C_5H_7 (m/z 67), C_6H_7 (m/z 79), C_7H_7 (m/z 91) which emerged by a difference of 12 units only.

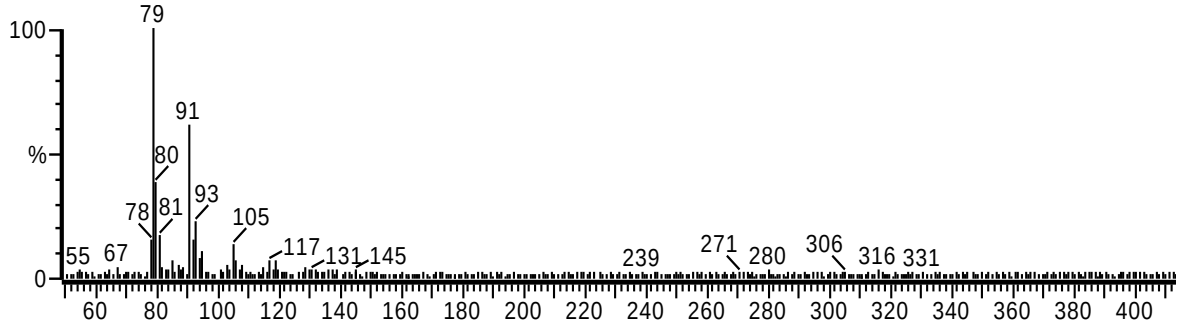


Figure 1. Mass spectrum of methyl eicosanoate present in marine fishes

Fig. 2 shows the mass spectra of methyl docosahexaenoate (C22:6). The compound did not show the molecular mass peak at m/z 243. As mentioned in eicosapentaenoic acid, the common diagnostic peaks such as at m/z 55,67, 79, and 91 were prevalent in this compound, m/z 79 being the base peak of the spectrum. The ions at m/z 105 (91 + CH_2), 119 (105 + CH_2), 133 (119 + CH_2) and 147 (133 + CH_2) were also observed, the regular

difference being 14 units between each subsequent peak. At m/z 99 (M^+-43) a small intensity peak could be seen in the spectrum.

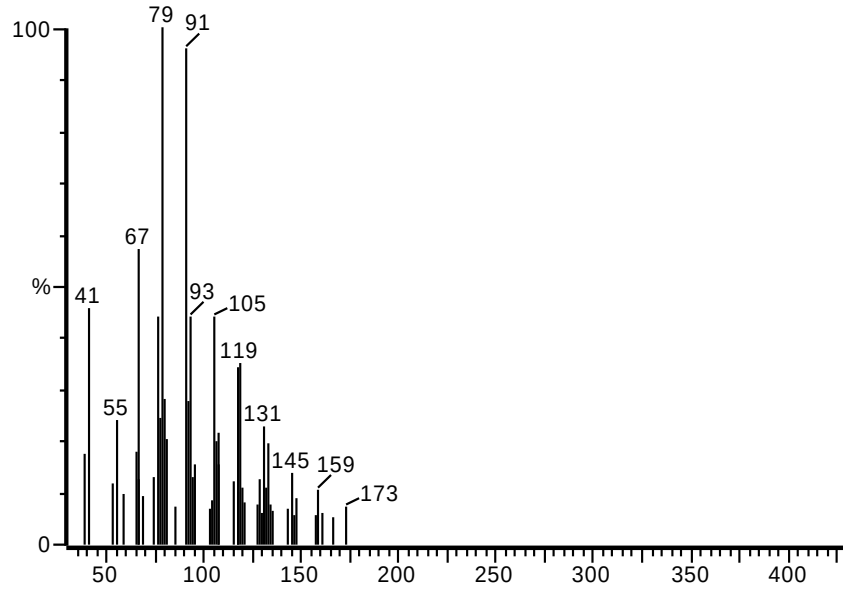


Figure 2. Mass spectrum of methyl docosahexaenoate present in marine fishes

The identities of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in marine fish oils by co-chromatography with reference standards as well as through their mass spectra clearly indicates that these fatty acids are present in marine fish oils samples extracted from the muscle and the orbital. The diagnostic fragment of each identified fatty acid methyl ester is summarized in Table 16.

Table 16. Mass and their fragmentation pattern of fatty acids from L. Naivasha and Kenyan coast selected fish species

Systemic name	Common name	Molecular formula	Molecular weight	Mass, fragmentation pattern and % abudance
Saturated Fatty Acids				
Methyl tetradecanoate	Methyl myristate	$C_{15}H_{30}O_2$	242	GC-MS, m/z 242 (M^+ $C_{15}H_{30}O_2$, 13%), 211 (M^+ -31, 14%), 199 (M^+ -43, 11%), 185 (2%), 157 (2%), 143 (M^+ -99, 12%), 129 (5%), 111 (2%), 107 (7%), 87 (65%), 74 (100%), 55 (22%).
Methyl *n*-pentadecanoate	Methyl pentadecanoate	$C_{16}H_{32}O_2$	256	GC-MS, m/z 256 (M^+, $C_{16}H_{32}O_2$, 12%), 225 (M^+ -31, 7%), 213 (M^+ -43, 10%), 185 (2%), 171 (1%), 157 (5%), 143 (14%), 129 (4%), 115 (2%), 101 (5%), 87 (77%), 74 (100%).
Methyl *n*-hexadecanoate	Methyl palmitate	$C_{17}H_{34}O_2$	270	GC-MS, m/z 270 (M^+, $C_{17}H_{34}O_2$, 56%), 239 (M^+ -31, 29%), 227 (M^+ - 43, 39%), 199 (11%), 185 (17%), 171 (14%), 157 (7%), 143 ($C_8H_{15}O_2$, 62%), 129 (26%), 101 (20%), 87 (100%), 74 ($C_3H_6O_2$, 92%), 55 (73%).
Methyl *n*-heptadecanoate	Methyl margarate	$C_{18}H_{36}O_2$	284	GC-MS, m/z 284 (M^+, $C_{18}H_{36}O_2$, 32%), 253 (M^+ -31, 10%), 241 (M^+ -43, 17%), 227 (2%), 213 (1%), 199 (7%), 185 (7%), 171 (1%), 157 (1.5 %), 143 (24%), 129 (10%), 115 (2.5%), 101 (9%), 87 (100%), 74 (99%), 55(42%).

Unsaturated fatty acids				
Methyl hexadecanoate	Methyl palmitoleate	$C_{17}H_{32}O_2$	268	GC-MS, m/z 268 (M^+, $C_{17}H_{32}O_2$, 7%), 236 (M^+ -32, 25%), 194 (M^+ -74,17%), 180 (2.5%), 166 (4%), 152 (17%), 138 (10%), 124 (13%), 110 (22%), 96 (33%), 82 (47%), 74 (64%), 55 (100%).
Methyl *n*-octadecanoate	Methyl oleate	$C_{19}H_{36}O_2$	296	GC-MS, m/z 296 (M^+, $C_{19}H_{36}O_2$, 6%), 264 (M^+ -32, 25%), 222 (M^+ -74, 12.5%), 180 (10%), 166 (7%), 152 (3.5%), 138 (8%), 124 (8.5%), 110 (17.5%), 97 (40%), 83 (42%), 69 (76%), 55 (100%).
(all-cis)-octadecatrienoate 9,12,15	Methyl linolenoate	$C_{19}H_{32}O_2$	292	GC-MS, m/z 292 (M^+, $C_{19}H_{32}O_2$, 61%), 261 (M+ -31, 18%), 236 (M+ 56, 20%), 223 (M+ 69, 7%), 191 (8%), 175 (7%), 161 (9%), 147 (27%), 133 (17%), 119 (32%), 105 (73%), 91 (94%), 79 (100%), 67 (53%), 55 (43%).
(all -cis)-eicosapentaenoate 5,8,11,14,17	Methyl eicosapentaenoate	$C_{21}H_{32}O_2$	316	GC-MS, m/z 316 (M^+, $C_{21}H_{32}O_2$, 0.2%,), 273 (M^+ -43, 0.5%), 242 (M^+ 74, 0.2%), 217 (5%), 203 (4.5%), 189 (0.2%), 175 (8%), 161 (7%), 147 (13.5%), 133 (25%), 119 (43%), 105 (38%), 91 (75%), 79 (100%), 67 (62%), 55 (33%).
(all-cis)-docosahexaenoate 4,7,10,13,16,19	Methyl docosahexaenoate	$C_{23}H_{34}O_2$	342	GC-MS, m/z 342 (M^+, $C_{23}H_{34}O_2$,), 311 (M^+ -31, 0.2%), 236 (M^+ -106, 0.5%), 222 (1%), 208 (5.5%), 194 (1%), 180 (2.5%), 175 (5%), 161 (12%), 147 (12%), 133 (22%), 119 (43%), 105 (43%), 91 (86%), 79 (100%), 67 (65%), 55 (33%).

4.5 Quality Assurance

Results of the recoveries of spiked fish muscle samples are given in Table 17. Percentage recoveries obtained for the metals under investigation (Pb, Zn, Cu, Ni and Cd) varied between 92.25 ± 3.28 – 101.56 ± 4.03%. Acceptable recoveries were obtained in all cases, which show that the digestion method used for fish tissues samples and the FAAS analysis were reliable.

Table 17. Percentage recoveries of trace metals from spiked fish muscle (Mean ± SD)

Trace metal	**Spike level**	**Expected concentration in spiked sample digest (mgL^{-1})**	**Observed concentration in spiked sample digest*(mgL^{-1})**	**% Recovery±SD**
Pb	30 mL of (1.8 mg L^{-1} of Pb and Cd; 3 mg L^{-1} of Ni; 5 mg L^{-1} of Zn and Cu) standard solution	1.080	1.012	93.69 ± 1.95
Zn		3.000	3.047	101.56 ± 4.03
Cu		3.000	2.929	97.62 ± 2.92
Ni		1.800	1.701	94.48 ± 3.37
Cd		1.080	0.996	92.25 ± 3.28

4.6 Heavy metal levels in fish muscle from L. Naivasha and the Kenyan coast

Heavy metal levels in the freshwater and marine fishes were determined. These metals included; cadmium, copper, lead, nickel and zinc. Results indicated that, there were significant differences (ANOVA, $p < 0.05$). High levels of zinc were recorded in all the specimens under study compared to other heavy metals studied. The zinc levels in the muscle tissue of the selected L. Naivasha fishes were higher than the selected Kenya coast fishes. However, they were not significantly different ($p > 0.05$). Table 18 shows the mean concentrations ± SD ($n = 6$) in mg/Kg dry weight of metals in the selected L. Naivasha and Kenya coast fish muscles.

Table 18. The concentration (mg/Kg dry weight) of metals in selected fish species from L. Naivasha and Kenyan coast. Mean ± SD (n=6)

	L. Naivasha fishes				Kenyan coast fishes		
Metal	***M. salmoides***	***C. carpio***	***C. specularis***	***O. leucostictus***	***S. luridus***	***L. campechanus***	***M. niger***
Copper (Cu)	$1.061\pm0.01_{b}^{BC1}$	$1.162\pm0.04_{c}^{A}$	$1.133\pm0.01_{b}^{AB}$	$1.123\pm0.05_{c}^{AB}$	$1.008\pm0.00_{c}^{C}$	$1.101\pm0.02_{c}^{AB}$	$1.069\pm0.01_{c}^{BC}$
Lead (Pb)	$1.634\pm0.26_{b2}^{A}$	$1.563\pm0.27_{bc}^{A}$	$1.325\pm0.31_{b}^{AB}$	$1.738\pm0.06_{b}^{A}$	$0.679\pm0.04_{c}^{B}$	$0.897\pm0.04_{c}^{B}$	$1.667\pm0.11_{b}^{A}$
Nickel (Ni)	$1.660\pm0.02_{b}^{AB}$	$1.700\pm0.04_{b}^{A}$	$1.634\pm0.02_{b}^{AB}$	$1.614\pm0.06_{b}^{AB}$	$1.495\pm0.05_{b}^{B}$	$1.687\pm0.03_{b}^{A}$	$1.634\pm0.13_{b}^{AB}$
Zinc (Zn)	$4.922\pm0.46_{a}^{B}$	$7.752\pm0.13_{a}^{A}$	$7.678\pm1.04_{a}^{A}$	$4.917\pm0.27_{a}^{B}$	$5.093\pm0.11_{a}^{B}$	$3.062\pm0.24_{a}^{C}$	$4.246\pm0.05_{a}^{BC}$
Cadmium (Cd)	$0.141\pm0.01_{c}^{BC}$	$0.203\pm0.00_{d}^{A}$	$0.169\pm0.12_{b}^{B}$	$0.135\pm0.02_{d}^{C}$	$0.164\pm0.01_{d}^{BC}$	$0.133\pm0.01_{d}^{C}$	$0.154\pm0.00_{d}^{BC}$

[1] Different means with different capital letters within a row are significantly different at the $P < 0.05$ level (Tukey's Least Significant Differences).

[2]Different means with different small letters within a column are significantly different at the $P < 0.05$ level (Tukey's Least Significant Differences).

Lead, zinc and nickel metal concentration were significantly higher than other metals. However, there were no significant differences between them. Cadmium was low in concentration in every muscle of the selected fish species studied.

The levels of metals were consistently high in both *C. carpio* and *C. specularis* as shown in Table 18. This could be attributed to the fact that the carp are bottom feeders (rooters) who prove to be quite destructive. Evidence has also proven that the carp prey on the eggs of other fishes and their foraging activities can destroy spawning beds of more desirable species (Koehn, 2004). Therefore, *C. carpio* and *C. specularis* are responsible for the decline of some native fish species such as the *O. leucostictus* in L. Naivasha. The mature carps are omnivorous that specialize on invertebrates which live on the sediment while the young juvenile carps feed on zooplankton. There could be accumulation of heavy metals from the water to the algae which is food to the juvenile carps and later bio-magnify to the mature carps who feed on both the algae and the smaller fishes.

The concentrations of copper in the samples analyzed ranged from 1.008 to 1.162 mg/kg meat, with the highest concentrations in *C. carpio* (1.162 ± 0.04 mg/kg). But the concentrations in the samples were much below the toxic limit (30 mg/kg in FAO, 1983). The concentration of Cu in fish muscle tissue both of marine and freshwater showed an appreciable amount of absorption of Cu in relation to the Cu content in the waters of the various locations. Most probably the affluents to the Kenya coast are not carrying higher content of Cu than the affluents in L. Naivasha because of poor

industrial development at the South coast where the samples were obtained and the main source could be from anti-fouling agents which are used in marine paints. It appears that Cu could be partly taken up from the sediments where the fish flourish.

Copper is an essential part of several enzymes and it is necessary for the synthesis of hemoglobin. The richest sources of copper are shellfish, especially oysters and crustaceans (Underwood, 1977). No deficiencies of copper in adults have been reported but, in infants, anemia and hypoproteinemia are common (Underwood, 1977).

There were no significant differences between the levels of lead in the muscle tissue of the *M. salmoides*, *C. carpio*, *O. leucostictus* and *M. niger* at ANOVA, $p < 0.05$. The least concentration was detected in two marine fishes; the *S. luridus* (0.679 ± 0.04 mg/kg) and the *L. campechanus* (0.897 ± 0.04 mg/kg). However, all the samples contained lead above 0.4 mg/kg (EC, 2001) and also above the FAO recommended level of 0.5 mg/kg (FAO, 1983). Lead concentration in the fish muscle of the locations under study may be attributed to the inpouring effluents of industries consuming lead in different forms from the industrial products. The main sources of lead in the Kenya coast are the oil refinery and rivers like Athi and Tana which pass through industries like plastic, textile, dying and bleaching and glass industries. Lead causes renal failure and liver damage in humans (Emmerson, 1973).

The highest concentration of Nickel was noted in the muscle of *C. carpio* (1.7 ± 0.04 mg/kg) collected from L. Naivasha and *L. campechanus* (1.687 ± 0.03 mg/Kg) collected from the Kenya coast which was not significantly different at $p < 0.05$. Variable

concentration of nickel in the muscle of fishes most probably indicates the relative absorption capacity of the fish species in the environment where interactions with the pollutants of the water take place. The minimal risk level for nickel is 70 – 80 mg/Kg (USFDA, 1993c), and the samples analyzed showed concentrations only up to 1.70 mg/Kg meat. Therefore, the fish muscles under study are safe for consumption.

Zinc concentrations in the muscle of fishes under study showed a wide variation. The variation in the concentration of zinc in the fish species was generally higher in selected fishes from L. Naivasha than those from the Kenya coast. The highest concentration was detected in *C. specularis* (7.678 ± 1.04 mg/Kg) and *C. carpio* (7.752 ± 0.13 mg/Kg) which were not statistically different while the least concentration was noted in *L. campechanus* (3.062 ± 0.24 mg/Kg). These variations in zinc content in the fish species under study may be attributed to the relative abundance of organic constituents in water and sediments for which zinc has a higher affinity and the fishes consume them.

Zinc (Zn) is an essential trace element for both animals and humans. The recommended daily allowance is 10 mg/day in growing children and 15 mg/day for adults (NAS-NRC, 1974). A deficiency of zinc is marked by retarded growth, loss of taste and hypogonadism, leading to decreased fertility. Zinc toxicity is rare but, at concentrations in water up to 40 mg/kg, may induce toxicity, characterized by symptoms of irritability, muscular stiffness and pain, loss of appetite, and nausea (NAS-NRC, 1974).

Cadmium (Cd) was significantly the least in *L. campechanus* (0.13 ± 0.01 mg/Kg) and *O. leucostictus* (0.14 ± 0.02 mg/Kg). The cadmium content in all the fish species under study were much below the legal limit of 1 mg/kg meat (EC, 2001).

Different fish species contained strikingly different metal levels in their tissues. This may be related to the differences in ecological needs, swimming behaviors and the metabolic activities among different fish species. The differences in metal concentrations of the muscle tissues might be as a result of their capacity to induce metal-binding proteins such as metallothioneins.

Table 19 shows heavy metal levels in fish from different regions that have been reported by other researchers. Figures obtained in this study for cadmium, copper, lead, zinc and nickel in the muscle tissue of the different freshwater and marine fish species are within the values reported in literature.

Table 19. Comparison of heavy metal levels in fish muscles with values reported in literature

Sample area	Cd	Cu	Ni	Pb	Zn	References
Middle Black Sea[1]	0.09–0.48	1.28–2.93	-	0.22–0.85	9.5–22.9	Tüzen , 2002
Black Sea Coast[1]	<0.02–0.24	1.01–4.54	<0.01–2.04	<0.05–0.06	25.7–44.2	Topcuoğlu, *et al.,* 2002
Kerguelen Islands[1]	0.01–0.1	0.5–2.5	-	-	9.2–33.2	Bustamante, *et al.,* 2003
Masan Bay, Korea[1]	0.01	0.18–0.25	0.02	0.04–0.15	6.33–12.9	Kwon and Lee, 2001
California Lagoons[1]	0.1–0.3	1.9–7.5	0.61–12	0.8–4.1	36–150	Tamira, *et al.,* 2001
Mediterranean Sea[1], 1996	1.07–1.43	3.40–5.88	4.25–6.07	7.33–9.11	16.1–31.4	Kalay, *et al.,* 1999
Mediterranean Sea[1], 2000	0.37–0.79	2.19–4.4	-	2.98–6.12	16.5–37.4	Canlı and Atlı, 2003
Iskenderun Bay[2], 2001	-	0.66–1.98	0.32–1.72	-	8.99–42.18	Yılmaz, 2003
Iskenderun Bay[1], 2003	0.95	1.57	2.90	2.32	4.36	Türkmen, *et al.,* 2005

[1]Values represent the ranges or mean expressed as mg/kg dry wt.
[2]Values represent the ranges or mean expressed as μg/g wet wt.

4.6 Heavy metal concentration in water samples from L. Naivasha

Water samples from different sampling points was analysed and concentrations recorded. Five points were sampled and results were statistically different from each other ($p < 0.05$). In selecting the sampling points, R. Malewa and R. Gilgil inlets represented the water source. Kihoto sampling site was chosen because of its nearness to the flower farms that directly use water from the lake and later discharge it back to the lake. Generally, sampling points had significantly high cadmium concentration compared to other metals as shown in Table 20. Zinc metal was second highest in all sampling points in terms of concentration after cadmium.

Nickel and lead concentrations were significantly lower compared to other metals in all sampling points. All the sampling points had significantly different concentrations of zinc, cadmium and lead metals. The metal with the least concentration in L. Naivasha waters was lead.

Table 20. Mean concentrations (mg/l) of metals in the waters of L. Naivasha

Metal	Sampling sites				
	R. Malewa inlet	**Lake centre**	**R. Gilgil inlet**	**Kihoto**	**Central beach**
Cadmium	$1.531 \pm 0.1_{b}^{A1}$	$1.035 \pm 0.13_{b}^{D}$	$1.515 \pm 0.15_{b}^{AB}$	$1.503 \pm 0.15_{a}^{AB}$	$1.135 \pm 0.13_{b}^{C}$
Copper	$0.975 \pm 0.015_{c2}^{A}$	$0.952 \pm 0.008_{c}^{AB}$	$0.961 \pm 0.011_{c}^{AB}$	$0.888 \pm 0.002_{c}^{C}$	$0.919 \pm 0.025_{c}^{BC}$
Lead	$0.158 \pm 0.04_{e}^{C}$	$0.183 \pm 0.06_{e}^{AB}$	$0.166 \pm 0.04_{e}^{B}$	$0.175 \pm 0.05_{e}^{AB}$	$0.239 \pm 0.09_{e}^{A}$
Nickel	$0.473 \pm 0.16_{d}^{D}$	$0.559 \pm 0.15_{d}^{B}$	$0.493 \pm 0.16_{d}^{C}$	$0.561 \pm 0.09_{d}^{B}$	$0.62 \pm 0.01_{d}^{A}$
Zinc	$1.671 \pm 0.21_{a}^{C}$	$1.947 \pm 0.13_{a}^{B}$	$2.403 \pm 0.13_{a}^{A}$	$1.344 \pm 0.16_{b}^{D}$	$1.239 \pm 0.2_{a}^{E}$

[1] Different means with different capital letters within a row are significantly different at the $P < 0.05$ level (Tukey's Least Significant Differences).

[2]Different means with different small letters within a column are significantly different at the $P < 0.05$ level (Tukey's Least Significant Differences).

The results obtained in this study were compared to the World Health Organization (WHO) and European Union (EU) for drinking water as shown in Table 21. All heavy metals analyzed in the study except for zinc and copper had higher concentrations than what is recommended by the WHO and the EU.

Table 21. WHO and EU standard limits for drinking water (mg/l)

PARAMETER	UNIT	WHO LIMIT	EC LIMIT
Nickel	Ni	0.02	0.02
Cadmium	Cd	0.003	0.005
Copper	Cu	2.0	2.0
Lead	Pb	0.01	0.01
Zinc	Zn	5.0	5.0

The most probable source of nickel metal may be contributed by natural rocks as well as anthropogenic sources (Adamo *et al.,* 1996). Romic and Romic (2003) reported that basic and ultrabasic magmatic rocks can be a source of nickel. They further reported that sources such as combustion of fuel may have some effect on the Ni content especially along highways (Romic and Romic, 2003). This can consequently be carried to the lakes as runoff during rainy seasons. Adamo *et al.* (1996) on the other hand, reported that Ni often occurs as inclusion within the silicate sphere rather than as separate grains. In this way, therefore, Ni is protected against natural decomposition as well as reagents alteration and it is only the dissolution of the silicate through digestion with hydrofluoric acid and other strong acids that would ensure their extraction.

The lead content in the lake is suggestive of an anthropogenic source. The lake is situated along the busy national/ international highway (Nairobi - Nakuru, The Great

North Road). Vehicles sources could therefore, be the major contributor of Pb in this lake. High lead concentration in the air and soil in urban areas has been attributed to ever increasing automobiles especially those using leaded gasoline (Chow *et al.,* 1974). Environmental lead pollution and consequent poisoning are severe and increasing rapidly in developing countries (Nriagu *et al.,* 1996) and Kenya is no exception.

There are several sources of cadmium in the aquatic systems which include runoff from agricultural sector where phosphate fertilizer which most probably contains cadmium (Huang *et al.,* 2004) is applied to the farms situated around Lake Naivasha. The presence of cadmium could also be as a result of road traffic (wear of tyres), which has been described as an important source of cadmium emission (Alloway, 1990). The high mobility of cadmium (weak in binding to soil/sediment) (Lowler and Tippings, 2003) could also contribute to most if not all of it.

4.7 Heavy metal concentration in water samples from the Kenyan coast

Coastal waters were sampled for analysis of possible presence of heavy metals. The results indicate significant differences in concentrations of the metals that were found as shown in Table 22.

The stretch from Shimoni mainland to Wasini island is approximately 3 Km. The first sampling point was at the creek just before boarding a boat to the island. The other three sampling points were collected by after approximately 750m from the previous sampling point. The fifth sampling point was at the shallow waters of Wasini.

Table 22. Mean concentrations (mg/l) of metals in the waters of the Kenyan coast

Metal	Sampling sites				
	Shimoni (creek)	Shimoni (750 m)	Shimoni (1500 m)	Wasini (750 m)	Wasini (shore)
Cadmium	$1.824\pm0.17_{a}^{A1}$	$1.112\pm0.11_{b}^{D}$	$1.415\pm0.13_{b}^{C}$	$1.05\pm0.09_{b}^{D}$	$1.548\pm0.11_{a}^{B}$
Copper	$0.311\pm0.09_{d2}^{A}$	$0.245\pm0.06_{d}^{C}$	$0.275\pm0.03_{d}^{BC}$	$0.298\pm0.04_{d}^{AB}$	$0.265\pm0.09_{d}^{C}$
Lead	$0.76\pm0.03_{c}^{A}$	$0.683\pm0.14_{c}^{B}$	$0.757\pm0.1_{c}^{A}$	$0.59\pm0.1_{c}^{C}$	$0.777\pm0.12_{c}^{A}$
Nickel	$0.356\pm0.11_{d}^{A}$	$0.221\pm0.04_{d}^{C}$	$0.285\pm0.09_{d}^{B}$	$0.217\pm0.04_{e}^{C}$	$0.283\pm0.13_{d}^{B}$
Zinc	$1.597\pm0.10_{b}^{A}$	$1.341\pm0.11_{a}^{B}$	$1.444\pm0.07_{a}^{AB}$	$1.102\pm0.06_{a}^{C}$	$1.119\pm0.03_{b}^{C}$

[1] Different means with different capital letters within a row are significantly different at the $P < 0.05$ level (Tukey's Least Significant Differences).

[2] Different means with different small letters within a column are significantly different at the $P < 0.05$ level (Tukey's Least Significant Differences).

Cadmium metal was significantly higher compared to other metals in all the sampling points as shown in Table 22. However, at Shimoni creek cadmium metal had the highest concentration compared to other metals including lead. Copper and nickel metals were not significantly different from each other ($p > 0.05$) at Wasini shore. The results were compared to the WHO and EU recommended limits for drinking water. All metal concentration in all sampling sites except zinc and copper exceeded the recommended limits.

Zinc was present in all sampling points and was significantly higher than other metals except cadmium. Nickel was in low concentrations in every point except at the Shimoni creek. Generally, cadmium and zinc metals were exceptionally in higher concentrations than other metals. There were little variations in lead metal concentration among the sampled points unlike other metals.

High concentration of metals in water can retard fish development causing possible alterations in fish size (Friedmann *et al.,* 1996). Fish development can be affected by the presence of heavy metals in water and especially the early life stages such as hatching time, larval development and juvenile growth as they are more sensitive than the mature stages. Although both essential and non-essential metals may retard fish development, some metals like mercury seems to be more effective than the others. Both essential and non-essential metals could alter embryonic development of fish embryos causing retardation of normal development, disability of organs or mortality.

4.8 Fish consumption habits of Juja, Naivasha and Mombasa inhabitants

4.8.1 Frequency of fish consumption

A questionnaire was developed to determine the fish feeding habits of Juja, Naivasha and Mombasa residents. When the question on "how often do you consume fish?" was posed to the respondents, it was noted that only 12% of Juja respondents consume fish more than once in a week while 34% consume fish very few times in a year. 35% of Naivasha respondents and a whooping more than 60% of Mombasa respondents would eat fish more than once in a week as shown in Fig. 3. There were higher percentages of respondents who consume fish more than once in a week in Naivasha and Mombasa probably due to the fact that fish is readily available as opposed to the Juja respondents who have to wait for the catch from both L. Victoria and L. Naivasha via Nairobi or the Sagana fish farm.

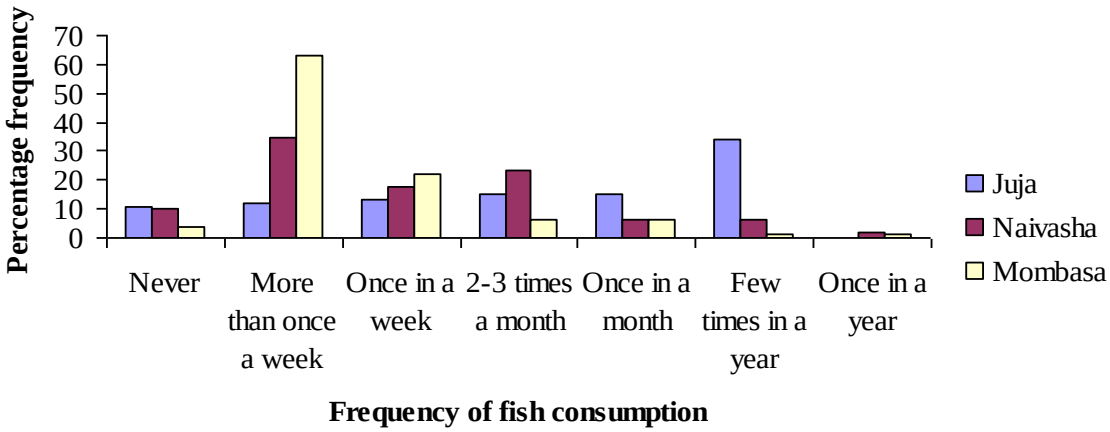


Figure 3. Frequency of fish consumption by Juja, Naivasha and Mombasa respondents

4.8.2 Reasons for not consuming fish

Fig. 4 shows the reasons why the respondents in different locations do not consume fish. When asked what was the, reason for not consuming fish, 15% of the respondents in Naivasha said they were allergic to fish meat while 13% and 9% of the respondents from both Juja and Mombasa respectively were also allergic to the fish meat. Juja and Naivasha residents had a problem of the fish meat not being readily available. For the Naivasha case, most of the catch is sold to the big hotels around the lake while the rest is transported to Nairobi where there is ready market. The surplus, if any is taken to the Naivasha municipal market where the Naivasha inhabitants can obtain their desired fish meat.

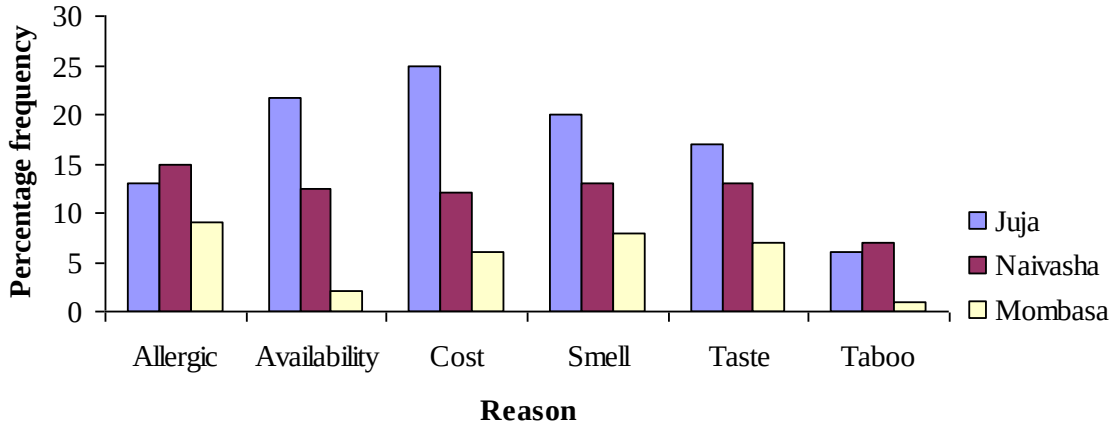


Figure 4. Reasons for not consuming fish

The price of fish meat is also another reason why 25% of Juja residents and 12% of Naivasha respondents do not consume fish more often unlike the Mombasa respondents with a 6% problem of price of fish meat. The price of one fish, approximately one kilogram goes for about KShs. 350/= (US$ 5.0) in Juja town. This could probably be

because of the many middle men involved in the sale of fish. Only 8% of Mombasa respondents had a problem with the smell and 7% did not consume fish because of the taste. 20% and 13% of Juja and Naivasha respondents respectively do not consume fish because they do not like the smell of fish meat. More than 5% of both Juja and Naivasha do not consume fish because it is a taboo in their community as they associated the appearance of the skin of fish to that of the reptile family such as the snakes.

4.8.3 Probable consumption of fish provided cheaply

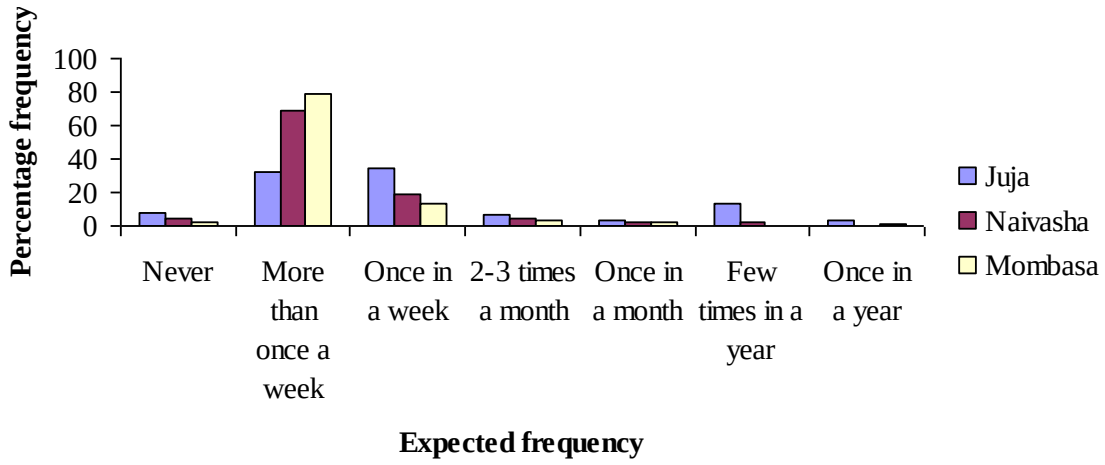


Figure 5. Expected frequency of fish consumption if provided at cheaply

From Fig. 5, more than 50% of the Naivasha and Mombasa respondents were willing to consume fish more than once in a week while only 34% of Juja respondents are willing to do so weekly if the fish meat is provided at an affordable price. This shows that there are other factors other than the cost of fish meat that influences the fish feeding habits of these communities.

4.8.4 Prioritized meat consumption of Juja respondents

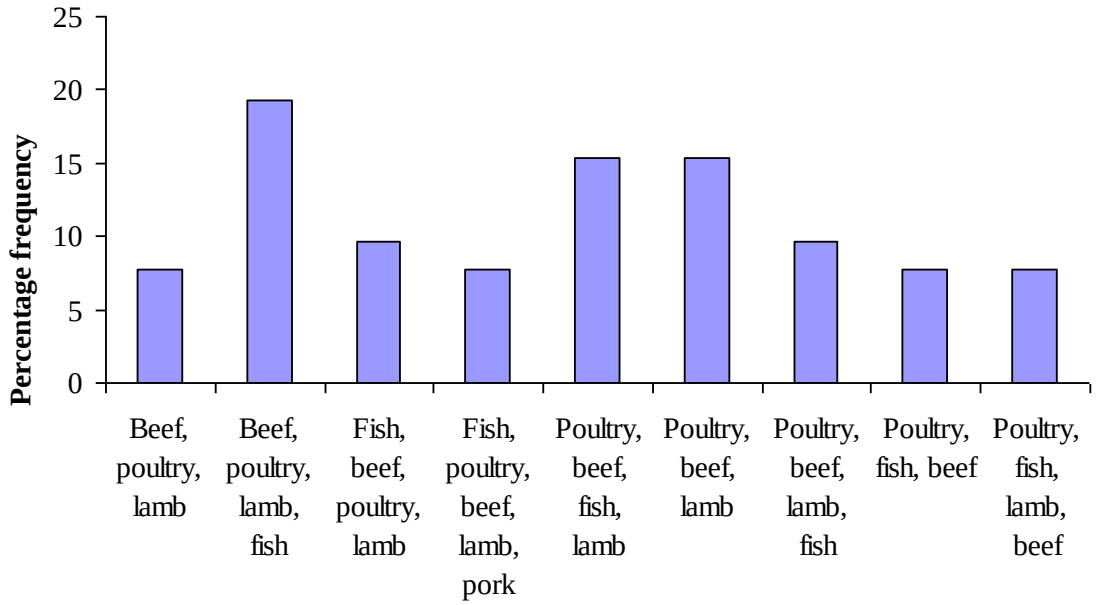


Figure 6. Prioritized meat consumption of Juja respondents

Beef, poultry, lamb and fish is the most preferred order of meat consumption by Juja respondents (Fig. 6). Most of the respondents prefer beef and poultry as their first or second option because they are readily available and the cost is affordable. One kilogram of beef is KShs. 280/= while that of poultry is KShs. 300/=.

4.8.5 Prioritized meat consumption of Naivasha respondents

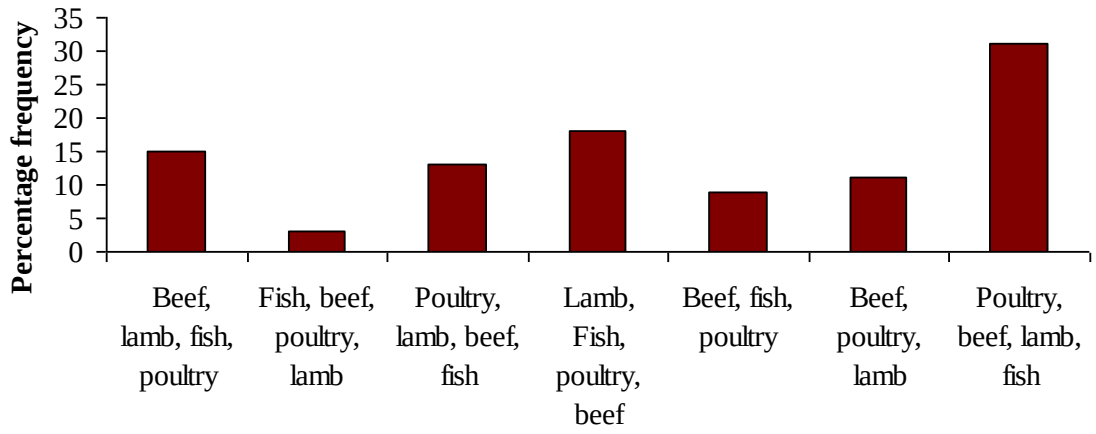


Figure 7. Prioritized meat consumption of Naivasha respondents

The Naivasha respondents do not like fish as compared to other types of meat. 31% of the respondents preferred poultry, beef, lamb and fish in order of priority while 18% would prefer, lamb, fish poultry, beef and only a small number of respondents (3%) would prefer fish as a first priority, beef, poultry, lamb in that order (Fig. 7). In general, fish only comes as a second, third or fourth option and those respondents are willing to consume more fish if they become more available and cheaper.

4.8.6 Prioritized meat consumption of Mombasa respondents

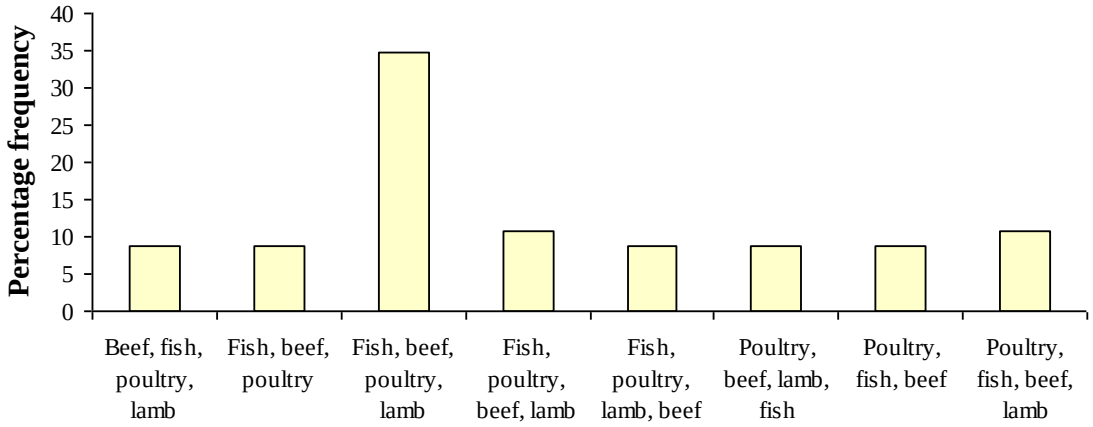


Figure 8. Priotitized meat comsumption of Mombasa respondents

Fish meat only comes as a first option to the inhabitants of Mombasa. A whooping 68% of the respondents in Mombasa consume fish as a first priority followed by other orders of prioritized neat consumption. Beef and poultry meat are also popular among the inhabitants of Mombasa as shown in Fig. 8. Fish meat is readily available to the inhabitants and they are not affected by the smell or taste of the fish as compared to the respondents in Juja and Naivasha.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

5.1.1 Total lipid content

The total lipid (TL) contents in the muscle tissue of all the fish species under study were highest in *O. leucostictus*. Apparently, it was the smallest fish species with respect to its physical parameters compared to the other selected fishes both from L. Naivasha and the Kenyan coast. The probable reason could be that, at the time of sampling it was involved in intense feeding and very little swimming activities. The selected marine fishes did not show any significant difference in the average percentage lipid content in their muscle tissues.

Fishes can be classified by their lipid storage sites. The results obtained shows that fish species from L. Naivasha store most of their lipids in the liver and pyloric ceacum than in the muscle tissue hence, they are lean type of fishes.

5.1.2 Lipid classes

The total lipids comprised mostly of the neutral and polar lipids. The neutral lipids are composed of the wax esters, triacylglyceride and diacylglyceride and sterols. Triacylglyceride was the highest component of the neutral lipids in all the tissues examined. Polar lipids which are also the phospholipids include the phosphatidylcholine and phosphatidylethanolamine, inositol and sphingolipids were a major lipid classes in the muscle, pyloric ceacum, liver and orbital tissues of the specimen under study. The

TAG lipid class composition in the muscle tissue of all the seven species both the marine and freshwater were quite low compared to the phospholipids class composition. Based on the high levels of TAG and phospholipids in the liver, pyloric ceacum and orbital tissues, we suggest that the high proportion of free fatty acids (FFA) in the mentioned tissues of all the fishes was as a result of enzymatic degradation of the glyceride derivatives. Decomposition could have occurred during storage.

5.1.4 Fatty acid composition

The fish oil isolated from different tissues of the selected fish species provided interesting data regarding the fatty acid composition of the TAG class. In all fish species under study, different fatty acid groups (saturated, monounsaturated and polyunsaturated) were identified. Palmitic fatty acid was found to be the most abundant fatty acid in all fish species. This is a common characteristic for selected L. Naivasha fish species.

Essential fatty acids such as eicosapentaenoic and docosahexaenoic were identified and were present in selected Kenya coast fishes. *S. luridus* was found to be the richest fish with the presence of many essential fatty acids especially those which are essential for human nutrition. From the data obtained, higher peaks of omega-6 PUFA (linoleic, C18:2 and arachidonic, C20:4) were observed in selected L. Naivasha fishes. Therefore, the results obtained from this study reveal that selected marine fishes are better sources of omega-3 essential fatty acids while L. Naivasha freshwater fish are good sources of omega-6 essential fatty acids.

5.1.5 Heavy metal contamination

The results obtained in this study, showed that the fish muscle accumulated levels of cadmium, lead, nickel and zinc. In L. Naivasha and Kenyan coast waters, concentration of cadmium, lead, and nickel were recorded but the levels did not exceed the World Health Organization (WHO) limits, European Commission (EC) limits and the Ministry of Water Resource and Management Department (Kenya). Heavy metal concentrations under study in the edible tissue of the fish specimen were in the safety permissible levels for human use.

5.1.6 Fish consumption habits

Fish consumption habits of Juja, Naivasha and Mombasa inhabitants was established through the questionnaire method. Results obtained indicate that respondents are willing to consume more fish if made readily available and at subsidized prices.

5.2 RECOMMENDATIONS

i. Further research should be carried out on qualitative and quantitative characterization of the fatty acid composition of the triacylglyceride (TAG), free fatty acid (FFA), Phoshatidylcholine (PC) and phosphatidylethanolamine (PE) classes of the various organs of the marine and freshwater fishes.

ii. There is little or no information on the effect of cooking process on the total lipid and the long chain polyunsaturated fatty acid content of the available fish species in Kenya. Further research should be carried out on the effects of cooking process on the total lipid and ω-3 long chain poly unsaturated fatty acid content.

iii. Contaminant information on the broad range of metals in fish is generally not available to the public. Thus there is need for more information on contaminant levels in fish from specific regions of the country and that the public should be provided with on exact specific species identification, collection location and growth method (farmed or wild caught). These data will help people make informed decisions on which fish to eat to reduce their risk of contaminants.

iv. Studies should be carried out to find out if the lipid contents, lipid classes and fatty acid composition of the selected fish species are influenced by season and spawning.

v. Studies on fish development are generally carried out in laboratory conditions with excess levels of metal exposures. However, fish growth and its relationship with metal concentration in the aquatic environment should also be monitored

occasionally in the field to better understand the effects of metals on fish development and the current situation of population dynamics.

vi. It is necessary to build up local demand by vigorous campaigns and awareness, pointing to the nutritional benefits of fish, and to follow the campaign up by making available supplies of fish samples, if necessary, initially at subsidized prices.

REFERENCES

Abuodha, P. A. W. and Kairo, J. G. (2001). Human-induced stresses on mangrove swamps along the Kenyan coast. *Hydrobiologia* **458**: 255 - 265.

Ackman, R. G. and Eaton, C. A. (1979). Mackerel lipids and fatty acids. *Canadian Institute of food Technology Journal* **4**: 169 - 174.

Ackman, R. G.; Ratnayake, W. M. and Napolitano, G. E. (1988). Fatty acid components of larval *Ostera edulis* (L): Importance of triacylglycerols as a fatty acid reserve. *Comparative biochemistry* **90**(4): 875 - 883.

Ackman, R. G. (1989). Fatty Acids In: *Marine Biogenic Lipids, Fats and Oils*, R.G. Ackman (Ed.), CRC Press, Boca Raton pp. 145 - 178.

Adamo, P.; Dudka, S.; Wilson, M. J. and McHardy, W. J. (1996). Chemical and mineralogy forms of Cu and Ni in contaminated soils from Sudbary Mining and Smelting Region. *Canada Environmental Pollution* **91**(1): 11 - 19.

Albert, C. M.; Hennekens, C. H. and O'Donnell, C. J. (1998). Fish consumption and risk of sudden cardiac death. *Journal of the American Medical Association* **279**: 23 - 28.

Alloway, B. J. (1990). Cadmium In: Alloway, B. J. (ed.), Heavy metals in soils, pp. 100 - 124. Blackie, Glagow.

Association of Official Analytical Chemists (1970, 1975). Official Methods of analysis. 12th edition. AOAC, Washington, D.C. pp. 1116 - 1125.

Association of Official Analytical Chemists, (1994). *Official Methods of Analysis of the Association of Official Analytical Chemists*, Vols. I & II, Association of Analytical Chemists, Arlington. pp. 1298 - 1312.

Bligh, E. G. and Scott, M. A. (1996). Lipids of cod muscle and the effect of frozen storage. *Bulletin of Fish Research Board of Canada* **23**: 1025 - 1036.

Buchtova, H.; Smutna, M.; Vorlova, L.; Svobodova, Z. and Flajsans, M. (2004). Fatty Acid Composition of Diploid and Triploid populations of Trench (*Tinca tinca* L.). *Journal of the University of Veterinary and Pharmaceutical Sciences in Brno , Czech Republic* **73**, 235 - 245.

Burger, J.; Stern, A. H.; Dixon, C.; Jeitner, C.; Shukla, S.; Burke, S. and Gochfeld, M. (2004). Fish availability in supermarkets and fish markets in New Jersey Science. *Science of the Total Environment* **333**: 89 - 97.

Bustamante, P.; Bocher, P.; Chérel, Y.; Miramand, P. and Caurant, F. (2003). Distribution of trace elements in the tissues of benthic and pelagic fish from the Kerguelen Islands. *The Science of the Total Environment* **313**: 25 - 39.

Canli, M and Atli, G. (2003). The relationship between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. *Environmental pollution* **121** (1): 129 - 136.

Catherine M. (2006). "Effects of Omega-3 Fatty Acids on Cancer Risk". *Journal of the American Medical Association* **295** (4): 403 - 415.

Chen, M. H. And Chen, C. Y. (1999). Bioaccumulation of sediment-bound heavy metals in grey mullet, *Liza macrolepis*. *Mar. Pollut. Bull*. **39**: 239 - 244.

Chow, T. Y.; Snyder, C. B. and Earl, J. L. (1974). Isotope ration of lead as pollution source indicators. Symposium STIPyPO3 Conf-741-120 IAEA. *International Atomic Energy Agency*. pp. 5 - 108.

Clearwater, S. (2002). Metals in the aquatic food web: bioavailability and toxicity to fish. Fact Sheet on Environmental Risk Assessment. Int. Council Mining Met. **6**: 1 - 7.

Cranwell, P. A.; Creighton, M. E. and Jaworski, G. M. (1988). Lipids of four species of freshwater *Chrysophytes*. *Phytochemistry* **27**: 1053 - 1059.

Daviglus, M. L.; Stamler, J. and Orencia, A. J. (1997). Fish consumption and the 30-year risk of fatal myocardial infarction. *New England Journal of Medicine* **336**: 1046 -1053.

Daviglus, M.; Sheeshka, J. and Murkin, E. (2002). Health benefits from eating fish. *Comments Toxicology* **8**: 345 - 374.

Diplock, A. T. (1993). Indexes of selenium status in human populations. *American Journal of Clinical Nutrition* **57**: 256 - 258.

Dittmer, J. C. and Lester, R. L. (1964). A simple, specific spray for the detection of phospolipids on thin-layer chromatograms. *Journal of Lipid Research* **5**: 126 - 127.

Dyerberg, J.; Bang, H. O.; Stoffersen, H. O.; Moncada, S. and Vane, J. R. (1978). Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis. *Lancet* **2**: 117 - 118.

Emmerson, B. T. (1973). Chronic lead nephropathy (editorial). *Kidney Int*. **4**: 1 - 5.

European Commission (EC), (2001). Commission Regulation No. 466/2001 of 8 March 2001, *Official Journal of European communities* **1**: 77/1, pp. 1 - 13.

Folch, J.; Lee, M. and Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistrty* **226**: 497 - 509.

Food and Agricultural Organization (FAO). (1983). Compilation of legal limits for hazardous substance in fish and fishery products. *FAO fishery circular* **464**: 5 -100.

Food and Agricultural Organization (FAO). (1999). Land-based sources and activities affecting the marine, coastal and associated freshwater environment in Comoros, Kenya, Mozambique, Seychelles and United Republic of Tanzania. EAF/5. Waruinge D and Ouya D (eds). Nairobi. Kenya, pp. 42 - 58.

Food and Agricultural Organization (FAO)/World Health Organisation (WHO). (1986). Maximum limits for pesticide residues. Codex Alimentarius Vol. XIII, 2nd edn. Rome. pp. 85 - 98.

Friedmann, A. S., Watzin, M. C., Brinck-Johnsen, T. and Leiter, J. C. (1996). Low levels of dietary methylmercury inhibit growth and gonadal development in juvenile walleye (*Stizostedion vitreum*). *Aquatic Toxicology* **35**: 265 - 278.

Guallar, E.; Sanz-Gallardo, I.; Van't Veer, P.; Bode, P.; Aro, A.; Gomez-Aracena, J.; Kark, J. D.; Riemersma, R. A.; Martin- Moreno, J. M. and Kok, F. J. (2002). Heavy metals and Myocardial Infarction Study Group: Mercury, fish oils, and the risk of myocardial infarction. *New England Journal of Medicine* **347**: 1747 - 1754.

Gulson, B. L.; Jameson, C. S.; Mahaffey, K. R.; Mizon, K. J.; Korsch, M. J. and Vimpani, G. (1997). Pregnancy increases mobilization of lead from maternal skeleton. *Journal of Laboratory and Clinical Medicine* **130**: 51 - 62.

Hardersen, S. and Wratten, S. D. (1998). The effects of carbaryl exposure of the penultimate larval instars of *Xathocnemis zealandica* on emergence and fluctuating asymmetry. *Ecotoxicology* **7**: 297 - 304.

Heath, A.G. (1987). Water Pollution and Fish Physiology. CRC press, Florida, USA. pp 34 - 89.

Helfman G.; Collette B. and Facey D. (1997). The Diversity of Fishes, Blackwell Publishing, pp. 53 - 106.

Hightower, J. M. and Moore, D. (2003). Mercury levels in high-end consumers of fish. *Environmental Health Perspect*ives **111**: 604 - 608.

Hossain. M. A.; Fsana K. A. and Azad S. A. K. M. (1999). Nutritional value of some small indeginous fish species of Bangladesh. *Bangladesh Journal of Fish* **3**: 77 - 85.

Hradec, J., Dusek, Z. and Mack, O. (1974). Influence of cholesteryl 14-methylhexadecanoate on some ribosomal functions required for peptide elongation. *Journal of Biochemistry* **138**: 147 - 154.

Hu, F. B.; Bronner, L. and Willett, W. C. (2002). Fish and omega-3 fatty acid intake and risk of coronary heart disease in women. *Journal of the American Medical Association* **287**: 1815 - 1821.

Huang, B.; Kuo, S. and Bembenek, R. (2004). Availability of cadmium in some phosphorous fertilizers to field grown lettuce. *Journal of Water, Air, Soil pollution* **158**(1): 35 - 51

Huss, H. H. (1995). Quality and changes in fresh fish. FAO Fisheries Technical Paper Nairobi, Kenya. **348**:195 - 203.

International Fish Oil and Fish Meal Organisation Fish Oil Bulletin. No.**18**,Young, F. V. K. (Ed), Liverpool, UK.pp. 131 - 156.

Jacobson, J. L. and Jacobson, S. W. (1996). Intellectual impairment in children exposed to polychlorinated biphenyls in uterus. *New England Journal of Medicine* **335**: 783 - 789.

Kalay, M.; Ay, Ö. and Canli, M. (1999). Heavy metal concentrations in fish tissues from the Northeast Mediterrenean Sea. *Bulletin of Environmental Contamimants Toxicol*ogy **63**: 673 - 681.

Kamal, D.; Khan, N. A.; Rahman, M. A. and Ahamed, F. (2007). Biochemical Composition of Some Small Indigenous Freshwater Fishes from the River Mouri, Khulna, Banglagesh. *Pakistan Journal of Biological Sciences* **10**(9): 1559 - 1561.

Kamau, J. N. (2001). Heavy metals distribution in sediments along the Kilindini and Makupa Creeks, Kenya. *Hydrobiologia* **459**: 235 - 240.

Keli, S. O.; Feskens, E. J. and Kromhout, D. (1994). Fish consumption and risk of stroke: the Zutphen Study. *Stroke* **25**: 328 - 332.

Knuth, B.; Connelly, N. A.; Sheeshka, J. and Patterson, J. (2003). Weighing health benefits and health risk information when consuming sport caught fish. *Risk Analysis* **23**: 1185 - 1197.

Koehn, J. D. (2004). Carp (*Cyprinus carpio*) as a powerful invader in Australian waterways. *Freshwater Biology* **49**: 882 – 894.

Kwon, T. T. and Lee, C. W. (2001). Ecological risk assessment of sediment in wastewater discharging area by means of metal speciation. *Microchemical Journal* **70**: 255 - 264.

Lee, H. (2006). Risks and benefits of omega 3 fats for mortality, cardiovascular disease, and cancer: systematic review. *British Medical Journal* **332**: 752 - 760.

Linden, O. and Lundin, C. G. (1997). The journey from Arusha to Seychelles. Proceedings of the Second Policy Conference on Integrated Coastal Zone Management in East African and Island States, Seychelles, 23-25 October, 1996. Land, Water and Natural Habitats Division Environment Department, The World Bank. Washington.

Lohne, P. (1976). Fat separation – New knowledge can open new processing abilities. *Meld. Fra. S. S. F.* **3**: 9 - 14.

Lonky, E.; Reihman, J.; Darvill, T.; Mather Sr., J. and Daly, H. (1996). Neonatal behavioral assessment scale performance in humans influenced by maternal consumption of environmentally contaminated fish. *Journal of Great Lakes Research* **22**: 198 - 212.

Lowler, A. J. and Tipping, E. (2003). Metals in bulk deposition and surface waters at two upland locations in Northern England. *Environmental Pollution* **121**: 153 - 167.

Manal, S. T. (2009). Proximate Composition and Fatty Acids Profiles in Most Common Available Fish Species in Saudi Market. *Asian Journal of Clinical Nutrition* **1**(1): 50 - 57.

Meisner, A. and Burns, J. (1997). Viviparity in the Halfbeak Genera *Dermogenys* and *Nomorhamphus* (Teleostei: Hemiramphidae). *Journal of Morphology* **234**: 295 - 317.

Mizushima, S.; Moriguchi, E. H. and Ishikawa, P. (1997). Fish intake and cardiovascular risk among middle-aged Japanese in Japan and Brazil. *Journal of Cardiovascular Risk* **4**: 191 -199.

Morris, M. C.; Manson, J. E. and Rosner, B. (1995). Fish consumption and cardiovascular disease in the Physicians' Health Study: a prospective study. *American Journal of Epidemiology* **142**: 166 -175.

Munga, D. (1993). The impact of pollution on the mangrove ecosystem in Kenya. National Workshop for improved management and conservation of the Kenyan mangroves. pp. 255 - 272.

Mwaguni, S. and Munga, D. (1997). Land based sources and activities affecting the quality and uses of the marine coastal and associated freshwater environments along the Kenya coast. Coastal Development Authority, Mombasa. pp. 104 - 111.

Mwashote B. M. (2003). Levels of Cadmium and Lead in Water, Sediments and Selected Fish Species in Mombasa, Kenya. *Western Indian Ocean J. Mar. Sci.* **2**: 25 - 34.

NAS-NRC, (1974). National Academy of Sciences-National Research Council. Food and Nutrition Board. Recommended Dietary allowances. Washington DC: National Academic Press, pp 265 - 322

Neuringer, M.; Anderson, G. J. and Connor, W. E. (1988). *Annual Review of Nutrition* **8**: 517 - 541.

Nriagu, J. O., Jinabhai, C. C., Naidoo, R. and Coutsoudis, A. (1996). Atmospheric lead pollution in Kwa Zulu/Natal, South Africa. *Science of the Total Environment* **191**: 67 – 76.

Olsen, S. F.; and Secher, N. J. (2002). Low consumption of seafood in early pregnancy as a risk factor for preterm delivery: prospective cohort study. *Journal of Biomedical Discovery and Collaboration* **324**: 1–5.

Onyari, J. (1981). The need for aquatic pollution studies in Kenyan inland waters. Proceedings of the Workshop of KMFRl on Aquatic Resources of Kenya, July 13 - 19, 1981. pp 28 - 35.

Phleger, C. F.; Nichols, P. D. and Virtue P. (1997). The lipid, fatty acid and fatty alcohol composition of the myctophid fish *Electrona antarctica:* high level of wax esters and food-chain implications. *Antarctic Science* **9** (3): 258 - 265.

Puri, B. (2006). Proton and 31- phosphorus neurospectroscopy in the study of membrane phospholipids and fatty acid intervention in schizophrenia, depression, chronic fatigue syndrome (mylagic encephalomyelitis) and dyslexia. *International Review of Psychiatry* **18**(2): 145 – 147.

Rahman, A. A.; Haider, R.; Huq, S. and Jansen, E. G. (1994). Environment and Development of Bangaldesh. University Press Ltd., Dhaka Bangladesh, pp. 71 - 93.

Rajotte, J.; Pyle, G. and Couture, P. (2003). Indicators of chronic metal stress in wild yellow perch from metal-contaminated environments. In: Conference Presentations, Mining and Environment, 28th Annual Meeting, pp. 67 - 89.

Rees, J. G.; Williams, T. M.; Nguli, M. M.; Kairu, K. K. and Yobe, A. C. (1996). Contaminant transport and storage in the estuarine creek systems of

Mombasa, Kenya. British Geological Survey Technical Report WC/96/42, pp. 34 - 117.

Robins, C. R., Bailey, R. M., Bond, C. E., Brooker, J. R., Lachner, E. R., Lea, R. N. and Scott, W. B. (1991). World fishes important to North Americans. Exclusive of fishes from the continental waters of the United States of America and Canada. *Am. Fish. Soc. Spec. Publ*. **21**: 243 - 249.

Romic, M. and Romic, D. (2003). Heavy metals distribution in agricultural top-soils in urban area. *Environmental Geology* **43**: 795 - 805.

Rouser, G.; Siakotos, A. N. and Fleischer, S. (1966). Quantitative analysis of phospholipids by thin-layer chromatography and phosphorus analysis of spots. *Lipids* **1**: 85 - 86.

Saito, H.; Ishihara, K and Murase, T. (1997). The fatty acid composition in Tuna (Bonito, *Euthynnus pelamis*) caught at three different localities from tropics to temperate. *Journal of Science in Food Agriculture* **73**: 53 - 59.

Saito, H. and Murata, M. (1998). The origin of the monoene fats in the lipid of mid-water fishes: relationships between the lipids of myctophids and those of their prey. *Marine Ecology: Progress Series* **168**: 21 - 33.

Saito, H.; Keriko, J. M. and Yoshida, K. (2002). Utilization of the deep-sea grenadier: the high content of ceramide derivatives in the internal organ lipids of genus *Corypaenoides*. *Fisheries Science-Tokyo* **68**: 1426 -1429.

Salem, N.; Kim, H. Y. and Yergey, J. A. (1986). Docosahexaenoic acid: Membrane function and metabolism. In health effects of Polyunsaturated Fatty Acids in Seafood. Academic Press, Orlando, pp. 263 - 317.

Sekhar, K. C.; Chary, N. S.; Kamala, C. T.; Raj, D. S.and Rao, A. S. (2003). Fractionation studies and bioaccumulation of sediment-bound heavy metals in Kolleru lake by edible fish. *Environ. Int*. **29**: 1001 - 1008.

Shirai, N.; Suzuki, H.; Tokairin, S. and Wada, S. (2001). Spawning and season affect lipid content and fatty acid composition of ovary and liver in Japanese catfish (*Silurus asotus*). *Comparative Biochemistry Physiology* **129B**, 185 - 195.

Shirai, N.; Terayama, M. and Takeda, H. (2002). Effect of season on the fatty acid composition and free amino acid content of the sardine Sardinops melanostictus. *Comparative Biochemistry Physiology* **131B**: 387 - 393.

Simopoulos, A. P. (1999). Essential fatty acids in health and chronic disease. *Am. J. Clin. Nutr.* **70**: 560 - 569.

Siscovick, D. S.; Raghunathan, T. E. and King, I. (1995). Dietary intake and cell membrane levels of long-chain n-3 polyunsaturated fatty acids and the risk of primary cardiac arrest. *Journal of the American Medical Association* **274**: 1363 - 1367.

Stansby, M. E. (1967). Fish oils chemistry technology, stability, nutritional properties and uses. Avi. Publishing Co., USA. Eds M. E. Stansby. pp. 4 - 16.

Stansby, M. E. (1982). Properties of fish oils and their application to handling of fish and to nutritional and industrial use. In: Martin, R. E., Flick G. J., Hebard, C. E., & Ward, D. R. Eds *Chemistry and Biochemistry of Marine Food Products*. pp. 75 - 92.

Stone, N. J. (1996). Fish consumption, fish oil, lipids, and coronary heart disease. *Circulation* **94**: 2337 - 2340.

Suzuki, H.; Morikawa, Y. and Takahashi, H. (2001). Effect of DHA oil supplementation on intelligence and visual acuity in the elderly. Fatty acids and lipids – *New findings World Review of Nutrition and Dietics Basel, Karger* **88**: 68 - 71.

Suzuki, H.; Park, S. J.; Tamura, M. and Ando, S. (1998a). Effect of the long term feeding of dietary lipids on the learning ability, fatty acid composition of brain stem phospholipids and synapic membrane fluidity in adult mice: A comparison of sardine oil diet with palm oil diet: *Mechanism of Ageing and Development* **101**: 119 - 128.

Suzuki, H.; Takahashi, H. and Kataoka, H. (1998b). Effect of bread supplemented with DHA on the visual acuity in near-sighted patient (myopia). 3rd ISSFAL Congress. Abstract Book. pp. 254 - 259.

Talwar, P. K. and Jhingran, A. G. (1991). Inland fishes of India and adjacent countries. Volume 1. New Delhi: Oxford and IBH Publishing Co. pp. 256 - 267.

Tamira, C.; Shane, S. H. and Ambrose, R. F. (2001). Trace metals in fish and invertebrates of there California Coastal Wetlands. *Marine Pollution Bulletin* **42**(3): 224 - 232.

Topcuoğlu, S.; Kirbaşoğlu, Ç. and Güngör, N. (2002). Heavy metals in organisms and sediments from Turkish Coast of the Black Sea. *Environmental International* **1069**: 1 - 8.

Trewavas, E. (1983). Tilapine fishes of the genera *Sarotherodon*, *Oreochromis* and *Danakilia*. British Museum National History, London, U.K. pp. 583 - 586.

Türkmen , A.; Türkmen, M.; Tepe , Y. and Akyurt, İ. (2005). Heavy metals in three commercially valuable fish species from İskenderun Bay, Northern East Mediterranean Sea, Turkey. *Food Chemistry* **91**: 167 - 172.

Tüzen, M. (2002). Determination of heavy metals in fish samples of the middle Black Sea (Turkey) by graphite furnace atomic absorption spectrometry. *Food Chemistry* **27**: 521 - 526.

Underwood, E. J. (1977). Trace Elements in Human and Animal Nutrition (4th ed.). New York: Academic. pp. 51 - 117.

USFDA (1993c). Food and Drug Administration. Guidance Document for Nickel in Shellfish. DHHS/PHS/FDA/CFSAN/Office of Seafood, Washington, D.C. pp. 288 - 319.

Wandiga, S. and Onyari, J. (1987). The concentration of heavy metals: Mn, Fe, Cu, Zn, Cd and Pb in sediments and fish from Winam Gulf of Lake Victoria and fish bought in Mombasa market. *Kenya J. Sci*. **8**: 5 - 18.

Weis, J. S. and Weis, P. (1989). Effects of environmental pollutants on early fish development. *Rev. Aquat. Sci.* **1**: 45 - 73.

Welcomme, R. L. (1988). International introductions of inland aquatic species. *FAO Fish Technical Paper* **294**: 319 - 322.

Woodland, D. J. (1990). Revision of the family *Siganidae* with descriptions of the two new species and comments on distribution and biology. *Indo-Pacific fishes* **19**:136 - 140.

World Health Organization (WHO), (1982). Prevention of Coronary Heart Disease *Technical Report Series* **678**: 118 - 135.

Yılmaz, A. B. (2003). Levels of heavy metals (Fe, Cu, Ni, Cr, Pb, and Zn) in tissue of Mugil cephalus and Trachurus mediterraneus from İskenderun Bay, Turkey. *Environmental Research* **92**: 277 - 281.

Zhang, J.; Sasaki, S. and Amano, K. (1999). Fish consumption and mortality from all causes, ischemic heart disease, and stroke: an ecological study. *American Journal of Preventive Medicine* **28**: 520 - 529.

APPENDICES

Appendix 1. Questionnaire

Date: / / 2008

The name of the town where you live in…… Age……..

Sex……

S.1. How many people do live in your house?

Alone Two ☐ Three ☐ Four ☐ If more than four identify it with a number ☐

In group accommodation like a dormitory etc

S.2. What is your highest educational level?

No education ☐ Primary school ☐ Middle school ☐ High school ☐

College ☐ University ☐ Post graduate degree ☐

If you are still in school or university, give the name of the school or university & the class you are in (year): …….

S.3. How often do you consume fish?

More than once a week ☐ Once in a week ☐ 2-3 times in a month ☐ Once in a month ☐

Few times in a year ☐ Once in a year ☐ Never (Don't consume fish) ☐

S.4. If you don't consume fish, what is the reason?

……

S.5. If you didn't have any problem of buying fish, how often would you consume fish?

More than once a week ☐ Once a week ☐ 2-3 times a month ☐ Once a month ☐

Few times in a year ☐ Once in a year ☐ Never (Don't consume fish) ☐

S.6. Is fish beneficial for health? Yes ☐ No ☐

S.7. If 'yes' what kind of health benefits does it have?

……

S.8. If you believe in the health benefits of fish, what material(s) in fish that makes it healthy? ……

S.9. Give us the names of three fish types that you consume often starting from the one you like best. A…… **B** ……. **C** ……..

S.10. Do you consume processed seafood? Yes ☐ No ☐

S.11. If 'no' why? .....

S.12. If 'yes', which ones do you consume? Mark them with numbers starting from your first choice. Canned☐ Marinated ☐ Smoked ☐ Frozen ☐ Salted ☐ Surimi ☐

Others (Identify)

S.13. How often do you consume processed seafood ……

S.14. Do you consume seafood other than fish? Yes ☐ No ☐

S.15. If 'no', why? …….

S.16. If 'yes', which ones do you consume? Mark them with a number starting from your first choice.

Muscles ☐ Prawns ☐ Octopus ☐ Squid ☐ Lobster ☐ Other (Identify) ……

S.17. How often do you consume this type of seafood? ……..

S.18. Can you identify the good quality fish from the bad one? Yes ☐ No ☐

S.19. If 'yes' give a few criteria to show fresh fish ……

……..

S.20. Which of the following meat do you consume most. Number them starting from your first choice.

Fish or other seafood ☐ Poultry ☐ Beef ☐ Lamb ☐ Other

S.21. What is the reason for your first choice?

Health ☐ Taste ☐ Price ☐ Availability ☐ Other (Identify)

Appendix 2. Gas chromatograms of selected L. Naivasha and Kenyan coast fish oils

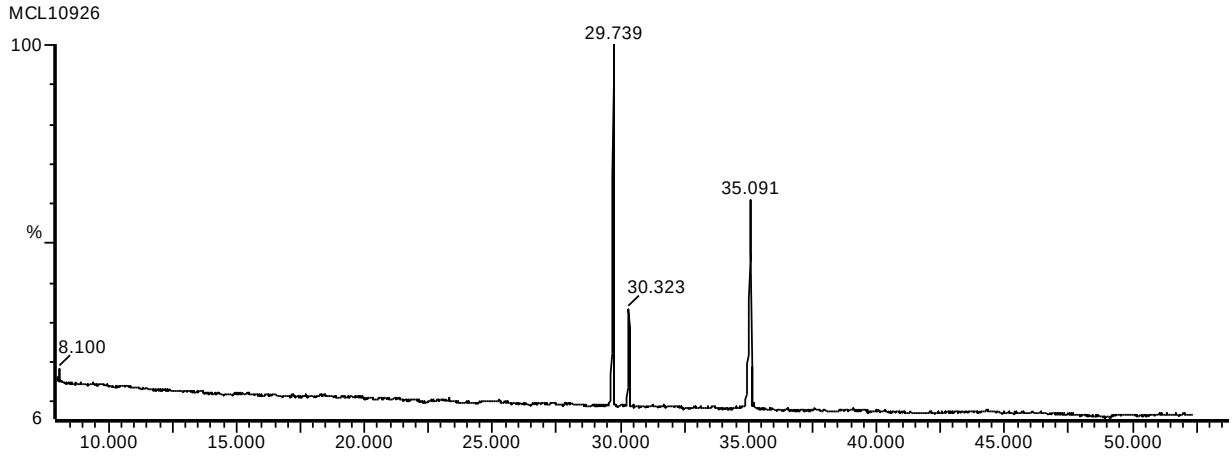


Figure 9. Gas - liquid chromatogram of fatty acid methyl esters of the liver of *C. specularis*

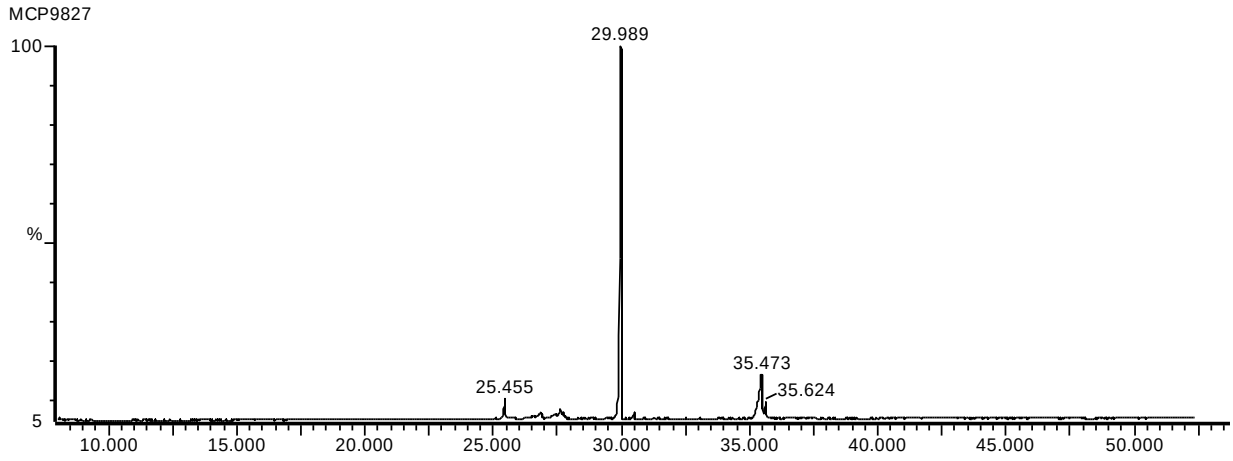


Figure 10. Gas - liquid chromatogram of fatty acid methyl esters of the pyloric ceacum of *C. specularis*

.

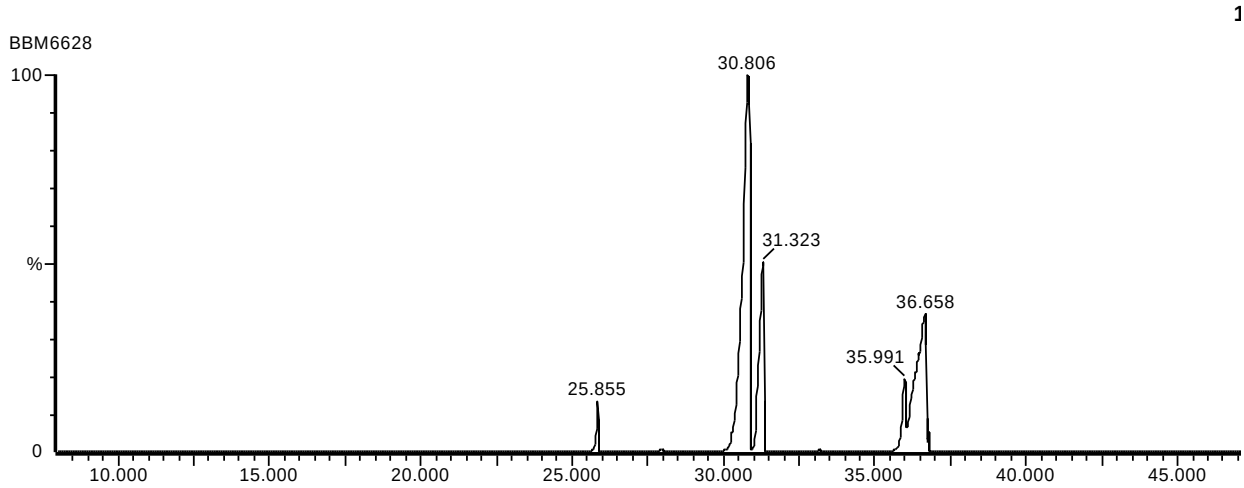


Figure 11. Gas - liquid chromatogram of fatty acid methyl esters of the muscle of *M. salmoides*

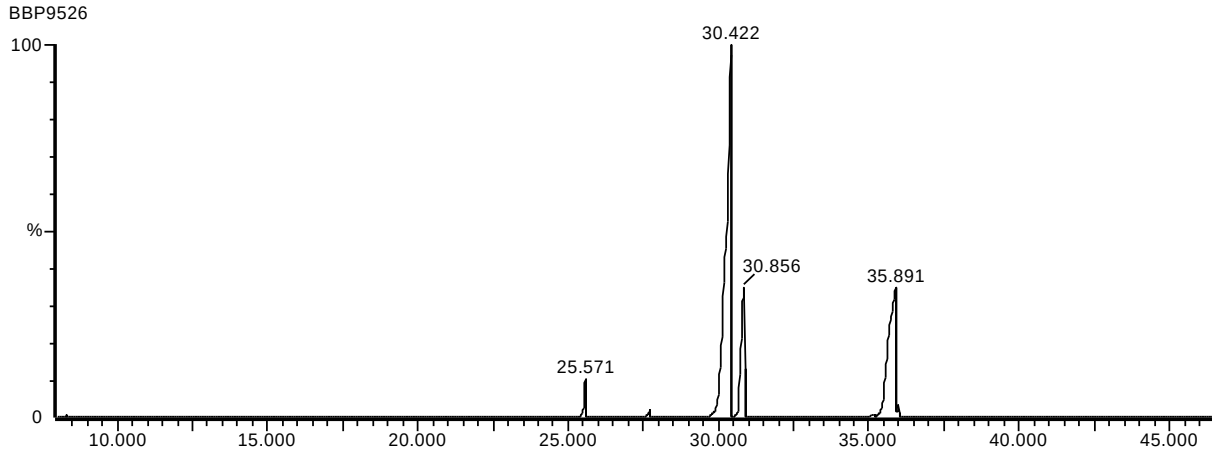


Figure 12. Gas - liquid chromatogram of fatty acid methyl esters of the pyloric ceacum of *M. salmoides*

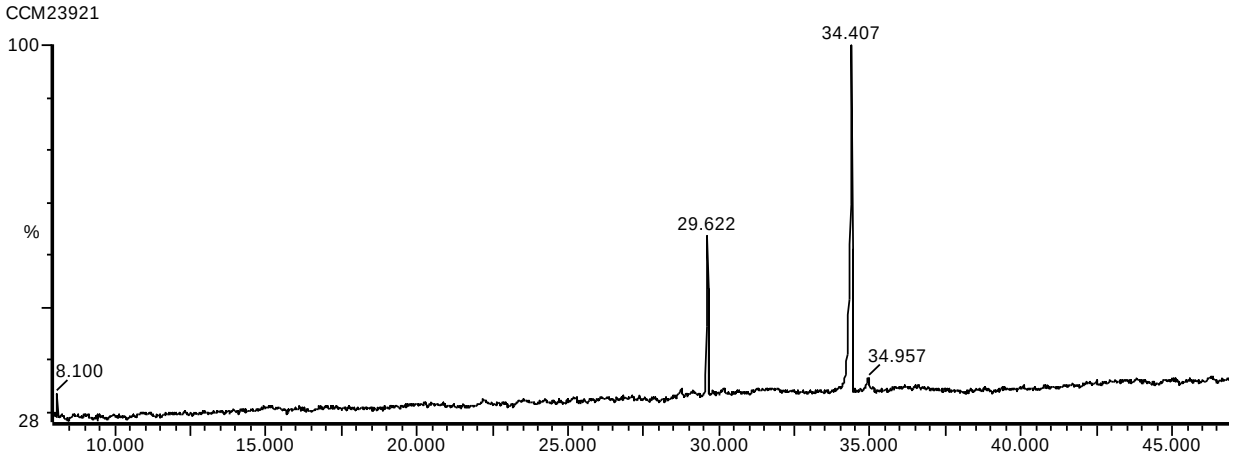


Figure 13. Gas - liquid chromatogram of fatty acid methyl esters of the muscles of *C. carpio*

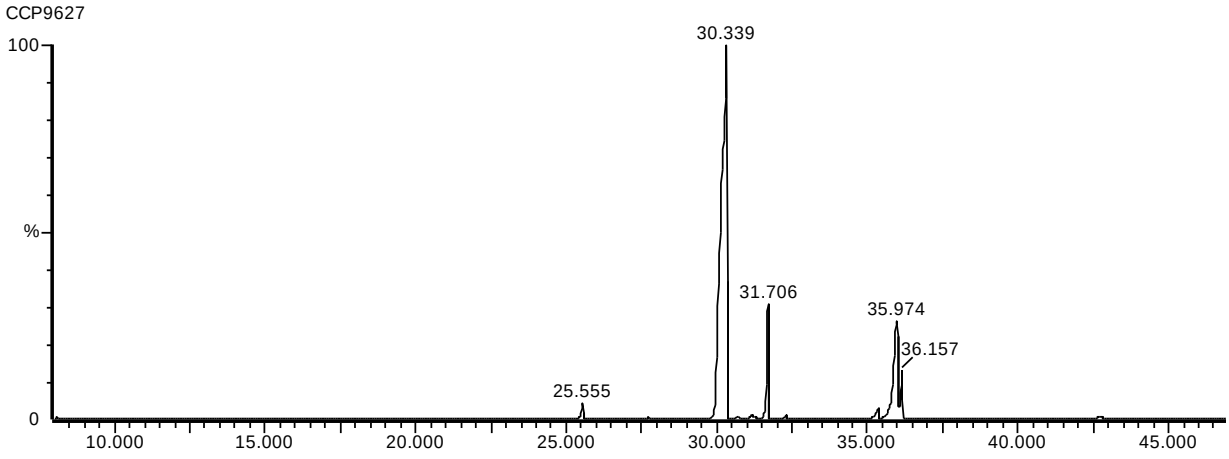


Figure 14. Gas - liquid chromatogram of fatty acid methyl esters of the pyloric ceacum of *C. carpio*

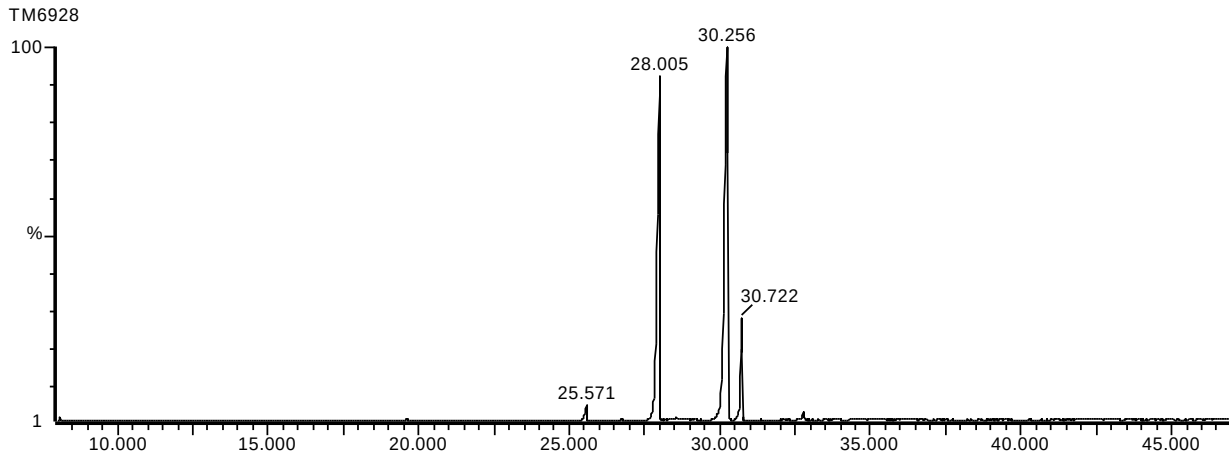


Figure 15. Gas - liquid chromatogram of fatty acid methyl esters of the muscles of *O. leucostictus*

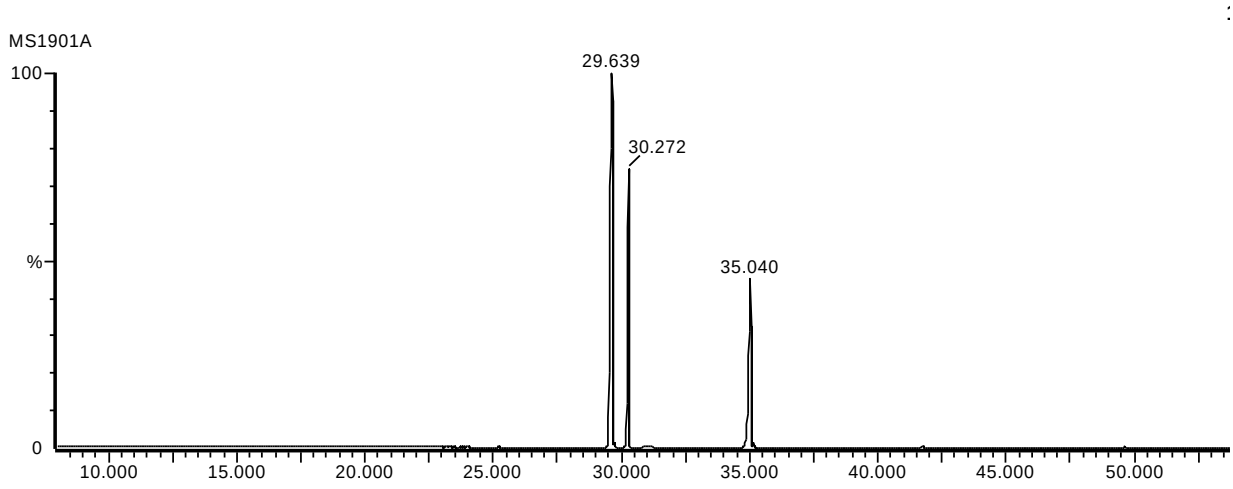


Figure 16. Gas - liquid chromatogram of fatty acid methyl esters of the muscle of *L. campechanus*

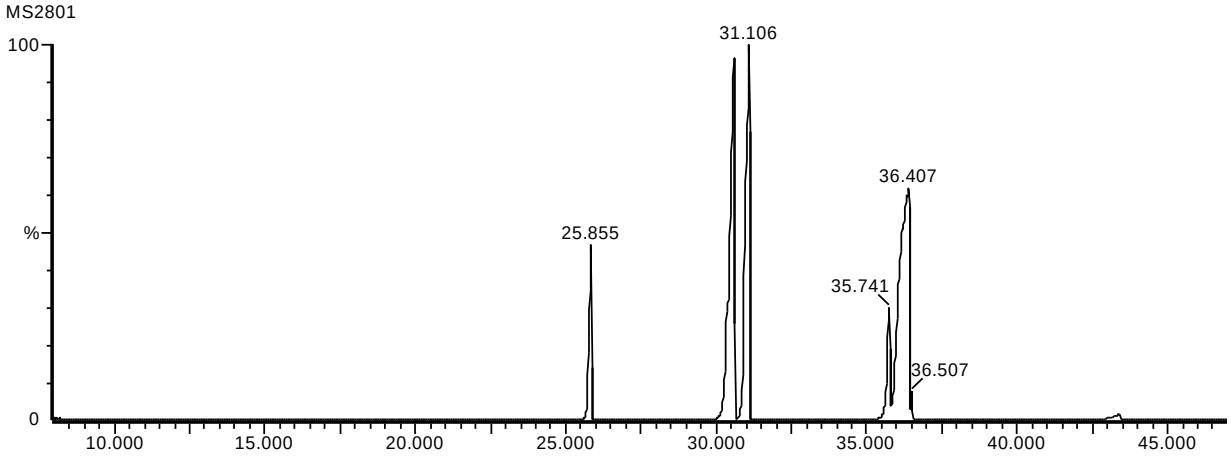


Figure 17. Gas - liquid chromatogram of fatty acid methyl esters of the muscle of *S. luridus*

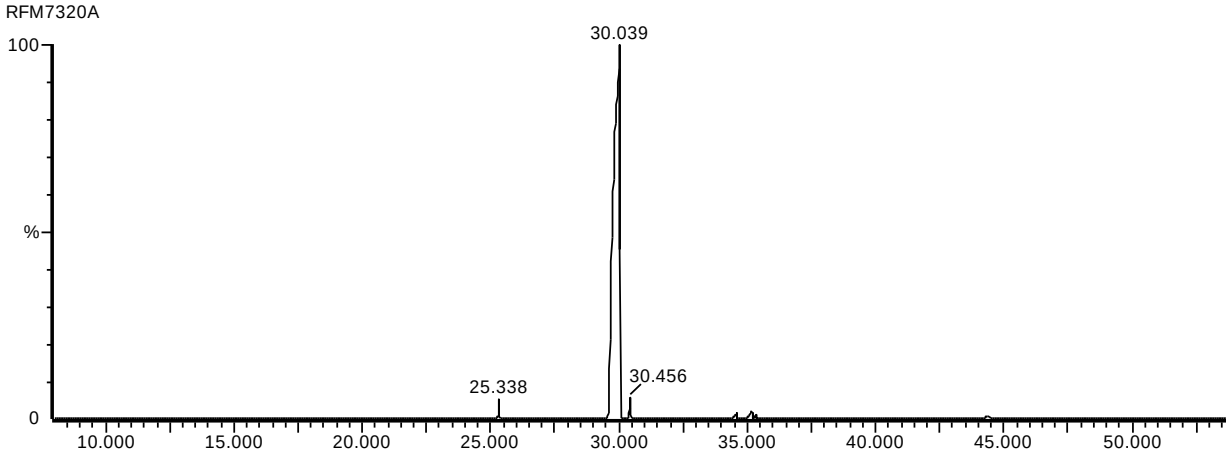


Figure 18. Gas - liquid chromatogram of fatty acid methyl esters of the muscle of *M. niger*

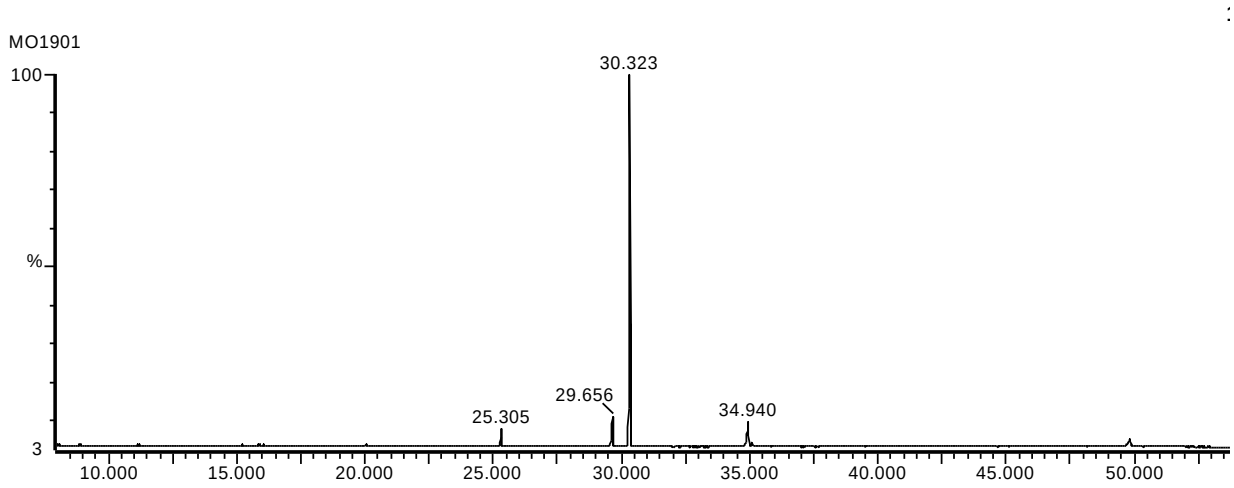


Figure 19. Gas - liquid chromatogram of fatty acid methyl esters of the orbital of *S. luridus*

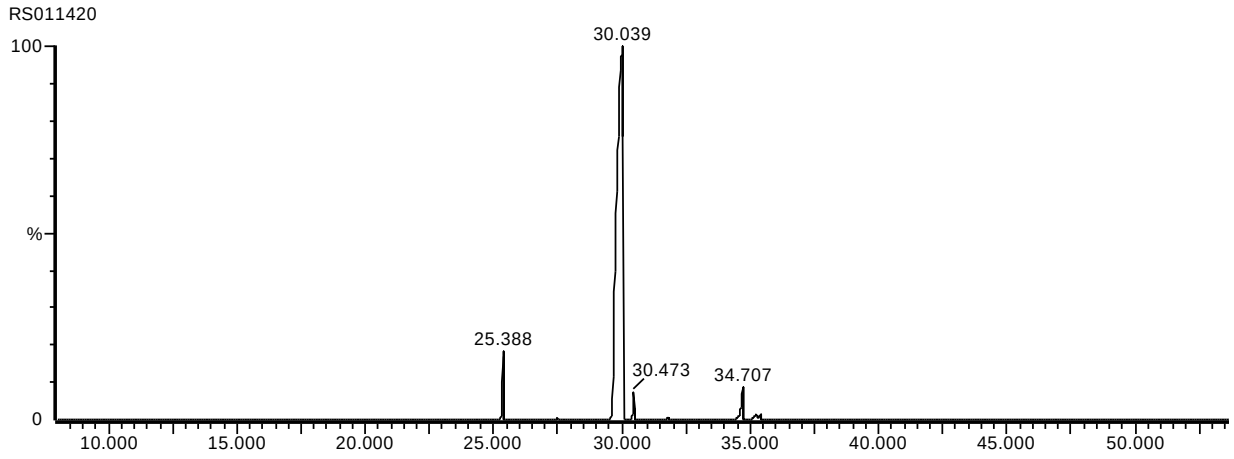


Figure 20. Gas - liquid chromatogram of fatt acid methyl esters of the orbital of *L. campechanus*

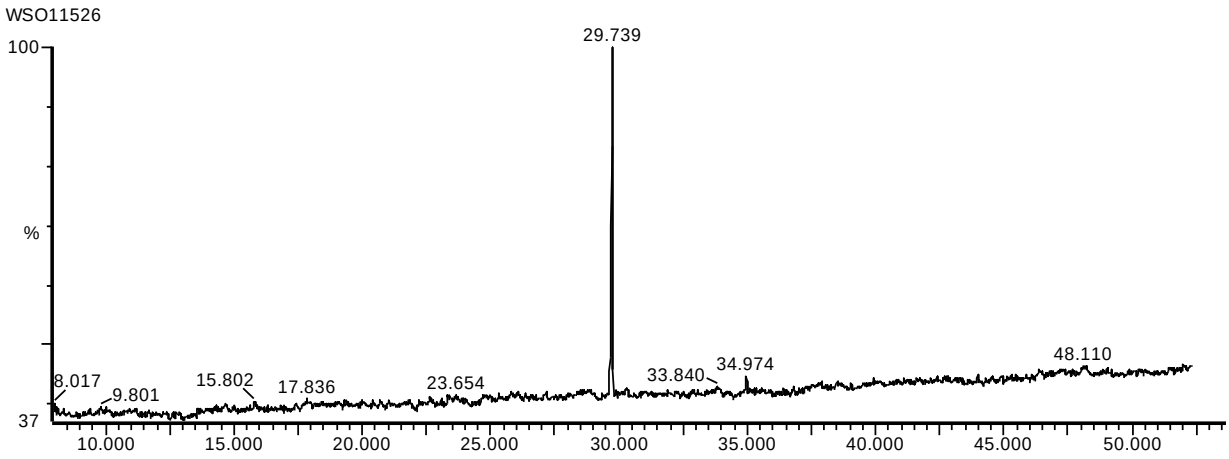


Figure 21. Gas - liquid chromatogram of fatty acid methyl esters of the orbital of *M. niger*

Appendix 3. Calibration curves for metal standards

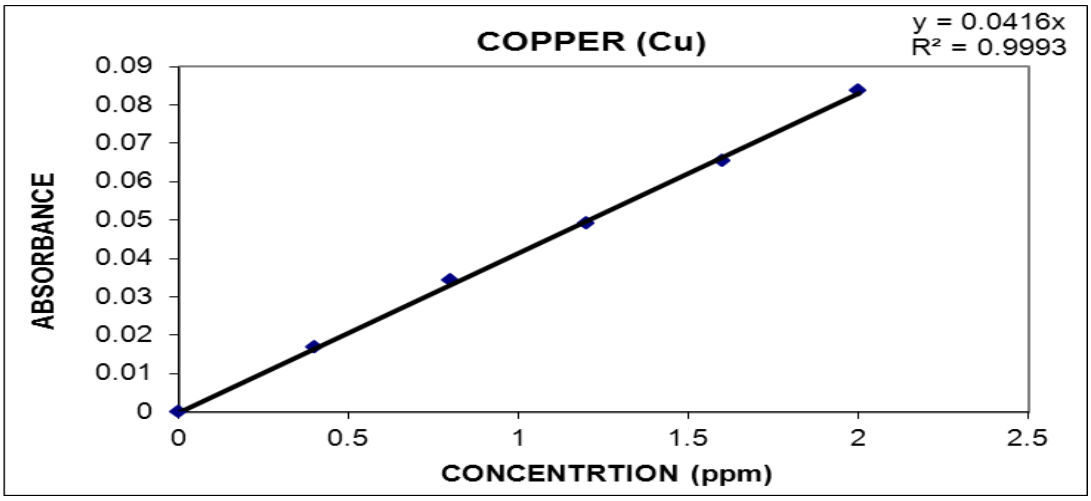


Figure 22. Copper (Cu) calibration curve

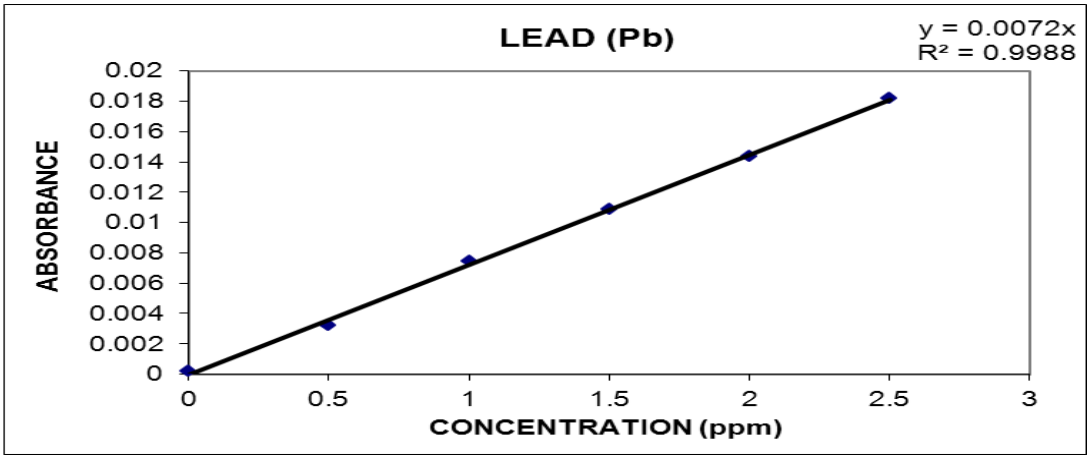


Figure 23. Lead (Pb) calibration curve

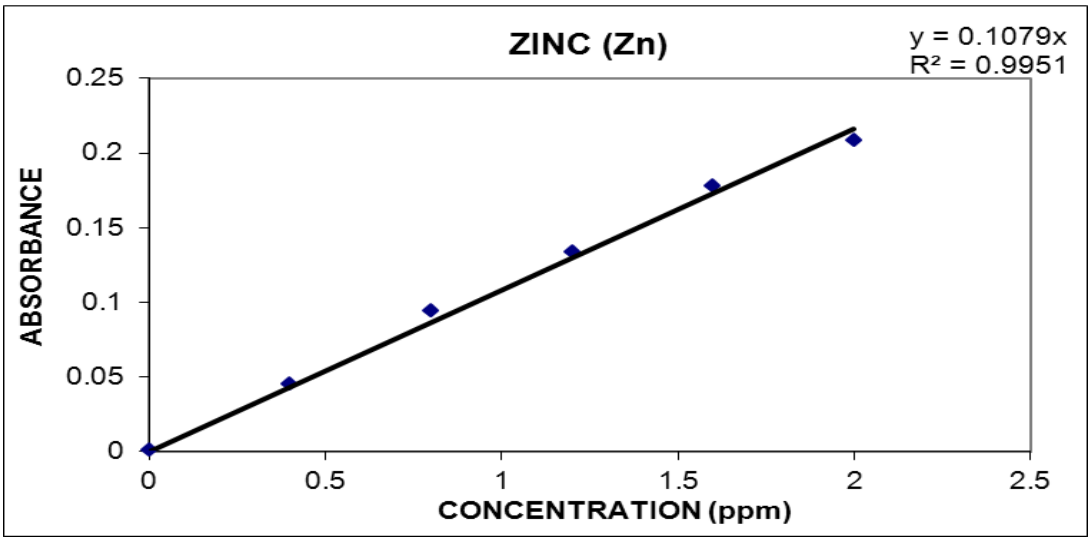


Figure 24. Zinc (Zn) calibration curve

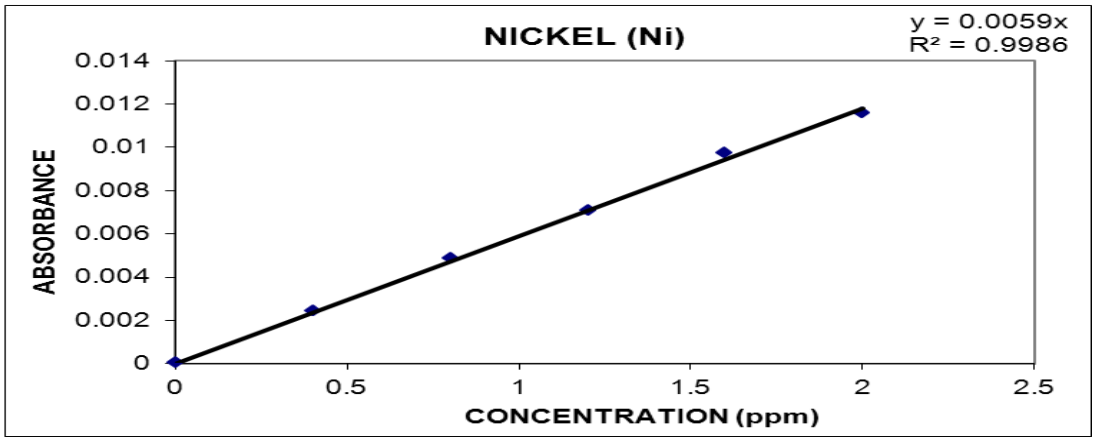


Figure 25. Nickel (Ni) calibration curve

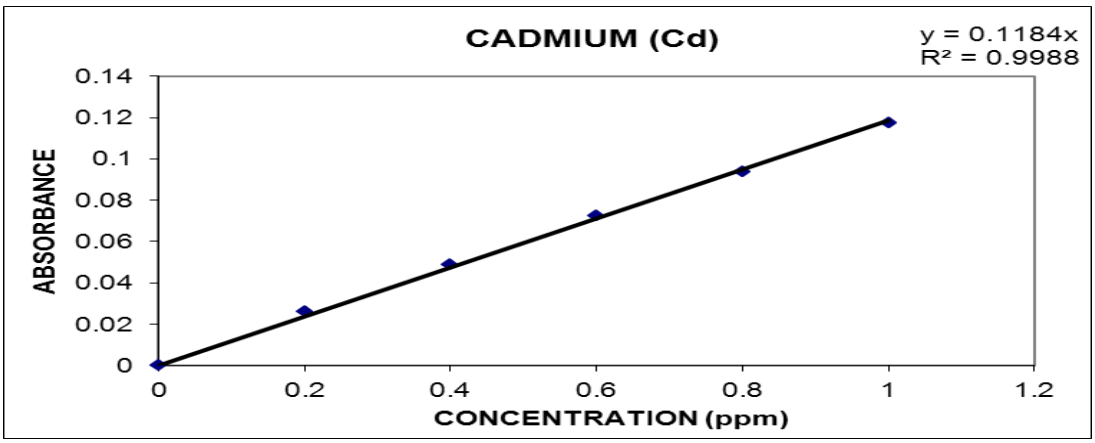


Figure 26. **Cadmium (Cd) calibration curve**

Appendix 4. Publication from the research work

African Journal of Pharmacy and Pharmacology Vol. 4(10), pp. 745-753, October 2010
Available online http://www.academicjournals.org/ajpp
ISSN 1996-0816 ©2010 Academic Journals

Full Length Research Paper

Fish lipid contents and classes of selected fish species found in Lake Naivasha (Kenya) and the fish feeding habits of the lake's inhabitants

Keriko, J. M.[1,2]*, Chege, C. W.[1], Magu, M. M.[2], Mwachiro, E. C.[3], Murigi, A. N.[1], Githua, M. N.[1] and Kareru, P. G.[1]

[1]Department of Chemistry, Jomo Kenyatta University of Agriculture and Technology. P. O. Box 62000-00200, Nairobi, Kenya.
[2]Institute of Energy and Environmental Technology (IEET), Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya.
[3]Institute of Tropical Medicine and Infectious Diseases (ITROMID), Jomo Kenyatta University of Agriculture and Technology (Currently at Pwani University College), Nairobi, Kenya.

Accepted 28 October, 2010

Consumption of marine natural products including fish is very beneficial to the health and development of the human body. These natural products provide essential nutrients that are unavailable in terrestrial plants and animal natural products. Data on the levels of lipid contents, lipid classes and fatty acid composition in the total lipid of fishes found in the fresh and marine waters of Kenya have not been determined. Thus, the aim of this work was mainly to determine the levels of the total lipid contents and lipid classes in some selected fish species of L. Naivasha, Kenya applying already known protocols with some minor modifications. The study also established the Lake Naivasha's inhabitant fish feeding habits by utilizing a questionnaire method. The following fish species commonly caught in the Lake were studied: Common carp (*Cyprinus carpio*), Mirror carp (*Cyprinus specularis*), Largemouth bass (*Micropterus salmoides*) and Tilapia (*Oreochromis leucosticus*). These fish specimen were obtained directly from the fishermen at the Central Landing in the Lake at 00° 44.369' N and 36° 24.133' E. The results of this study indicated that total lipid content was highest in the muscle tissue of the tilapia fish, in the pyloric caecum tissue of the largemouth bass and in the liver tissues of the common carp. The lipid content results obtained here indicated that the fish specimens were lean fishes. Phosphatidylcholine (PC) was the dominant lipid class in the muscle tissue of all the four fish species. In the pyloric caecum and the liver tissues of the four freshwater fish species, phospholipids [(PC and phosphatidylethanolamine (PE)] were the dominant lipid classes in both tissues. The fish eating habit results suggested that 36% of the respondents consume fish more than once per week while only 6% do so a few times a year because of its unavailability (24%) and high cost (25%). However, results suggested that respondents are willing to consume fish more frequently if they are made more available at subsidized prices. Thus, the positive change towards the consumption of fish products can lead to a healthier citizen whose diet will be free from reliance on red meat containing high levels of cholesterol (commonly associated with terrestrial animals) to white marine meat which contain high level of omega-3 fatty acids.

Key words: Total fish lipids, *Cyprinus carpio*, *Cyprinus specularis*, *Micropterus salmoides*, *Oreochromis leucosticus*, lipid content and classes, Lake Naivasha.