UNIVERSITY OF NAIROBI

DETERMINATION OF AFLATOXINS STRAINS AND NUTRITIVE LEVELS ASSOCIATED WITH *RASTRINEOBOLA ARGENTEA* IN SELECTED LOCALITIES IN KENYA

BY

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DECLARATION

I declare that this thesis is my original work and has not been submitted elsewhere for examination, award of a degree or publication. Where other people's work or my own work has been used, this has properly been acknowledged and referenced in accordance with the University of Nairobi's requirements.

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DEDICATION

This thesis is dedicated to, my late parents, my spouse and children for their encouragement and support throughout my studies.

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ABSTRACT

Rastrineobola argentea (Omena*)* is the second important fish catches after Nile Perch in Lake Victoria. Although rich in nutrients, huge postharvest losses are mainly reported during rainy seasons when large catches are made. This could be due to poor handling, processing and packaging practices causing infestation by fungi and subsequent production of aflatoxin under favourable conditions. Despite the economic importance, there are no studies in Kenya to quantify the aflatoxin associated with *Rastrineobola argentea* and its effects on micronutrient content. This study was therefore undertaken to determine public knowledge, diversity and content of aflatoxins in sun dried *Rastrineobola argentea* and assess its relationship with the moisture, calcium and iron content. To assess the public knowledge, a survey was carried out around Lake Victoria landing beaches in Kisumu, Migori and Siaya counties and fish markets in Nairobi. A total of 252 samples were collected in dry and wet seasons. The samples were blended and sub-sampled for proximate calcium and iron analysis as well as aflatoxin and moisture content determination. Iron and calcium levels were determined using Atomic Absorption Spectrometry. Total aflatoxin quantification was determined using fluorescence detector in High Performance Liquid Chromatography in reverse phase. Moisture content was quantified using an oven at 130 °C. There was female dominance in fish processing in the study sites. Illiteracy levels was high with majority of the respondents having attained only primary education. Sun drying was the most widely practiced method of fish preservation. There was a wide knowledge gap with 65.59 % unaware of aflatoxins contamination and its health implications. Proximate calcium levels ranged from 1,872.21 to 2,940.90 mg/kg compared to the recommended adult's daily intake of 1,000 – 1,300 mg. Proximate iron content ranged from 5.62 to 15.64 mg/kg. The total aflatoxin detected in singular samples ranged from 0.44 μg/kg – 4.42 μg/kg signifying Omena aflatoxin contamination. Moisture content was from 10.13 to 14.40 %. Although, total aflatoxin levels were within the accepted limits in Kenya, continued consumption can lead to chronic aflatoxicosis making one vulnerable to liver cancer, neurological area impairment, immunosuppression, and micronutrients deficiencies, stunted growth, child mortality and spontaneous abortion. Hazard analysis of critical control point and good hygiene ought to be operationalized from harvesting through processing and distribution channels of sun dried *Rastrineobola argentea* to reduce the moisture content as well as the levels of aflatoxin contamination. The national and county Governments of Kenya should educate the general

public on health hazards associated with consumption of contaminated Omena. The findings have contributed to science of analytical chemistry in mitigation of aflatoxin challenges while high calcium and iron content in omena helps in improved diet. The negative correlation of aflatoxin and moisture in Omena merits a further research study.

TABLE OF CONTENTS

DECLARATION .... **II**

DEDICATION .... **III**

ACKNOWLEDGEMENTS .... **IV**

LIST OF TABLES .... **X**

LIST OF FIGURES .... **XI**

LIST OF ABBREVIATIONS AND ACRONYMNS .... **XII**

CHAPTER ONE **1**

1. INTRODUCTION **1**

1.1 Background Information 1

1.2 Statement of the Problem 3

1.3 Research Hypotheses 4

1.4 Research Objectives 4

1.4.1 General objective 4

1.4.2 Specific Objectives 4

1.5 Justification and Significance of the Study 4

CHAPTER TWO **6**

2. LITERATURE REVIEW **6**

2.1 Fish Industry and its contribution to economy 6

2.2 Omena contribution to food and Nutrition security 9

2.3 Fish preservation methods and spoilage mechanisms 10

2.4 The role of fungi in microbial contamination of Omena fish 11

2.5 Mycotoxins in food contamination 12

2.6 Aflatoxins as food contaminants 13

2.6.1. Identification of aflatoxins 16

2.6.2 Aflatoxin food poisoning in the world 16

2.6.3 Aflatoxin food poisoning in Kenya 17

2.6.4 Effects of aflatoxicosis on human health 18

2.6.5 Control of Aflatoxins 19

2.6.6 Aflatoxin and percentage moisture content regulation limits 20

CHAPTER THREE **21**

3. MATERIALS AND METHODS **21**

3.1 Description of the study sites 21

3.2 Sample Collection 24

3.3 Assessment of public awareness on aflatoxin contamination in Omena fish 26

3.4 Determination and Quantification of aflatoxin and moisture levels in Omena samples 27

3.4.1 Materials and Equipments 27

3.4.2 Quantification of aflatoxin levels in Omena samples 27

3.4.3 Determination of moisture content in the Omena samples 35

3.4.4 Determination of proximate Calcium content in Omena samples 36

3.4.5 Determination of proximate Iron content in the Omena sample 36

3.4.6 Data analysis 37

CHAPTER FOUR **38**

4. RESULTS AND DISCUSSIONS **38**

4.1 Public knowledge on mycotoxins and aflatoxin contamination in Omena samples 38

4.1.1 Socio-economic characteristics of fish processors 38

4.1.2 Processing and drying methods of Omena 40

4.1.3 Knowledge and perception of aflatoxin contamination in Omena samples 44

4.2 Aflatoxin and moisture content levels in Omena samples 45

4.2.1 Aflatoxins strains and quantities in Omena sample 45

4.2.2 Percentage moisture content in Omena samples 48

4.3 Proximate calcium content in Omena samples 49

4.4. Proximate Iron content in Omena samples 50

4.5 Correlation between moisture, aflatoxin, calcium and iron levels 51

CHAPTER FIVE **55**

5. CONCLUSIONS AND RECOMMENDATIONS **55**

5.1 Conclusions 55

5.2 Recommendations 55

REFERENCES **57**

APPENDICES **72**

APPENDIX I QUESTIONNAIRE 72

APPENDIX II PROXIMATE QUANTITIES OF AFLATOXINS B1, B2, G1, AND G2 74

LIST OF TABLES

Table 3. 1 Lot weight and number of incremental sizes 24

Table 4. 1 Socio economic characteristics of Omena processors 39

Table 4. 2 Methods adopted for preservation and Packaging of Omena samples 43

Table 4.3 Aflatoxin knowledge/awareness and utilization methods of mouldy 44

Table 4. 4: Total aflatoxin levels in Lake Victoria Shores & Nairobi selling points 46

Table 4.5: Percentage moisture content around Lake Victoria Shores and Nairobi selling points 48

Table 4.6: Calcium levels in Omena around Lake Victoria Shores and Nairobi selling points ... 49

Table 4.7: Iron levels in the Omena samples for Lake Victoria and Nairobi outlets 50

Table 4.8: Correlations matrices between variables; moisture, aflatoxin, calcium and iron content 52

LIST OF FIGURES

Figure 3.1: Map of Kenya showing the sampling sites 22

Figure 3. 2 Chromatograms of Aflatoxins analysis 29

Figure 3.3 Chromatograms of Aflatoxins analysis 30

Figure 3.4 Chromatograms of Aflatoxins analysis 30

Figure 3.5 Calibration Curve of Aflatoxin B1 31

Figure 3.6 Calibration Curve of Aflatoxin B2 32

Figure 3.7 Calibration Curve of Aflatoxin G1 33

Figure 3.8 Calibration Curve of Aflatoxin G2 34

Figure 4.2: Unhygienic drying of Omena near a dumping area in Usenge beach 41

Figure 4.3: Unhygienic drying of Omena near stagnant dirty water in Usenge beach 42

Figure 4.4 The correlation between aflatoxin and moisture content during the wet season…….53

Figure 4.5 The correlation between aflatoxin and moisture content during the dry season……..53

LIST OF ABBREVIATIONS AND ACRONYMNS

µg	Microgram
AAS	Atomic Absorption Spectrophotometer
AFB1	Aflatoxin B1
ANOVA	Analysis of Variance
BMU	Beach management Unit
BW	Body Weight
CAST	Council for Agricultural Science and Technology
CBD	Central Business District
CDC	Centers for Disease Control and Prevention
CFU	Colony Forming Units
CPHR	Centre of Public Health Research
ELISA	Enzyme-Linked Immunosorbent Assay
EU	European Union
FAO	Food and Agriculture Organization (FAO)
FDA	Food and Drug Administration
FLD	Fluorescence Detector
FSNRL	Food Safety and Nutrition Reference Laboratory
GDP	Gross Domestic Product
HACCP	Hazard Analysis Critical Control Point
HPLC	High performance liquid chromatography
IAC	Immunoaffinity Column
IARC	International Agency for Research on Cancer
JECFA	Joint FAO/WHO Expert Committee on Food Additives (JECFA)
IUCN	International Union for Conservation of Nature
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KEMRI	Kenya Medical Research Institute
Kg	Kilogram
M	Million

MLs	Maximum Levels
MT	Metric tonnes
NPHL	National Public Health Laboratory
sec	second
POPs	Persistent organic pollutants
SPSS	Statistical Programme for Social Scientists
WHO	World Health Organization

CHAPTER ONE

1. INTRODUCTION

1.1 Background Information

Fishing industry holds a key position in the economy as a food source for nutrition and income security. Worldwide production capacity is around 171,000,000 tonnes/year where 88 per cent was consumed by human (Vannuccini, 2018). China is the major fish exporter and biggest producer of fish and fish products to the EU which forms the biggest destination for these products (FAO, 2018; Obiero *et al.*, 2019). In the Africa Continent, fish contributes up to 22 per cent of protein from animals consumed in the East and Central African (FAO, 2018; Jay *et al*., 2005).

The aquaculture in Kenya and fisheries' value chain contributes an estimated 0.54 percent to the country's gross domestic product (Farm Africa, 2016). It plays a key role in the country as a security in nutrition, food source and provides income plus employment (Obiero *et al*., 2019). Most common species for human consumption in Kenya include tilapia and *Rastrineobola argentea* (also referred to as "*Omena*" or "*Dagaa*"). Completing the list are Nile perch (or "*Mbuta*") and catfish (Farm Africa, 2016). Production of catfish, Nile perch, tilapia Omena*,* in Kenya was 11,398, 43,399, 47,555 and 69,561 respectively, in 2016 (FAO, 2016).

Interests in the safety of food and protection of the consumer have ensured strict hygiene measures at the international trade arena like the Code of Practice for Codex for Fishery Products and also as the Codex Alimentarius Commission 2016 (FAO, 2018). The Kenya Bureau of Standards 2011 Dried Rastrineobola Argentea Omena/dagaa/mukene — specification 2nd edition provides the protocol ensuring good Hazard Analysis Critical Control Point (HACCP) and hygienic practices for food safety administration system. Despite these concerns, the most common method of Omena fish preservation in Kenya is sun drying on used fishing nets or on bare ground (Jumbe *et al.,* 2010). However, huge post-harvest losses are incurred in Omena mainly in rainy season where there are huge catches. This is mainly due to improper handling, processing and packaging ways resulting in pathogenic contamination of fish by fungi and other

microorganisms (Owaga *et al*., 2009). Under favorable conditions of high humidity and high temperatures, these fungi multiply and produce secondary toxins referred to as mycotoxins that affects food quality and impacting negatively on human and animal health (Bennet and Klinch, 2003; Nyamwaka *et al*., 2017)

Aflatoxins are produced under conducive conditions like temperature, moisture content and high humidity that enhances fungal multiplication and toxins generation (Hassan *et al*., 2011). Notably, aflatoxins are compounds that are thermally stable that resist deterioration and have extended effect toxigenically during food production activity (Moss, 2002; Karan *et al.,* 2005). The aflatoxin can enter human by consuming food which is contaminated (Sorensen et al., 2010) resulting in aflatoxin poisoning referred to as aflatoxicosis. It causes cancer and damage liver in human beings, decreases milk production in animals and affects fertility in birds (Agag, 2004). Poorly stored foods, feeds and feeds with inferior quality of ingredients can have fungi sprouting in them. Aflatoxin occurs in various crops plus animal products. Food items have been confirmed to carry residues of the toxin. Therefore, it is undeniable that there is an exposure by human to aflatoxins by way of contaminated food commodities with an important constituent being fish (Murjani, 2003).

Aflatoxin B1 is regarded as the most notable subtype that inflicts high risk to animals and human health (Murjani, 2003). Aflatoxin B1 is the most potent and therefore potentially fatal metabolite and a known carcinogen for human (Lalah *et al.,* 2019). To reiterate, no other natural product has human carcinogenicity data so enthralling. The International Agency for Research on Cancer (IARC) ranked aflatoxin B1 as a group I carcinogen. Prevalence of aflatoxins are in regions situated within latitudes 40 ºN and 40 ºS (WHO, 2005). Aflatoxins, are toxic metabolites produced by some *Aspergillus* sp. species and have been established to be mutagenic, teratogenic and carcinogenic to numerous experimental animals (Sarma *et al*., 2017). The melting point of aflatoxins is as high as 250 °C.

Aflatoxicosis fall into two categories depending on the level of exposure as either acute aflatoxicosis or chronic aflatoxicosis (Karan *et al*., 2005). Acute aflatoxicosis occurs when the consumer intake large quantities at once whereas chronic aflatoxicosis occurs due to frequent intake of smaller amounts of aflatoxins. The health hazardous effects of aflatoxicosis for human

comprise carcinogenicity, mutagenicity hepatotoxicity, teratogenicity, immunosuppression, genotoxicity, nephrotoxicity and neurotoxicity, and. Symptoms of acute aflatoxicosis in human encountered include pulmonary oedema, fever, convulsions, jaundice, vomiting, abdominal pain, coma leading to death.Cerebral oedema and fatty involvement of liver, kidney and heart in man and other animals (Turner *et al*., 2009; Makun *et al*., 2010). On the other hand, chronic form aflatoxicosis can cause liver cancer, neuro and reproductive-disorder like abortion, stunted growth, immunosuppression and micronutrient deficiency (Horn and Domer, 2002).

Microbiological studies of microbes associated with Omena in Gucha, Kisii County, Kenya reported presence of the thirteen species of moulds in the Omena including different *Aspergillus* species of *niger, flavus, fumigatus*, *Mucor* sp.,Yeasts, *Absidia* sp., *Aureobasidium* sp., *Trichoderma* sp., *Alternaria sp Cladosporium sp*., Fusarium sp., *Penicillium* sp., *Rhizopus* sp., (Nyamwaka *et al.,* 2017). Although some of these microorganisms are responsible for mycotoxins production, there are no studies to this effect. Additionally, various studies have reported presence of mycotoxins in smoked fish in Nigeria and many parts of the world. This present study assesses the public awareness of aflatoxins and quantifies aflatoxins strains associated with Omena at the main landing beaches in Kisumu, Migori and Siaya and major consumer outlets in Nairobi. We further assess the effect aflatoxins accumulation on iron and calcium content in Omena.

1.2 Statement of the Problem

Whereas sun drying is the most widely practiced method of fish preservation in Kenya, unfortunately, Omena fish best catches are in the rainy season when drying is difficult resulting in poor hygienic handling due to large harvest (Onyango *et al.,* 2017). Due to insufficient drying, there is a rise in percentage moisture levels on sun dried *Rastrineobola argentea* enhancing the micro-fungal development a number of which produce toxins like *Fusarium*, *Peculium and Aspergillus*. The molds lead to post-harvest degradation of *R. argentea*, reducing the quality (Nyamwaka, 2014). Therefore, continued consumption of these *Omena* fish infested with moulds constitutes a health risk. The aflatoxins produced by these fungi can lead to chronic and acute effects in human (Turner *et al*., 2009). Previous studies dwelt on fruits, cereals and salted meat (Turner *et al*., 2009; Makun *et al*., 2010). However, there is little information on the public

awareness and the type and levels of aflatoxins in Omena fish highly consumed in Nairobi, Migori, Siaya and Kisumu counties and the calcium iron and moisture content.

1.3 Research Hypotheses

There no significant difference in total aflatoxins and iron, calcium and moisture contents in *R. argentea* sold in different counties in Kenya

1.4 Research Objectives

1.4.1 General objective

The general objective was to determine the diversity, abundance and public awareness of aflatoxin associated risks in sun dried *Rastrineobola argentea* and determine its relationship with the moisture, calcium and iron content.

1.4.2 Specific Objectives

1) To assess public awareness, knowledge and practices involved in *Rastrineobola argentea* handling, preservation and level of knowledge regarding aflatoxin contamination
2) To determine the prevalence of aflatoxins in *Rastrineobola argentea* from selected localities in Kenya and its relationship with moisture content
3) To quantify the content of calcium content in *Rastrineobola argentea* from selected localities in Kenya.
4) To quantify the iron content in *Rastrineobola argentea* from selected localities in Kenya.

1.5 Justification and Significance of the Study

Aflatoxins contamination of foods is a worldwide health problem and food security threat in both first and third world countries (Liu and Wu, 2010). Aflatoxins plus other naturally present toxins is of specific concern in rural fraternity of third world countries. Fish is highly susceptible to aflatoxin contamination. Despite Omena fish being one of the one highly consumed source of proteins and susceptible to aflatoxin contamination, there is little study on the public awareness on aflatoxin contamination in Omena fish in Kenya. Similarly, there are no studies on quantities of calcium and iron content in Omena and their relationship with aflatoxin contamination.

Findings of this study can inform advice to fish processors, retailers on recommended handling plus storage of sun dried Omena. The data gathered on connection between aflatoxin levels and percentage moisture content on sun dried Omena will be employed in establishing the moisture content that will be suitable in reducing the development of micro-fungi and toxin production on such fish.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Fish Industry and its contribution to economy

The fisheries industry provides food and nutrition security, and offers jobs, specifically for coastal people, who are mostly among the poorest and most exposed. On average worldwide, fishery and fishery products constitute 18 percent of animal protein intake. Following the population growth coupled with shrinking per capita income, need for fishery is contemplated to rise to 30 percent in 2030 (World Bank, 2019). The highest total world fish production was 171 million tonnes in 2016 and the highest export of fish amounted to USD 152 billion in 2017 (FAO, 2018).

Inland catches in Africa forms an important source of food for most states (FAO, 2018) with the ratio of cured fish greater than the global average. Inadequate roads, power supply, ice plants, potable water cold rooms, refrigerated transport and suitable storage and processing facilities in addition to the Africa tropical temperatures give rise to big post-harvest losses, of up to 50 percent, and deterioration in quality going to even 70 percent of the waste (Akande and Diei-Ouadi, 2010).

Kenya ranks fourth aquaculture producer in Africa recording a growth from 4,218 metric tonnes (MT) in 2006 to 24,096 MT in 2014, a 15 percent of national total fish production (KMFRI, 2017). Kenya's Fishery sector is categorized into freshwater aquaculture and mariculture. In 2018 Kenyan aquaculture, stood at 14,952 composed of freshwater and mariculture (rearing of shellfish,seaweeds,finfish,oysters,grey mullets and shrimps with combined production over 100 MT not as much as freshwater aquaculture currently standing at 14,852 MT (Opiyo *et al.,* 2018). Consumption of freshwater fish was estimated to be 195,206 tonnes in 2014. Nevertheless, considering the negative trade balance and post-harvest food losses, the overall consumption of fish may have been less (Farm Africa, 2016) The imports to Kenya is 5900 MT yearly from Pakistan, China, Uganda, Japan, Korea and India and to satisfy the deficit between fish locally produced and the rising food fish demand for (Opiyo *et al.*, 2018). In 2013 most imports

originated from China in the form of frozen tilapia (14%). The following year 2014 had overall fish imports surpassing 5853 MT, with Nile tilapia rising from 14% (2013) to 30.8% (2014) (Opiyo *et al.*, 2018; Farm Africa, 2016). Overall fishery and aquaculture yield was 186,700 tonnes in 2013, having 83 percent originating from inland capture fisheries from which Lake Victoria supplied almost 90% (FAO, 2015). Lake Victoria in the Kenyan side ordinarily has the most fresh water fishery in the country, with a 2006 production of 143, 900 tonnes. Lake Victoria is home to a range of 170 to 350 fish species, the three most important economically being the Nile perch (*Lates niloticus*), the silver cyprinid '*dagaa*' or '*Omena*' (*Rastineobola argentea*) and the Nile tilapia ('*ngege*'), all having a widespread presence in the lake. One breed of major lucrative importance is the ever-ubiquitous tiny endemic silver cyprinid *Rastrineobola argentea* (known diversely as '*Omena', 'mukene'* or '*dagaa*') which swim in colossal shoals and are targeted for human consumption and production of animal feed. The silver cyprinid *Rastrineobola argentea* adults stay at the lake bed over daylight and get to the top at night, where they are fished through light attraction by use of pressure lamps (Wandera, 1991). As of 2013 over 129, 300 Kenyans had their livelihoods depending on fishing and fish farming activities (FAO, 2015). The fish export in the country in 2012 reached USD 62.9 million, while the fish imports were a meagre 20% of exports at USD 12.3 million (FAO, 2015).

Additionally, Kenya's total exports of fish and fishery products for 2015 was 33.688M USD, 23.244 M USD in 2016 and in 2017 was 24.980M USD (FAO,2020). Tourists numbering over 1.6 million tour Kenya annually, a bigger proportion of who are allured by the leisure fishery, which has a numerous types of fish species near the shore. It is a leading destination for recreational-fishing tourists who do angling, troll and deep sea diving in the coastal deep sea (FAO, 2020).

The most common method of Omena preservation is the traditional sun drying which relies on reduction of moisture content to a level not able to sustain microbial and enzymatic activity (Jumbe *et al.,* 2010; Omojowo *et al.,* 2010) However, best catches of Omena fish occurs during the rainy season hence subject to insufficient drying possibly leading to high moisture accumulation resulting in microbial infestation including moulds and yeasts. There are strains of molds resulting in contamination and producing specific toxic secondary metabolites referred to as mycotoxins under favourable condition (Essien *et al.,* 2006). Mycotoxins are poisonous

metabolites occurring naturally and excreted extracellularly inside or on foods and feeds by toxigenic molds. Mycotoxins occurs in various forms including ochratoxin, zearalenone, tricothecenes, fumonisins and aflatoxin. Key important mycotoxin in Kenya is Aflatoxin. Aflatoxins result from strains of parasiticus, Aspergillus flatus, and the less common species Aspergillus monies (Hassan *et al.,* 2011). Aflatoxins occurs in various subtypes as AFB1, AFB2, AFG1, and AFG2. Aflatoxin B1 is converted into Aflatoxin M1 and Aflatoxin B2 are into AflatoxinM2 subtypes respectively which can be expressed in products such as breast milk for example if a mother consumes AFB1. Among these subtypes aflatoxin B1 is regarded the most potent to human in terms of public health (Herzallah, 2009) and categorized as class 1 carcinogen (IARC, 2002).

Incidences of human acute aflatoxicosis in have occurred from various regions worldwide mainly in subtropical and tropical areas of the World including Uganda, India, Taiwan, and Kenya (Strosnider *et al*., 2006). Circumstances aggravating the possibility of human acute aflatoxicosis are food scarcity, ecological situations conducive to mould growth in plants and commodities, and absence of regulation for aflatoxin control and monitoring (Bankole and Adebanjo, 2003). The year 2004, had hundreds of Kenyans very ill of which 125 died (Centre for Disease Control, 2004), of acute aflatoxicosis: a liver failure disease caused by consumption of extremely high aflatoxin levels in food (Strosnider *et al*., 2006; Lewis *et al*., 2005). From that time more attention worldwide is directed aflatoxin related health concern. Although this acute was destructive, much more people were ailing from ailment related with chronic levels of aflatoxin uptake in maize, groundnuts among others.

A substantial amount of Kenya's total fish catch provides for the protein needs of the community around. In 2009, 2008 and 2007 92.2%, 86.7% and 86.6%, respectively of all the fish catch were used as domestic consumption, hence providing for the community's food security (FAO, 2015). Kenya's per capita consumption of fish in 2011 stood at 5kg, and carried on similarly in 2018 and average lower than FAO proposed average of 20 kg /person annually (Opiyo *et al.*, 2018). Fish overall protein intake is low at 7.6% since cultural or historical reasons make most Kenyans not to consume fish. Nevertheless, Kenya's fishing folk rely majorly on fish as a bumper source of protein.

Kenyan consumption of fish is about 4.5 kg per capita/year (Farm Africa, 2016).The rural poor in East and Central Africa has an important animal protein source in Omena fish (Obiero *et al*., 2019). This is due to its richness in vitamins, minerals such as calcium, iron, phosphorous and polyunsaturated fatty acids that are crucial to a healthy diet (Heshmati *et al*., 2019). Additionally, compared to Tilapia or Nile Perch, Omena is cheap and readily available and drying preserves it for a long time (Mozola, 2001).

2.2 Omena contribution to food and Nutrition security

Food security is one of the pillars of the Kenya's Big Four Agendas that are anchored on the United Nations' Sustainable Development Goals (SDG's) to transform Kenya to middle income country (FAO, 2018). Fish industry contribute significantly towards this due to its richness in vitamins A, B, D, high quality protein, a variety of minerals such as calcium, potassium, phosphorus, iron, copper, essential free fatty acids, and iodine (Agile *et al*., 2007). *Rastrineobola argentea* accounts for 62.9% overall resources in fisheries from L. Victoria, Kenya and ranks the second largest by volume after Nile perch (Sablani *et al*., 2009). Fish constitute a major source of protein of most people in the third world countries Kenya included. Both fresh and salt water fish contain high level of proteins (Jay *et al*., 2005).

Survey findings in the community showed that *Omena* is the fish mostly consumed as it is easily obtainable in most in Kenya (Kariuki, 2011), cost effective in comparison to Nile Perch or Tilapia and drying can keep it preserved for long. (Mozola, 2001). Properly dried *Omena* can go for even two years without spoilage in storage. (Ofulla *et al*., 2007). One kilogram of *Rastrineobola argentea* goes for an estimated Kshs. 150 as opposed to tilapia the similar weight of costing Kshs. 400. Fish supply and distribution business is the next blue-chip enterprise that's in the offing in the Nairobi market, waiting to be tapped by the young, innovative and competitive entrepreneurs (Buluma, 2012). With most health experts recommending white meat to red meat, the people of Nairobi are slowly adopting the fish eating culture.

The *R. argentea* catch are big though the landings' worth are quite low. The post-harvest losses from the *R. argentea* sub-sector are approximately 20-30 % and goes to even 50 % when it is rainy (Ofulla *et al*., 2007). Owing to the fast loss in quality when sun-drying, *R. argentea* is more

and more being utilized in the feed industry for fish meal production other than for consumption by human (Bille and Shemkai, 2006). However, the need for Rastrineobola argentea in this area has increase in the past decade (Fisheries Annual Report, 2010). This is due to it being an important of proteins' source to livestock (Buere, 2005).

2.3 Fish preservation methods and spoilage mechanisms

Fish preservation refers to methods in which food is prevented from spoilage after harvest. The practices started in ancient times. Some of the earliest ways of preservation fish are sun drying, smoking and refrigeration (Thomas, 2008). Drying method relies on the reduction of water content principle to level where micro-organisms can no longer multiply. Products of sun-drying have quality slightly less because of disintegration of some vitamins in sunlight. Fish smoking is alternatively based basis of reduction of both internal water content and particles from smoke give an extra taste to the by-product (Steel, 2009). Currently modern methods of fish preservation are being deployed which include freezing, canning, irradiation, and chemicals added (Oduor *et al*., 2010). Marked progress in in packaging have contributed important step in modern preservation fish.

Improvement of the drying of Omena in the basin of Lake Victoria has been realized through the initiation of racks like in Coastal region and Suba District, (Aguilo *et al*., 2007). Drying racks, usually raised ventilated platforms, are based on circulation of air all over the product to vaporize the extra moisture and their application decreases fish soiled while drying. Nonetheless, aerial contamination, insect's infestation and rain still pose a challenge during drying on racks (Chamberlain and Titili, 2001). An additional advancement of drying with racks is the employment of solar dryers in which drying in the confined chamber prevents rain and insect infestation ,thereby ensuring fish is dried quickly and more hygienically (Bille and Shemkai, 2006).

Unfortunately, Omena is caught in very large quantities posing difficulty to handle hygienically on board. During fishing, initial consignment may stay longer in the boat, spoil faster and cross-contaminate the rest of the consignment and with the artisanal conditions used, it becomes hard

to preserve Omena using ice (FAO, 2015). After the fish catch, spoilage continues swiftly. The tropical high ambient temperatures, causes fish to decompose in 12 hours (Kathyryn, 2007).

Fish spoilage is the deterioration of food due development of off-flavours, deterioration of texture, and the loss of nutrients that renders food unfit for human consumption (Report on Public Health, 2006). These changes may be brought about by a variety various aspects, such as contamination by degradation by endogenous enzymes (those present naturally in the food), infestation by insects and microorganism (Singh and Desrosier, 2018). The distinctive microorganisms that give rise spoilage of food are molds (e.g., *Rhizopus)*, yeasts (e.g., Saccharomyces) and bacteria (e.g., *Lactobacillus*). Contamination of fish by fungi is regarded as the major source of indication of spoilage as stale and unappetising taste may lead to economic losses and a public health hazard as a result of aflatoxins poisoning (Hassan *et al*., 2011).

2.4 The role of fungi in microbial contamination of Omena fish

Fungi (yeasts and molds) and bacteria are the major microorganisms causing food-borne illnesses and fish spoilage . Contamination of fish with microorganisms can occur at storage, harvest, processing, handling, distribution, or preparation. The chief causes of contamination by microbes are food processing machinery, sewage, soil, air and utensils. Two fungal types which are important in spoilage of fish are molds and yeasts . Moulds are in form of multicellular fungi which form spores on reproduction.

Spores' formation are in big numbers and can be dispersed in air. Spores can land on a food substrate, grow and reproduce when the situations are conducive. Yeasts, the unicellular fungi are bigger than bacterial cells. Their reproduction is through budding or by cell division (binary fission). Molds and yeasts can grow in an acidic condition (pH less than 7). Yeast grows between pH of 3.5 to 4.5 while molds grow between pH 3.5 to 8.0. The amount of available water in a food product is also critical for the growth of fungi. Yeasts will not grow at a water activity below 0.9, while molds will not grow at a water activity less than 0.8.

Study of *Omena* in Gucha, Kisii County, Kenya by Nyamwaka (2014) discovered thirteen fungal species in the *R. argentea*.The fungal spores will germinate if favourable conditions are

available, sporulate and can even produce mycotoxins. Considering the fungal species isolated, *Aspergillus flavus, Fusarium* and *Penicillium* species are causative agents of mycotoxins (Nyamwaka *et al.,* 2017).

2.5 Mycotoxins in food contamination

Mycotoxins are poisonous secondary metabolites brought about by toxigenic fungi that contaminate feed materials and food in pre- and post-harvest periods under favorable conditions of temperature, moisture and relative humidity eliciting toxic response in the host organisms (Pinotti *et al.,* 2016; Stone, 2002). The toxic response they elicit is termed as mycotoxicosis which is further classified acute mycotoxicosis or chronic mycotoxicosis. There are 300 -400 compounds recognized as mycotoxins. Mycotoxins are fungi made, poisonous to vertebrates and other groups of animals in little amounts (Cimbalo *et al*., 2020; Bennett and Klich, 2003).

Mycotoxicoses are the animal diseases brought about by mycotoxins (Wallace, 2014). As opposed to mycoses, mycotoxicoses are illustrations of "poisoning by natural means" thereby are similar to the pathologies due to heavy metal or pesticides residues. The symptoms depend on mycotoxin, exposure duration, health, age, sex of the victim hence toxicity of mycotoxin poisoning is aggravated by deficiency in vitamins, deprivation in calories, condition of the infectious disease and alcohol abuse. (Bennett and Klich, 2003).

The dietary mycotoxins majorly encountered having global presence are, trichothecenes, zearalenone, patulin ochratoxins, fumonisins, and aflatoxins produced by the fungal genera *Penicillium, Fusarium* and *Aspergillus,* (Alshannaq and Yu, 2017). Children within Africa get exposed to 46 dietary mycotoxins (Turner *et al.,* 2009). Chronic exposure aggravates disease pathogenesis in laboratory trial animals and humans (Kibugu *et al.*, 2009) reduction in animal productivity, and impaired nutrition in animal (WHO, 2002). Mycotoxins can also be mutagenic, teratogenic, immunosuppressive, estrogenic,carcinogenic,hepatotoxic and nephrotoxic,in humans and animals (Williams *et al.,* 2004; Turner *et al.,* 2009). They arise from inhalation of spore-borne toxins ,eating contaminated foods, and skin contact with substrates which are mold-infested (Prasanna and Dharani, 2018).

Acute toxicity is exhibits has a quick onset with a toxic response, while chronic toxicity is manifested in cases of low-dose exposure over a long time period, leading to cancers and other someirreversible conditions. Almost definitely, the main veterinary and human health burden of mycotoxin exposure is associated to chronic exposure like immune suppression, kidney toxicity and cancer induction. The food-borne mycotoxins expected to be of major importance for health of human in developing tropical countries are the aflatoxins and fumonisins (Wilson *et al*., 2002).

2.6 Aflatoxins as food contaminants

According to Akande and Tobor (1992) freshly caught fish are covered with damp sacks or mixed with either water weeds or wet grass to lower the temperature. This method of fish treatment results in the fish being susceptible to contamination by bacteria and fungi. This shows that fish spoilage begins basically at the aquatic ecosystem. In artisanal fishery, handling fishes also make them vulnerable to be attacked by microbes due to unhygienic temperature reduction methods (Adejola, 2006). The environment where *Omena* is laid out in the market is rarely hygienic opening creating another window for contamination by aflatoxin. Quite often fish retailers display fish near the gutter (Figure 3.2) on heaps of refuse encouraging attack by fungi and subsequent aflatoxin production (Akande and Tobor, 1992).

Aflatoxins are low-molecular-weight natural products generated by filamentous fungi as secondary metabolites. . Aflatoxin term was forged in 1962 , in England, near Londonwhen an estimated 100,000 turkey poultry were killed due to a mystifying“Turkey X” disease associted to a Brazilian peanut (groundnut) meal which had secondary metabolites from Aspergillus flavus (aflatoxins) contamination (Chukwuka *et al.*, 2010). Aflatoxins are highly toxic compounds synthesized mainly by the moulds *A. parasiticus A. flavus,* and *A. nomius* (Lawley, 2013; Kumar *et al.,* 2016). Most notable and commonly occurring aflatoxin causingstrains are Aspergillus parasiticus and *Aspergillus flavus* (Kang’ethe *et al.,* 2007). Organic chemists classify aflatoxins as per chemical structures e.g., lactones, coumarins (Bennett and Klich, 2003). Figure 2.1 shows the structures of aflatoxins B1, B2, G1 and G2 (Bianchini and Bullerman, 2014).

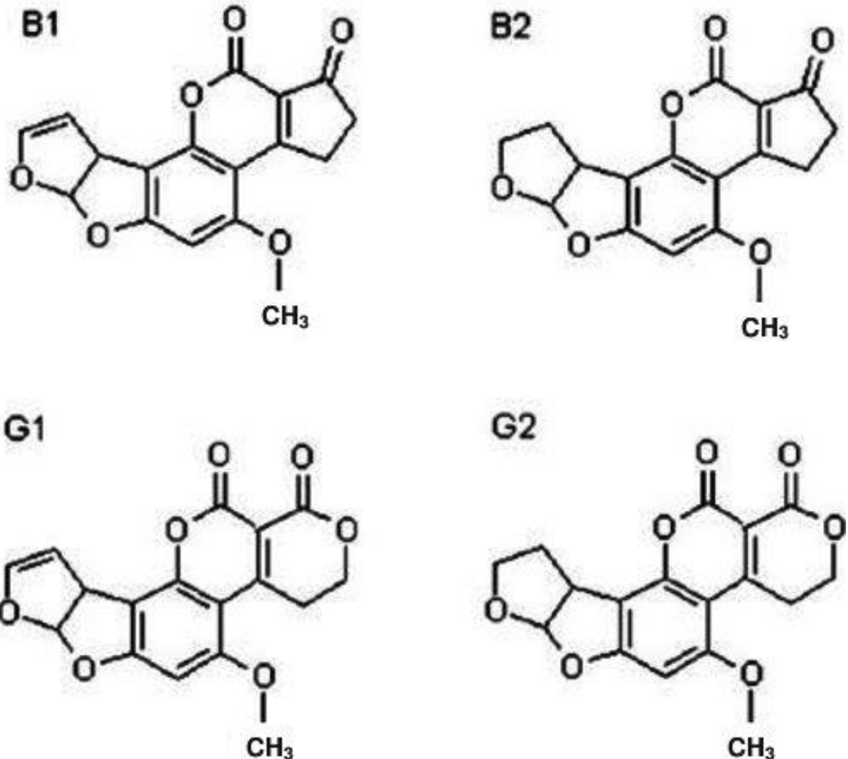

Figure 2.1 Structures of Aflatoxins B1, B2, G1 and G2

Food subtypes at high risk of aflatoxin effects include meat ,milk,cereals, fish,legumes , and fish (ICRI, 2000). Other products including fish can be contaminated with aflatoxin if stored in an environment which are favorable for mould production with relative humidity of 70 % and temperature ranging 10 $^{\circ}$C to 40 $^{\circ}$C and pH of 4-8.

There are various aflatoxins sub-types known so far including P_1. Q_1, B_{2a}, and G_{2a} G1, G2 B1, B2, with types B2, B1, G2 and G1 being the major ones in cereals, oil seeds, spices and tree nuts. These aflatoxins are classified depending on the color of their fluorescence under UV light; B (B1, B2) exhibits blue fluorescence on thin layer chromatography while G produces Green (G1 and G2) thin-layer chromatography relative chromatographic mobility (Barrett, 2000; Bennett and Klich, 2003). Aflatoxins M1 and M2 are products of metabolism of aflatoxin B1 and B2 generated when animals take B1 and B2, and they are excreted in urine and faeces and secreted in milk of both human and animal (Kang'ethe *et al.,* 2007).

The stability of aflatoxins in foods and the ability of aflatoxin-producing fungi multiply on a variety of food mean that control is best realized by ways devised to avert the contamination and on storage, or detection and removal of material contaminated from the food value chain (Lawley, 2013). Aflatoxins exposure may precipitate underweight and child stunting , immunosuppression child mortality and neurological impairment, according WHO Expert Group Meeting in July 2005 (Henry *et al.,* 2001; WHO, 2005).

Studies have found a probable link between growth faltering and aflatoxin exposure , specifically in children with kwashiorkor and stunting in young children (Gong *et al.,* 2008; Turner *et al.,* 2007). Exposure to aflatoxin may interfere with child growth through immune suppression leading to increased vulnerability to infectious disease, aflatoxin-induced disruption to RNA resulting in inhibition of protein synthesis or intestinal malabsorption (Williams *et al.,* 2004, Gong *et al.,* 2008). Stunting during childhood is linked to a reduced work capacity, reduced adult size and adverse outcomes reproduction.

Direct ingestion of high aflatoxin level.s of is fataf while chronic exposure is linked with immunosuppression, abortion (spontaneous), liver cancer, cirrhosis and other liver diseasesand can interfere with metabolism of micronutrient, causing malnutrition deficiencies in children below the age of 5 years. The rate of stunting among children below 5 years was 26.9% in Nyanza Province according to KNBS (2010). High stunting percentages have been found in all theareas in Nyanza Region with infants having a higher aflatoxins vulnerability as opposed to adults (Williams *et al.,* 2004) since infants possess a lower biotransformation capacity for aflatoxins than adults, giving rise to a longer time for circulation of the toxins. In West Africa, a study shows children who had chronic exposure to aflatoxin in foods were underweight and stunted, as compared to the World Health Organization (WHO) z-scores (Cardwell *et al.,* 2004).

The Dunga sampling site (Kisumu County) climatic conditions made of erratic rainfall (1,200 mm and 1,300 mm per annum), drought, high humidity (40-89%), high temperatures stretching between 20 ^{o}C - 35 ^{o}C provide conducive conditions for production of aflatoxins and growth of mould may constitute potential public health hazard (Onyango *et al.,* 2002).

2.6.1. Identification of aflatoxins

Currently available methodologies for aflatoxin determination have been summarized by Pascale and Visconti (2008), and include: Liquid Chromatography Mass Spectrometry (LC/MS), Gas Chromatography (GC), High Performance Liquid Chromatography (HPLC), Enzyme-Linked Imunosorbent Assay (ELISA) and Thin Layer Chromatography (TLC), and rapid tests. Verification of these methods have been carried out by Association of Analytical Chemists International (AOAC) (IARC, 1993; AOAC, 2000) and by various international committees (ISO, 1998). The test to be used is dependent on many elements like precision, cost effectiveness, and number of samples being tested. Some prefer analysis of aflatoxin using ELISA owing to its simplicity, speed, cost effectiveness, sensitivity and adaptability (International Crops Research Institute for the Semi-Arid Tropics, 2007) . It provides for testing of numerous samples which is perfect when screening. HPLC is most sutable for quantification and validation since it has has a good selectivity and highly sensitivity with ease of auotomation. Nonetheless the disadvantage with HPLC's is the high cost involved, rendering not ideal for routine procedures. Emerging techniques for analyzing mycotoxins are like Fluorescence Polarization Immunoassay (FPIA), lateral flow devices (LFDs),capillary electrophoresis Infrared Spectroscopy, molecularly imprinted polymers fibre-optic immunosensors and (Pascale and Visconti, 2008). No matter the method used but it should enable tolerance levels detection , to facilitate monitoring programs and ensure safety in international trade (Pascale and Visconti, 2008). High Performance Liquid Chromatography (HPLC) technique was chosen in this study over the other methods of detecting aflatoxins, because it is fast and gives aflatoxins detection results within a short and accurately time. HPLC is quantitative, with high precision, high sensitivity, and high automation. With FLD a detection sensitivity of as low as 0.1 ng/Kg has been reported (Herzallah, 2009).

2.6.2 Aflatoxin food poisoning in the world

The largest recorded aflatoxicosis outbreak occured in over 150 villages in neighbouring districts of two adjacent western Indian states in 1974. It took place in the contest of unreasonable rains while harvesting resulting in home grown maize stored in damp conditions being contaminated to approximately 55 µg/kg BW exposure leading to a 27% fatality rate (Lampel *et al.,* 2012).

Over 397 people were affected during the epidemic, with 108 people dying mainly from gastrointestinal haemorrhage. Contaminated corn was the main dietary constituent In this epidemic, with aflatoxin concentrations of 0.25 - 15 mg/kg of corn recorded. Day to day exposure to aflatoxin was approximated to be not less than 55 μg/kg BW for an unknown days (Cressey *et al.*, 2006).

In Philippines, aflatoxin B1 was discovered in the lungs of two agricultural and one textile worker who died from the pulmonary and intestinal fibrosis. These persons were possibly exposed occupationally to aflatoxin B1 through the respiratory route (Peraica, 1999; Dvorackova *et al.,* 1986). In the United Kingdom (UK), intravenous heroin users were exposed to aflatoxin B1 from heroin samples on sale (Hendrickse *et al.,* 1997; Peraica, 1999). In the Netherlands and the UK , 121 heroin addicts volunteered urine samples showed a larger percentage of test samples contaminated with aflatoxins B2, B1, M2, M1 and and aflatoxicol (20%) than the ones volunteered by normal adult (Hendrickse *et al*., 1997; Peraica, 1999).

2.6.3 Aflatoxin food poisoning in Kenya

Acute aflatoxicosis outbreaks from highly contaminated food have been recorded in Kenya (CAST, 2003). In the year 2004, acute hepatotoxicity outbreak was recognised among residents of Makueni, Thika Machakos and Counties. Epidemiologic analysis found that the outbreak resulted from aflatoxins poisoning through contaminated maize ingestion (Kang'ethe *et al.,* 2007). Within four months in 2004, 317 cases and 125 deaths had occurred; making it one of the largest and most severe acute aflatoxicosis outbreak recorded worldwide (Centre for Disease Control , 2004). Maize samples were analysed and establishedto have aflatoxin B1at levels up to 8 mg/kg (Cressey, 2006). Another aflatoxicosis outbreak of contaminated maize occurred in 1981, an in Makueni County (Centre for Disease Control, 2004). It had 20 hospital admissions and 60% case fatality rate (Cressey, 2006).

The two years 1981 and 2004, had shortages of food and drought succeded by unreasonable rains over harvest possibly favoring the multiplication of aflatoxigenic *Aspergillus* in maize in the household (Okoth *et al.*, 2012). Daniel *et al.*, (2011) studies found that nmore than 35 percent of sampled maize in the years 2005, 2006, and 2007 surpassed the 20 µg/kg Kenyan regulatory

maximum limit then (Kenya Bureau of Standards, 1988) also an also a United States FDA regulatory limit the (FDA, 2000) with levels as high as 48,000 µg/kg. Nonetheless Kenya has since lowered their regulatory limit to 10 µg/kg. Given the high maize consumption in Kenya of up to 36% of the day to day calories intake of Kenyans (Gitu, 2004), a lower limit for aflatoxin contamination in grains should be beneficial. Previous studies show the levels of aflatoxins in homegrown maize in this region of Kenya are 60 times greater than the maximum maize aflatoxin levels in other parts of the world. From the foregoing aflatoxin food contamination is a common feature in Eastern part of Kenya and occurs on cereals commonly used by many communities as staple food. These cereals can be stored in non-processed and processed and form (IUCN, 2014). However, there are no studies to screen other foods on the possible contamination with aflatoxin including *Omena.*

2.6.4 Effects of aflatoxicosis on human health

As per ICRI (2000), aflatoxins in humans exposure is through consumption of foods contaminated with fungal growth products. The exposure is hard to avoid since fungal growth prevention in foods is not easy. Even though heavily contaminated food stocks are not allowed in the market place in first world countries, there remains a possibility of severe effects from extended exposure to low levels of aflatoxins within the food value chain.

Aflatoxicosis on human health can be classified as either acute aflatoxicosis or chronic aflatoxicosis depending on the amount of aflatoxin ingested. Chronic toxicity is more likely than acute toxicity (ICRI, 2000). Acute aflatoxicosis in humans has occured in various areas globally including Kenya with the syndrome manifested through abdominal pain, vomiting, convulsions, coma pulmonary oedema, , and death with cerebral oedema and the liver’s fatty involvement of, heart and kidney. Conditions exacerbating acute aflatoxicosis are lack of regulatory aflatoxin monitoring and control systems, inadequate food and environmental conditions favouring fungal growth.

After aflatoxins invading the liver, hepatocytes are infiltrated by lipids leading to necrosis or liver cell deaths as aflatoxin metabolites react negatively with dissimilar proteins of the cell, resulting in inhibition of and protein synthesis also carbohydrate and lipid metabolism. Linked to

liver function decrease is jaundice,a derangement of the blood clotting mechanism, and a reduction in requisite serum proteins processed by the liver. JECFA reviewed the toxicity of aflatoxins lately in 1998 concluding that aflatoxins were human liver carcinogens and that aflatoxin B1was the most potent of the carcinogens, with aflatoxin M1 (AFM1) estimated at one order of magnitude less toxic. Hepatitis B positive persons have a substantially higher aflatoxins potency than those not hepapitic B. IARC deduced that there was adequate proof in humans for the carcinogenicity of naturally existing mix of aflatoxins and for AFB1 (IARC, 1993). IARC utilizes the ranking of “sufficient evidence of carcinogenicity” (Group 1) when it examines a set causal relationship and there can be reasonable confidence this association is not the result of chance, bias or confounding (IARC, 1993; Cressey, 2006). Based on the above evidence the aflatoxin B1 was reconfirmed as Group 1 carcinogen through assessment of naturally occurring aflatoxins (IARC, 2002).

Other symptoms of aflatoxicosis are abdominal pain, and vomiting oedema of the lower extremities. No particular treatment yet has been found for acute aflatoxicosis. Under supportive care, the case fatality rate for acute poisoning lies between 25 – 40 percent (Eaton and Groopman, 1994; Azziz- Baumgartner *et al*., 2005). Kenya’s Eastern province has had the most extreme exposure to aflatoxin recorded globally (Kaiming, 2020). Chronic aflatoxicosis results from consumption of sub-lethal amounts of aflatoxin for several days or weeks by animals leads to a sub-acute toxicity syndrome such as moderate upto severe liver damage. Chronic aflatoxicosis has been associated to be causative agents for liver cancer, stunted growth, and immunosuppression as well as micronutrient deficiencies.

2.6.5 Control of Aflatoxins

Properly dried foods cannot produce Fungi mycotoxins (Atalla *et al*., 2003). Efficient drying of food items and the dry state maintenance is therefore an successful control measure in preventing fungal growth and aflatoxin production (Isabel *et al*., 2007). To avert production of aflatoxins, drying ought to take place as rapidly as possible (Council for Agricultural Science and Technology, 2003). Though it is possible to manage fungal growth in stored food items by controlled atmospheres or use of natural inhibitors or preservatives, such procedures are more costly than effective drying and are therefore rarely feasible in the third world (Sage *et al*.,

2002). On a large scale, safe storage requires well-designed structures with floors and walls impermeable to moisture while on a small scale, polyethylene bags are effective (Mycotoxins in Grain, 1997).

2.6.6 Aflatoxin and percentage moisture content regulation limits

Most countries globally have put legal regulation for the control of aflatoxins in food residues. In the the EU, harmonized maximum levels (MLs) for some mycotoxins exist for feeds only, whereas in foods, MLs have been fixed (Rosner, 1998). *A. flavus* and *A. parasiticus* are extensively studied in both first and third world countries, because of the nature of their carcinogenicity and toxicity (Jonathan *et al.,* 2004). For preventive consumer protection reasons, efforts are put to arrive at MLs for genotoxic and carcinogenic mycotoxins which are technologically feasible and analytically detectable in the food ready for consumption but are as low as possible (Rosner, 1998). The EU has proposed the minimum possible aflatoxins concentrations in food products and foods . Dried fruits should have limit of 5 µg/kg, ,cereals 5 µg/kg, wine 1-2 µg/kg coffee 6-8 µg/kg and spices 10 µg/kg (Nyangaga, 2014). The Kenyan Standard specifies that the dried fish R*astrineobola argentea* shall contain no 10 µg/kg or less total aflatoxin and not more than 5µg/kg may be aflatoxin B1 (Kenya Bureau of Standards KS 1470:2011).

Food quality parameters, in which the taste,texture, appearance and stability of foods depends on the water content. Food processing operations, in which moisture content knowledge is often vital to predict the performance of foods during processing, including mixing, drying, flow through a pipe or packaging (Godwin, 2006).

The Kenya Bureau of Standards, National standard recommendeds a percentage moisture content of 5 to 7 and the Dried Silver Cyprinid to be a whole dried fresh water fish product not gutted, beheaded or split and washed and dried. Packaging material should be safe and not pass on substances or unpleasnt odour or flavour to the product free from physiological deterioration or adulteration/contamination affecting appearance, edibility dried fish quality. While drying the fish it should be protected from contamination by dirt, insects and sand and free from spoilage like mouldiness, colour change (Kenya Bureau of Standards KS 1470:2011).

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Description of the study sites

The study was carried out in 4 selected counties of Nairobi, Kisumu, Migori and Siaya focusing on the seven sampling sites based on the geographic locations and whether the catchment area of the market has enough vendors. The sampling sites were Nairobi fish markets of Gikomba (eastern side) and Toi (western side), Central Business District (CBD) (central side) and Karen Shoppng Centre (far western) and Lake Victoria main Omena landing beaches in Sori (southern part of the lake) in Migori county, Dunga (central part of the lake) in Kisumu county and Usenge (northern part of the lake) in Siaya county from where Omena are supplied from (Figure 3.1). The choice of the area of study was done based on their abundant rainfall in many months of the year giving high moisture content easy access to the markets coupled with availability of *Rastrineobola argentea.*

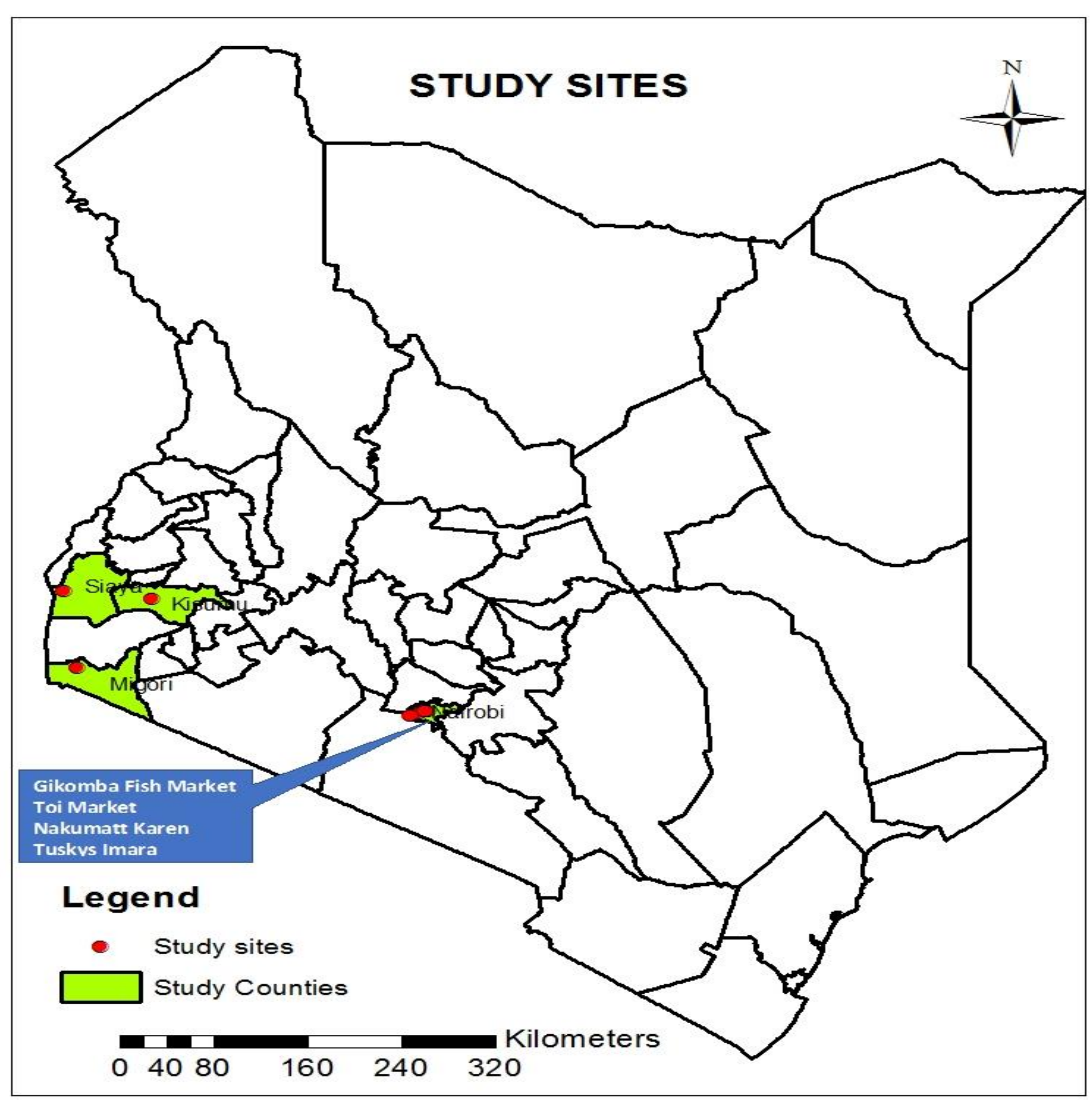

Figure 1.1: Map of Kenya showing the sampling sites

Sori beach in Migori County is situated on 0.8087° S, 34.1588° E. The altitude is 1,366 m and has averages of annual temperature of 21.2 °C and precipitation of 1369 mm. February is the warmest month averaging 22.0 °C while the average lowest month temperatue is in July temperature of 20.1°C (Merkel, 2020). The precipitation difference between the driest and wettest months is 188 mm (Aquastat, 2020; Merkel, 2020). The Migori County had a population was 1,116,436 (536,187 males 580,214 females) and a population density of 430/km^2 in 2019

national census compared to 917,170 in 2009 census (KNBS, 2019). Migori is located in the wetlands sugar belt within Western Kenya and in the tropics. It enjoys a pleasant climate due to the breeze from Lake Victoria and high altitude (Wikipedia, 2020).

Usenge (Siaya County) coordinates are 0.0677° S, 34.0558° E and has a tropical climate with significant rainfall in most months of the year with only a short dry season (Merkel, 2020; Aquastat, 2020). The average temperature in Usenge is 21.7 °C, the average annual rainfall is 1,572 mm and the driest month is January having 54 mm of precipitation (Merkel, 2020). Most precipitation averaging 253 mm falls in April, March is the warmest month with an average of 22.6 °C temperature averages (Aquastat, 2020). July has the lowest average temperature at 20.7 °C. The driest and the wettest month precipitation vary with 199 mm. There is a variation in average temperatures through the year by 1.9 °C (Merkel, 2020). Siaya County had a population of 993,183 with a population density of 398/km^2 in 2019 national census compared to 842,304 in 2009 census (KNBS, 2019).

Dunga (Kisumu County) coordinates are 0.1411° S; 34.7368° E with a tropical climate. It has a significant amount of rainfall even for the driest month. The average temperature in Kisumu is 22.9 °C (Merkel, 2020; Aquastat, 2020). The rainfall averages 1321 mm with the driest month being January. There is 62 mm of precipitation in January (Aquastat, 2020). The average of 228 mm, with the highest precipitation being in April. The precipitation varies by 166 mm between the wettest month and driest month (Aquastat, 2020; Merkel, 2020). Through the year, the average temperatures vary by 1.9 °C. The driest month is July having 14 mm of rainfall. At 1,131 m (3,711 ft.) elevation, Kisumu county population was at over 1,155,574 as of 2019 national census of county by county in Kenya (KNBS, 2019).

The latitudes and longitudes for the Nairobi sampling sites are Toi Market 1.3034° S, 36.7807° E, Gikomba Market 1.2856° S, 36.8412° E, Karen Shoppng Centre 1.3169° S, 36.6903° E and CBD, -1.334722; Longitude: 36.893889. Nairobi is the capital city of Kenya and is 695 km^2 in area with a population at over 4,397,073 and a population density of 6300/km^2 as of 2019 national census of county by county in Kenya up from population 3,138,369 and a population density of 4515/km^2 according to 2009 census. The elevation is 1,661 m (Merkel, 2020; Aquastat, 2020).

3.2 Sample Collection

Sampling of sun-dried *Omena* employed a combination of systematic sampling and cluster following the USDA Aflatoxin sampling procedure (Whitaker *et al.*, 2010) and Codex Sampling plan for Aflatoxin Samples (Codex, 1995) in which the consignment or batch held by the fisherman or trader or vendors in sacks,basins,baskets etc was considered as the lot. From the lot, incremental samples were picked. Several incremental samples whose number and size of varied with lot size were taken to make up 2 kilogram of aggregate sample (the combination of the incremental samples from the lot). The incremental samples varies from a minimum of 10 and to a maximum of 100 (Codex, 1995) see Table 3.1 below. For this study the lot sizes were less than 1 (one) tonne hence 10 incremental samples were taken.

Table 3. 1 Lot weight and number of incremental sizes

Lot weight tonnes – (Tonnes)	No. of incremental samples
Tonnes≤ 1	10.00 Samples
1 < Tonnes ≤ 5	40.00 Samples
5< Tonnes ≤ 10	60.00 Samples
10<Tonnes < 15	80.00 Samples

Table 3.1 is adapted from (Codex, 1995). The Omena lots were traded in individual packages, and according to Codex ,1995 the number of packages from which the incremental samples were taken from or the sampling frequency (SF weight of lot (LT) weight of incremental samples (IS), weight (AS) aggregate sample (IP) individual packing weight follows:

SF=(LT x IS) / (AS x IP). ……………………………….Equation (1)

(Equation adapted from Codex, 1995)

The frequency of sampling (SF) or the number of sampled packages. All the weights are in kg. Polypropylene sacks were the most used packaging, but the weights ranged between 85 kgs – 110 kgs depending on who is packing (Kariuki, 2011). Taking an average of 100 kgs for the 18

sacks sampled per site per season for the wet and dry seasons (LT=1800 kgs). The incremental sample weight (IS) averaged 0.2kg while aggregate sample (AS) picked was 2 kgs with an average number of packages (SF) from which the samples were taken from being 10.

Rearranging the Equation (1) above gives

IP=(LT x IS) / (AS x SF) ……………………….. Equation (2)

Substitung the values results in

IP=(1800 kgs x 0.2 kg) / (2 kgs x 10) = 18 samples per site per season

For 2 seasons across 7 sites

=18 x 2 x 7

=252 samples

The aggregate sample was as large like the laboratory sample.Each and every Omena fish had an equal chance of being picked. Mycotoxins are unevenly distributed hence high toxin concentrations could be in "hot spots" or "pockets" in bulk storage of items or else in a single commodity or in a group of commodities (Whitaker *et al.,* 2010).

Using the above process two hundred and fifty two Omena (252) samples of approximately 2kgs were collected randomly from major landing sites in Siaya Kisumu,Migori and Nairobi outlets. The samples were collected over the dry and wet seasons between April and November 2016 from each bag of consenting sellers. Two 1 kg (2 kg aggregate sample) samples of Omena from each fisherman or trader or vendor were picked after thoroughly mixing the upper and lower layers at various angles of the vendor's selling bags/containers. The samples were double packaged and sealed in sterile khaki bags to avoid cross contamination and moisture entry as described by Njapau e*t al*. (2008). The packages were labeled, sources recorded then the samples were transported in the khaki absorbent paper bags in carton boxes to the National Public Health Laboratory (NPHL) for storage, while kept away in dark environment because aflatoxins are light-sensitive and also kept away from dampness and at room temperature to prevent growth of fungi before analysis. Adopting the USDA Aflatoxin sampling procedure for aflatoxin where the lot is sampled then incremental samples were combined to make the

aggregate samples (Whitaker *et al.,* 2010). In each sampling site, 36 samples were collected over two seasons (18 each). Groups of six samples from same sampling site were mixed together forming 3 composite samples for each season in each location. The total composite samples size of 42 composite samples as recommended for research which is experimental (Kasomo, 2006). One kilogram of the sub sample was finely ground using 7 speeds Black and Decker Blender 120V, 60Hz, 600W and thoroughly mixed before aflatoxin, moisture, calcium and iron content analysis were done. The comminuted laboratory sample test portion was randomly picked,weighed before extraction of the aflatoxin was carried out.

3.3 Assessment of public awareness on aflatoxin contamination in Omena fish

Field survey data was by carrying out a face-to-face interview with the aid of a structured questionnaire (see Appendix IV) that had open and close ended questions. A total of 93 respondents which is 36.9% of the targeted population of 252 total samples were interviewed which agrees with Mugenda & Mugenda (2003) who recommended that a sample size of 10-50% is acceptable.The respondents comprised of traders, fishermen, boat owners BMU officials were interviewed across the study areas. Peer-reviewing and pilot-testing of questionnaire was done on twenty fish handlers across Nairobi and Kisumu County (not part of the sample), 2 weeks prior to start of the study thereafter slight changes were effected in expressing certain questions to ensure that meanings were unambiguous. The questionnaire (Appendix III) was tailored to accommodate the practices, understanding, demographics, and the perceived health hazard of food by fungal contaminated and mycotoxins amongst fishermen, boat owners, BMU officials and food sellers. The respondents were taken through the objective of the study and their verbal consent was sought through a consent form which was standardized. A total of 93 respondents took part in the study in their own accord. For maximum data collection, certain exploring interactive sessions out of the formal data collection sessions were done. The information collected in the questionnaire traversed demographics, mode of storage, duration of drying, how fish is deemed to be dry enough, fish handling practices, attitudes and awareness of respondents on aflatoxins contamination this was supported by field observation to verify information on the fish handling and preservation process.

3.4 Determination and Quantification of aflatoxin and moisture levels in Omena samples

3.4.1 Materials and Equipments

The analytical equipment used included High Performance Liquid Chromatography (UFLC Prominence Shimadzu), Atomic Absorption Spectrophotometer (AA-7000 Shimadzu), Dry Heat Sterilizers/Ovens (FN 400 from Nüve Sanayi Malzemeleri), Fluorescent detector SPD RF-20A (XS), Phenomenex Luna 5u C18 (2) 100A 250 X 4.6 mm column, Black and Decker Blender 7 speeds 120V, 60Hz, 600W and Immuno-affinity Columns (IAC)-Aflatest Vicam.

3.4.2 Quantification of aflatoxin levels in Omena samples

Sample preparation was done by, properly grinding, mixing and sub-sampling, and extraction carried out to get the aflatoxin out of the multiplex mix of substances; purification was then carried out to do away with interferents then lastly detection and quantification by employing HPLC (AOAC, 2000). The prepared samples were analysed individually by High Performance Liquid Chromatography for determination of aflatoxin levels by validated methods according to AOAC (2000).

3.4.2.1 Sample extraction

The Omena samples were thoroughly mixed then grinding done by use of 7 speeds Black and Decker Blender 120V, 60Hz, 600W. The ground sample was well homogenised and 50 g sample weighed into a blender jar then 200 millilitres of Water: Acetonitrile (1:9 v/v) added to the jar, incubated in a dark room while maintaining ambient temperature for 5 minutes. It was then blended for 2 minutes and the filtratrion of the extract done through Whatman No. 1 filter paper into a clean vessel.

3.4.2.2 Sample Cleanup for aflatoxin detection

A micropipette was used to add 8 mL of the filtered extract into a syringe then completely passed through the Immuno-affinity Column (IAC) (Scott and Trucksess, 1997) using Aflatest aflatoxin testing system from Vicam, USA at a speed of 1drop/sec till air passed through the column. The aflatoxin binds to the antibody in the column. Five millilitres of purified water was poured

through the column at 2 drops/second to rinse off impurities. This was repeated once more air passed trough the column**.** A glass cuvette was put beneath the Immuno-affinity column then 1 mL of HPLC grade methanol added into the glass syringe barrel. The retained aflatoxins eluting in the sequence G2, G1, B2 and B1, were eluted at a rate of 1drop / second by dripping the methanol in the column and collecting all of elute in a glass cuvette**.**

3.4.2.3 Sample Concentration and Derivatization

One milliliter of the sample elute was pipetted into a cuvette then concentrated by use of a gentle stream of Nitrogen gas to accelerate evaporation up to complete dryness by decreasing the partial vapor pressure of the solvent just above the surface of the liquid.The concentration was done at Persistent Organic Pollutants (POPs) Laboratory, Chemistry Department, Chiromo Campus. The derivatizer, 0.2 mL Trifluoroacetic acid (TFA) was added then taken in a dark room kept within ambient temperature lasting 15 minutes then 0.8 mL of Water / Acetonitrile (1:9 v/v) added and the derivatised solution collected through a syringe barrel by which it was then transferred into an HPLC vial while passing it through 0.45 μm microfibre filter then loaded into the HPLC for determination.

3.4.2.4 Characterisation and Quantification of the aflatoxin strains

The determination and characterization of aflatoxin strains G2, G1, B2 and B1 was carried out using HPLC (UFLC Prominence from Shimadzu Corporation Japan) with fluorescent detector SPD RF-20A (XS), at 365 nm and 450 nm wavelengths for both excitation then emission respectively. Phenomenex Luna 5u C18 (2) 100A 250 X 4.6 mm column was used and the mobile phase composed of Water : Methanol : Acetonitrile = 6 / 3 / 1 (v:v:v). The Mobile Phase pumped constantly at a rate of flow at 0.8 millitre permin. Twenty micro liter of sample reconstituted was injected into the HPLC at RF conventional cell temperature of 25 °C to attain optimal aflatoxins resolution , while the column temperature was 40 °C. Blanks samples (only methanol) and standards of aflatoxin solution were also injected. Sample assessments was done in triplicates with the sample viewed as positive for individual aflatoxin G2, G1, B2, B1, if the peak and retention time relate to the one for the standard. Calculations to arrive at the concentrations of aflatoxin G2, G1, B2 and B1 in the tested samples were done automatically.

3.4.2.5 Quality and HPLC Standards Preparation

Control Standards for Aflatoxin G2 B1, G1 and B2 were purchased from Sigma Aldrich St. Louis, USA then stored at -20°C. Careful handling measures and safety precautions were applied when preparing the aflatoxin standards due to their carcinogenicity. Verification of the aflatoxin levels and purity of standard solutions was done as per AOAC (2000) then mixed (B1, B2, G1, G2) working standards solutions were prepared by diluting 1000 µg/kg individual stock solutions with methanol to obtain solutions at 0, 0.2, 0.4, 1.0, 2.5, 5.0, 10.0 and 20 µg/kg concentrations.

All standard solutions were maintained in amber bottles to protect them from light and stored in refrigerator and equilibrated to room temperature before use. Evaporation was carried out by a gentle stream of nitrogen gas to complete dryness after which 0.2 mL Trifluoroacetic acid (TFA) was added and allowed to stand in the dark at room temperature for 15 min. ,0.8 mL of water / acetonitrile = 9/1 (v/v) was then added. The solution was then transferred to HPLC vial while passing it through 0.45 µm microfibre filter using a syringe barrel. The levels of aflatoxin were detected by HPLC through different peak areas at different retention times. The results obtained were used to draw standard calibration curves, from which trend lines with linear equations were obtained. Chromatograms of Aflatoxins analysis are shown in Figures 3.2-3.4.

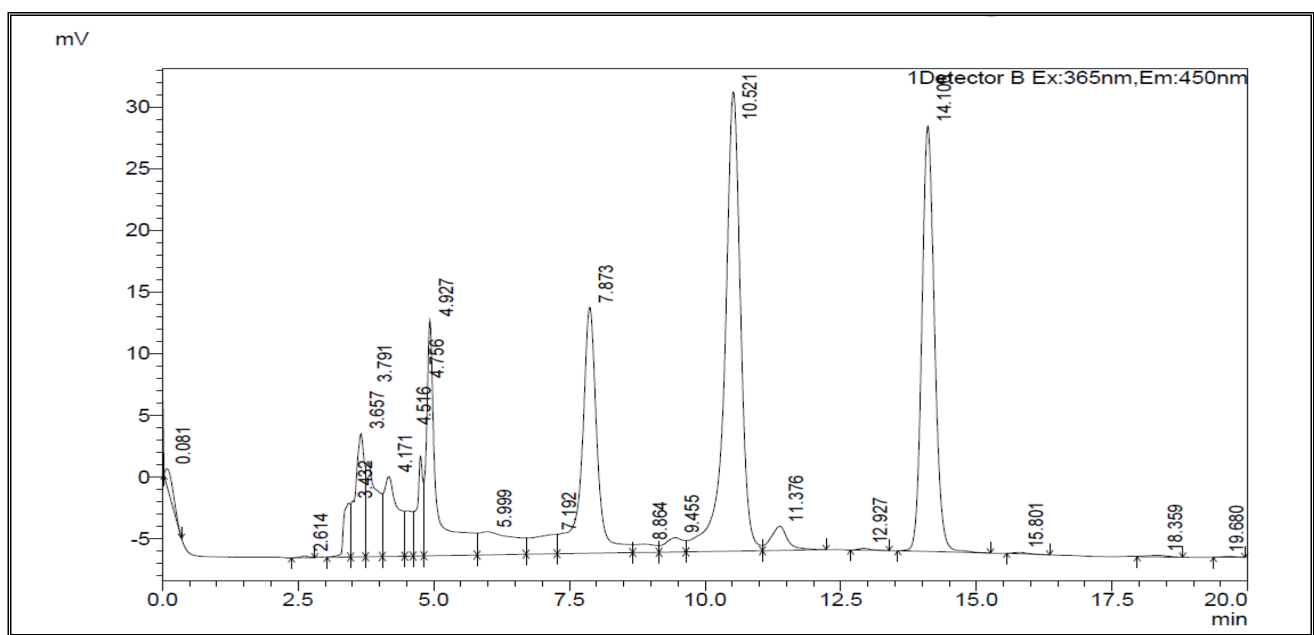

Figure 3. 2 Chromatograms of Aflatoxins analysis

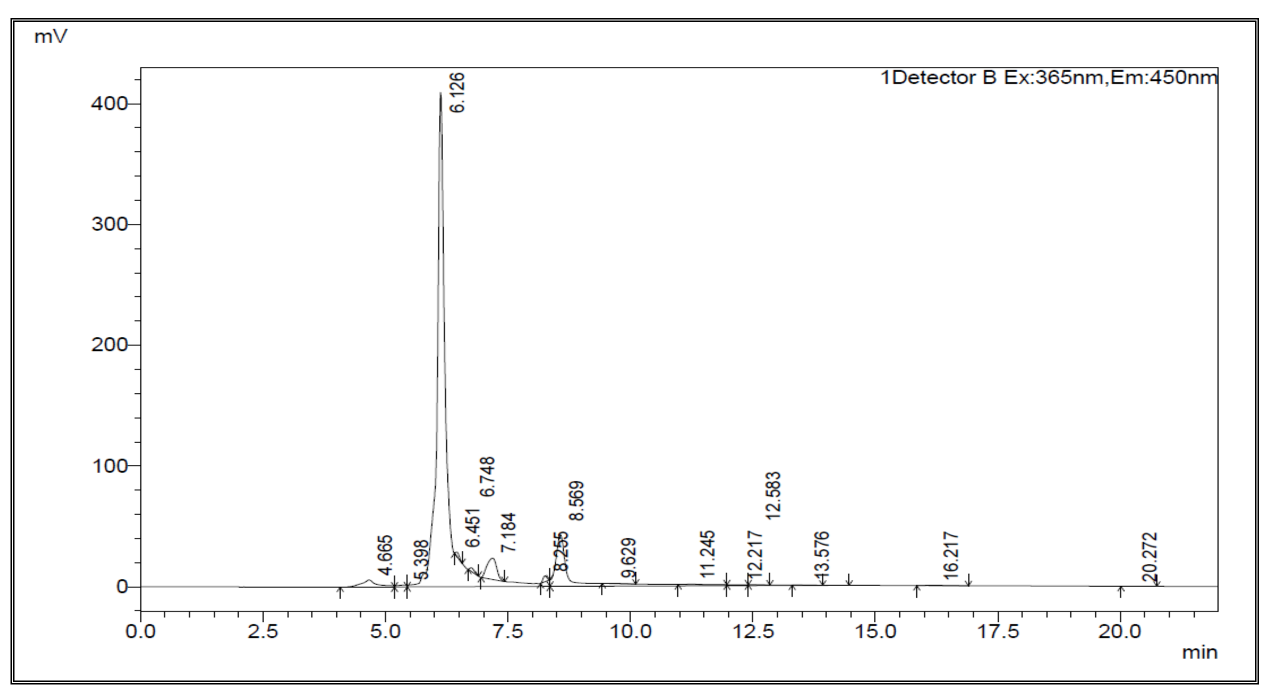

Figure 3.3 Chromatograms of Aflatoxins analysis

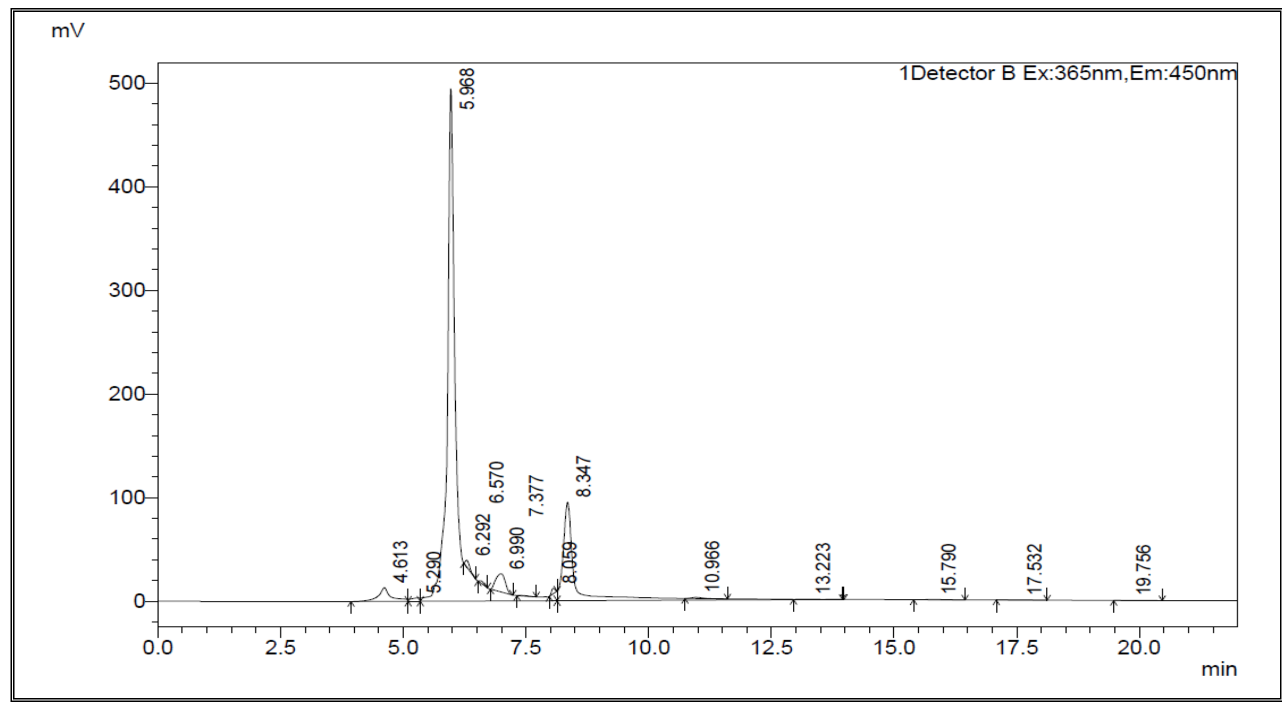

Figure 3.2 Chromatograms of Aflatoxins analysis

3.4.2.6 Calculation of results

Calibration curves of the aflatoxin strains that were determined in this study are given in Appendix II. Calibration curves with good R^2 regression values were derived from the peak areas versus the retention times for various known concentrations of the control standards. The linear equations obtained were then used to calculate the concentrations of analysed samples after obtaining respective aflatoxin peaks' area. Calibration Curves of Aflatoxins B1, B2, G1, G2 are shown in Figures 3.5-3.8.

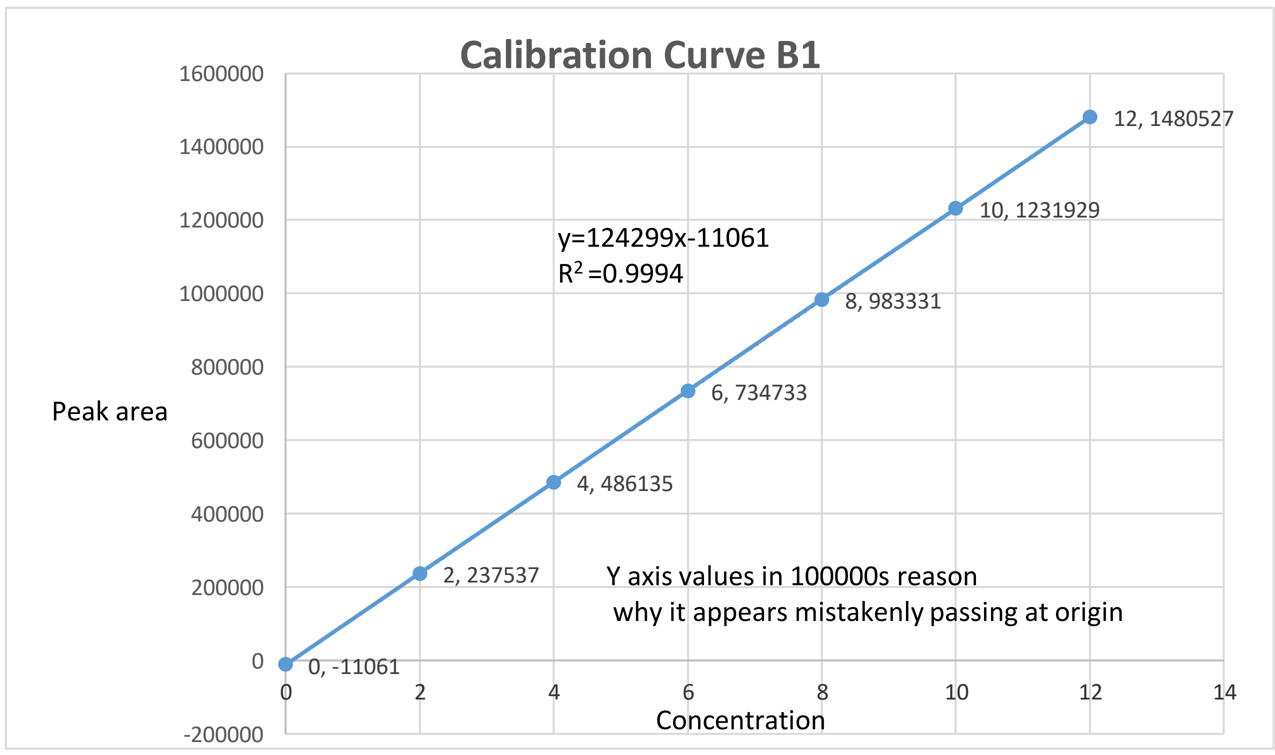

Figure 3.5 Calibration Curve of Aflatoxin B1

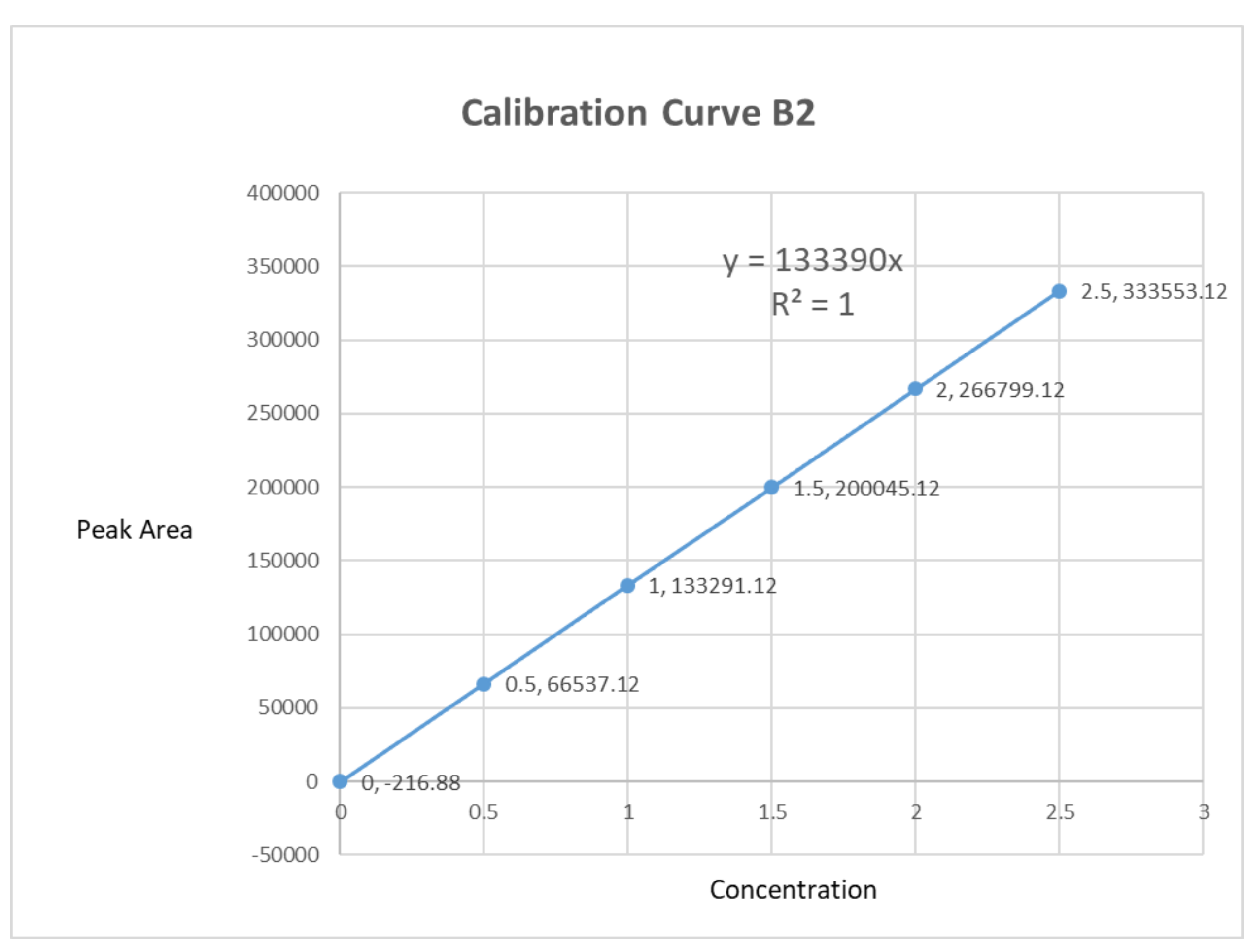

Figure 3.6 Calibration Curve of Aflatoxin B2

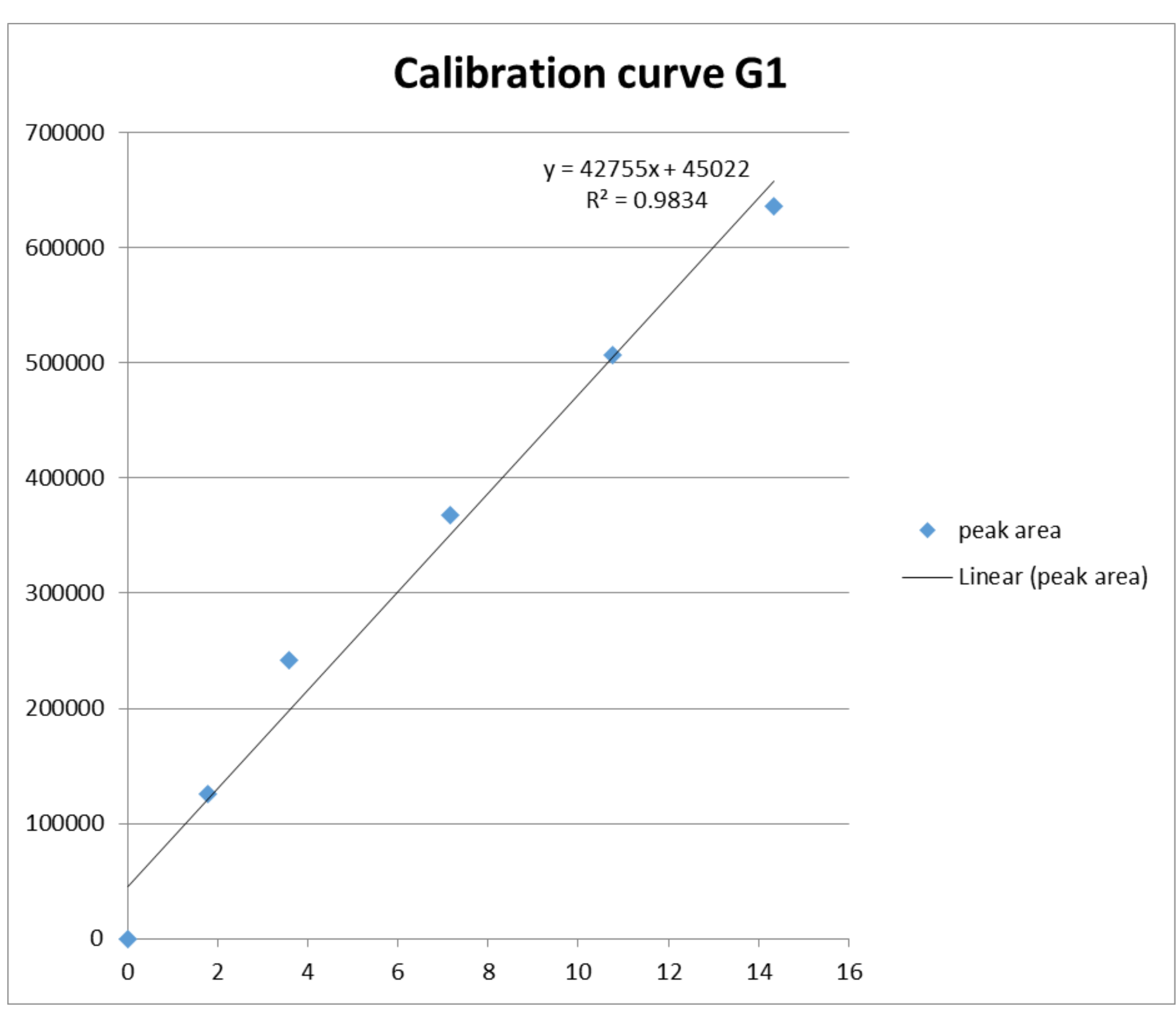

Figure 3.7 Calibration Curve of Aflatoxin G1

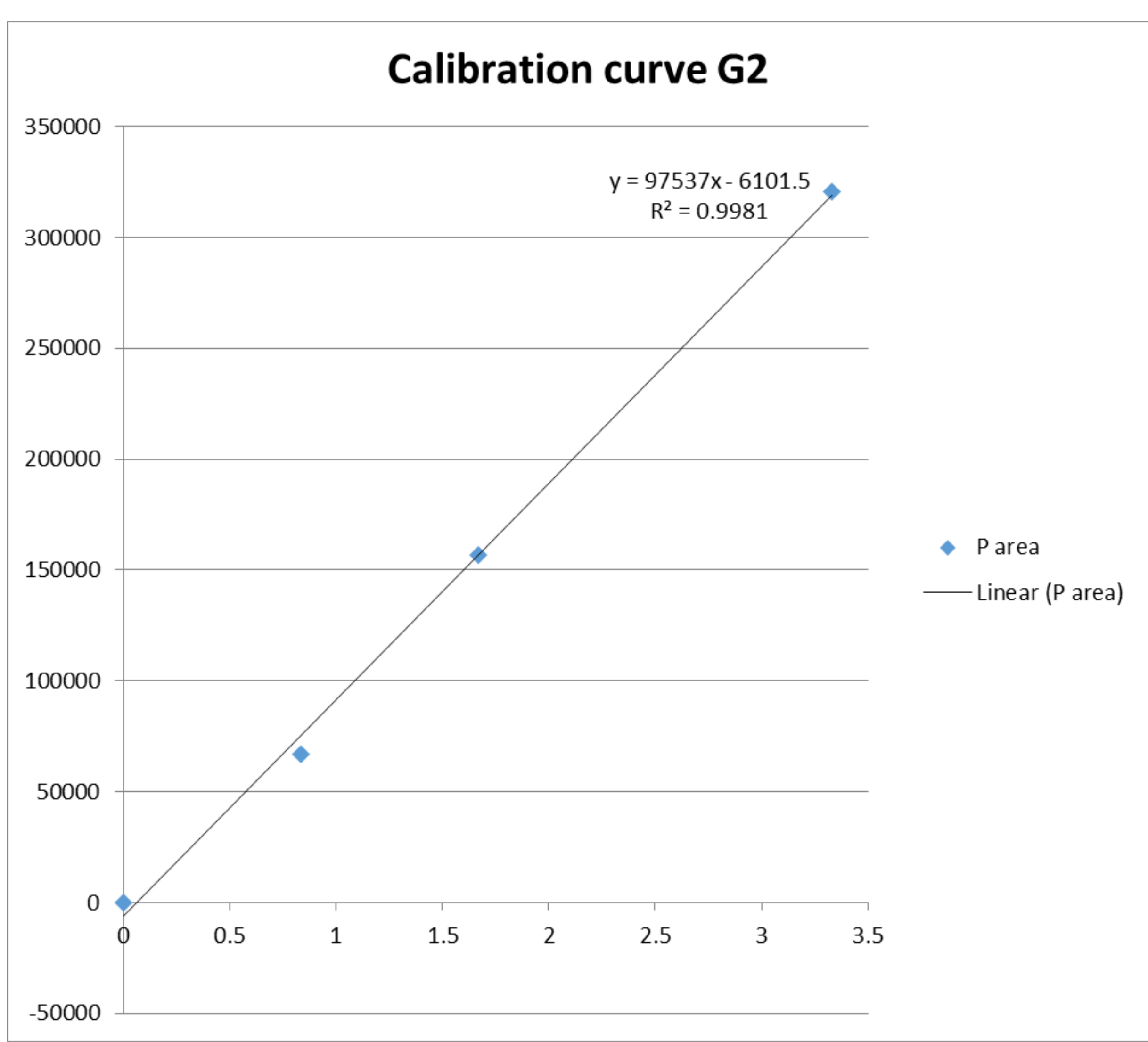

Figure 3.8 Calibration Curve of Aflatoxin G2

3.4.2.7 Quality Control and quality assurance

Analysis of Omena were done in triplicate samples to verify the presence of the analytes. Purchased standards, blank samples, spiked samples, purchased quality control samples, proficiency testing samples with known assigned values were included during sample extraction, cleanup and quantification to verify the performance of the method. All standard solutions were kept in amber bottles for protection from light and stored in refrigerator and equilibrated at room temperature before use. During sampling the *Omena* were scooped from various positions of the vessel to maximize on uniformity and while using ordinary vendor tools like cups, tins, bowls or

bare hands then double packaged and sealed in sterile khaki bags to avoid cross contamination and moisture entry as described by Njapau e*t al*. (2008). The samples were transported in the khaki absorbent paper bags in carton boxes to the National Public Health Laboratory (NPHL) for storage, while stored away in the dark condition since aflatoxins are light-sensitive and kept far away from damp conditions and at ordinary room temperature for prevention of fungal growth before testing.

A questionnaire was administered in a free environment and respondents were allowed to express themselves freely and a in a language they were comfortable in.

3.4.3 Determination of moisture content in the Omena samples

Empty aluminum dishes with the lid on were dried on FN 400 Dry Heat Sterilizers/Ovens from Nüve Sanayi Malzemeleri Turkey at 130 °C for I hour and cooled in the desiccators at room temperature for 15 minutes. The dishes with the lids on were labelled, weighed and weights recorded. The lid was removed and the ground sun-dried *Rastrineobola argentea* homogenized and 2 grams of the prepared Omena sample added to the dish well dispersed at the dish base after which it is covered with its specific lid then weighed (W1). The samples in the dish, partly enclosed by lid were dried in the oven at a 130 °C until an attainment of constant weight (Ocloo *et al*., 2011). Then tightened cover, cooled in the desiccators for 15 minutes then weighed quickly (W2). The sample end weight was subtracted from the original weight with the loss in weight being given expressed as a percentage (Ocloo *et al*., 2011) as per the following equation 3 (AOAC, 2000; Nielsen, 2010).

$$\text{Percentage moisture} = \frac{\text{Sample initial Weight (W1)} - \text{Weight of the sample after drying (W2)} \text{ X } 100\%}{\text{Initial weight of the sample (W1).}} \qquad \text{Equation (3)}$$

In Equation (3) W1 represents initial weight of the sample and W2 the sample's weight after drying.

3.4.4 Determination of proximate Calcium content in Omena samples

The Omena samples collected as earlier described in section 3.2 were ground by blending in 7 speeds Black and Decker Blender 120V, 60Hz, 600W. Approximately 3.8 grams of the homogenized sample placed in a crucible and dried in a furnace at 105 ^oC for 5 hours, charred until it ceased to smoke. This was then ashed in the furnace at 550 ^oC till whitish/greyish ash was obtained and 3 drops of nitric acid were added to achieve better ashing. The ash was treated with concentrated hydrochloric acid then transferred to a volumetric flask then topped up to 50 mL. The crucible was cleaned severally with 1N HNO_3 for full ash removal then filtered using Whatman filter paper No.1.It was diluted to the mark with 1N HNO_3. Tongs were used to handle crucibles.

An aliquot for each ash solution was used for calcium determination by employing AA-7000 Atomic Absorption Spectrophotometer (AAS) (Shimadzu Corporation Japan,2016) with an air-acetylene flame of 0.09 MPa and wavelength set to 248.3 nm and line search of 248.45 nm by using appropriate calcium hollow cathode lamp. Commercial calcium standard (AAS grade) was used to prepare the standard curves (within the analytical range) with appropriate dilutions to achieve concentrations of 0.0, 2.0, 4.0, 6.0 and 10.0 mg/kg (Tee *et al.*, 1989).

3.4.5 Determination of proximate Iron content in the Omena sample

The samples were ground by blending in 7 speed Black and Decker Blender 120V, 60Hz, 600W and 3.8 grams placed in a crucible then charred in a furnace at 105 ^oC for 5 hours until smoking ceased. Ashing was then done in the furnace at 525 ^oC for 4 hours till whitish/greyish ash was obtained. The furnace was turned off and the temperature allowed to drop to 250 ^oC. The crucible was removed and cooled. Five milliliter 1N Nitric acid was added to dissolve the ash and poured in a volumetric flask (50 mL). Washing was done to the crucible several times with 1N HNO_3 to warrant complete ash removal and filtered using Whatman filter paper. It was topped up to 50 mL mark with 1N HNO_3 (Hernandez *et al.,* 2004). An aliquot for each ash solution was used for iron determination by employing AA-7000 Atomic Absorption Spectrophotometer (AAS) from Shimadzu Corporation Japan. With an air-acetylene flame of 0.09 MPa and wavelength set to 248.3 nm and line search of 248.45 nm and using appropriate

iron hollow cathode lamp. Commercial standard, AAS grade, from Sigma Aldrich, St. Louis, USA was used to prepare the standard curves (within the analytical range) with serial dilutions to achieve concentrations of 0.0, 2.0, 4.0, 6.0 and 8.0 mg/kg (Tee *et al*., 1989).

3.4.6 Data analysis

The data from the survey was encoded and the respondents number other than the total sample formed the percentages. Instances with multiple responses had the total sample size being used. Statistical analyses were accomplished using the Statistical Programme for Social Scientists (SPSS) 16.0 for Windows Evaluation Version 16.0. Data on aflatoxin levels, iron and calcium nutritive content and moisture content were put through statistical analysis of variance (ANOVA) and means separated using Least significant difference test (LSD). Performance of Correlation was through Pearson Correlation Coefficients in order to determine whether there are significant links among the aflatoxin levels, moisture content and nutritive elements. The analysis was carried out at 95% level of significance.

CHAPTER FOUR

4. RESULTS AND DISCUSSIONS

4.1 Public knowledge on mycotoxins and aflatoxin contamination in Omena samples

4.1.1 Socio-economic characteristics of fish processors

A total of 93 respondents were interviewed from the three Lake Victoria landing sites and 4 marketing channels in Nairobi using semi structured questionnaire that had close and open ended questions. Fish processing is mainly dominated by females across all the sampling sites with minimal participation by youth and male. Middle aged respondents within the age bracket of 31-50 years in *Omena* business had the highest percentage at 44.09 % with female business people making up 71.15 % of the total while 66.66 % of the total respondents were primary school dropouts.

Those who have had been trained on fish processing or sanitation were a meagre 23.66 % while those without any training were 76.34% impacting heavily on hygienic drying and handling. Some Omena caught is sold locally in the respective counties (Migori 69.0%, Kisumu 50%, Siaya 33.3%) while others are transported in polythene or polypropylene sacks to Nairobi, Kisumu and Mombasa Counties by vehicles or buses. A high percentage of the Omena are meant for human consumption (Migori 51.72 %, Kisumu 75.00 %, Nairobi 75.70 %, and Siaya 73.30 %) (Table 4.1).

Table 4. 1 Socio economic characteristics of Omena processors

Variable		Migori N=29	Kisumu N=12	Nairobi N=37	Siaya N=15	Mean frequency N=93
Gender	Female	68.97	58.30	97.30	60.00	71.15
	Male	31.03	41.70	2.70	40.00	28.85
Age range(years)	<30	44.83	25.00	32.43	0	30.11
	31-50	41.38	58.33	43.24	40.00	44.09
	Above 50	13.79	16.67	24.32	60.00	25.81
Educational level	Primary	79.31	50.00	56.76	80.00	66.66
	Secondary	20.69	0.00	24.32	0.00	16.13
	College	0.00	0.00	8.11	0.00	3.23
	Not attended	0.00	50.00	10.81	20.00	13.98
Training on Processing attendance	Yes	34.48	0.00	35.14	0.00	23.66
	No	65.52	100.00	64.86	100.00	76.34
Purpose for fish processing	Consumption	51.72	75.00	75.70	73.30	67.74
	Sale	48.28	25.00	24.30	26.70	32.26

There was female dominance in Omena processing at the landing sites with less participation by men and youth. This could be attributed to the perception within the community that fishing industry was an activity preserved for women. This agrees with previous studies by Medard *et al*. (2001) who found out that women predominated Lake Victoria fisheries sector with 70% to 87% of them being the fish workers involved in this fisheries sector especially artisanal fish trade. The involvement of women in fish processing is spurred by cultural, social, economic and political factors. Other possible contributing factors include low initial capital requirements, profitability, easy accessibility to fish, easy storage, divisibility, low prices of fish compared to beef and family business heritage. Climatic changes have resulted in farm yields which are insufficient to maintain families throughout the year hence alternate income source. The findings of this study agreed with Medard *et al.* (2001) that more women chose fish business especially *Omena* which is easily stored at home, easily broken into small quantities and involves less migration compared to Nile perch. Secondly, fish is most readily available resource with immediate demand (Medard *et al.,* 2001; Olufemi and Daniella, 2019).

A socio-economic study in 2006 at the Okavango Delta's fishery found that most subsistence fishermen were single parent families with females as the head and families have a high number of young children up to 60 months old with fish as the source of protein to young children (Ngwenya *et al.*, 2012). Discussions with participants in focus group showed that women fishing reached its peak in the rainy season while river banks are flooded and the women are able to fish from the flooded areas by employing hand-woven baskets, when standing in the water. When the floods subside women fishing activity reduces though it continues in using boats in main waters.

Low numbers of youth participating in *Omena* processing could be due to widespread perception that fishing is not rewarding and the resulting benefits are long term. As a result, young people tend to search for urban salaried employment than fish processing. High participation of middle aged group (31-40 years) in fish processing in this study agrees with Olufemi (2019) who found out that the middle age group persons were active in taking up of effective measures in agriculture including fishing in both Sierra Leone's Goderich (37%) and Tombo (39%). This current study and the Sierra Leone's both had almost similar percentages of respondents' educational qualification as primary school was 66.6% agreeing with 58% in Goderich and secondary 16.13% matched the 17% in Tombo (Olufemi, 2019). This study also agrees with KMFRI (2019) which revealed that most of the fish traders are female in conformity to primary knowledge on typical small-scale artisanal fisheries in which fishing is dominated by men while women dominate fish trade and post-harvest activities.

4.1.2 Processing and drying methods of Omena

Fishing of Omena is done using nets, lamps according to 51.61% of respondents with highest catch being during rainy season at 32.26%. Drying of the fish by spreading on fishing or mosquito nets is purely by sunshine for an average of 1/2-2 days depending on the prevailing whether conditions. Omena is deemed dry enough by touching (65.59%), not wet/no flies on it (7.53%), has no smell (6.45%) or by eating to confirm (4.3%) or sweeping by broom (4.3%). These methods of which are quite subjective and might be a possible cause of poor drying ultimately leading to aflatoxin contamination. Omena drying was done on nets (38.71%), polythene/(*kapera) 47.31*% no drying was done on bare ground, a good practice which should be

encouraged. About 88.17 % of the respondents did not treat the Omena with anti-fungal increasing the chances of aflatoxin contamination.

Adherence to good storage practices are not done by wholesalers during storage of sun-dried fish products, with stores not well ventilated while pest gain access into the stores very easily. The environment in which fishes are displayed in the market is not usually hygienic forming another avenue for contamination by microbes (Figure 3.2 and Figure 3.3). Quite often, retailers parade the fish samples beside the gutter in open trays or refuse heaps (Figures 4.2 and Figures 4.3), as observed in sampling sites in Dunga, Usenge and Sori, Gikomba, Toi this also encourages fungal attack and subsequent toxins production. Customarily, Omena drying is by spreading them on bare ground possibly making it be polluted with animal waste and sand. This therefore piles it up with fungi and bacteria thus lowering the income and quality of the dried products (Table 4.2).

Figure 4.2: Unhygienic drying of Omena near a dumping area in Usenge beach

Figure 4.3: Unhygienic drying of Omena near stagnant dirty water in Usenge beach

Table 4. 2 Methods adopted for preservation and Packaging of Omena samples

Variable		Sampling sites (Frequency %)				
		Migori N=29	Kisumu N=12	Nairobi N=37	Siaya N=15	Mean frequency % N=93
How *Omena* fishing is done	Net	62.07	50.0	35.14	53.33	48.39
	Lamps/ Osram	10.34	0.00	0.00	0.00	3.22
	Others	27.59	50.00	64.86	46.67	48.39
Season with highest catch	Rainy	51.72	66.67	51.35	53.34	53.76
	Dry	13.80	0.00	18.92	13.33	13.98
	Dark nights/No moon	34.48	33.33	18.92	20.00	25.81
	No answer	0.00	0.00	10.81	13.33	6.45
How long drying is done (days)	12 hours	6.90	0.00	27.03	46.67	22.58
	1	86.21	100.00	56.76	26.67	66.67
	2	6.90	0.00	8.11	0.00	5.38
	3	0.00	0.00	8.11	0.00	3.23
	Depending on sunshine	0.00	0.00	0.00	26.67	4.30
How is detection of complete drying determined	Broom sweeping	13.79	0.00	0.00	0.00	4.30
	Touching	58.26	83.33	62.16	73.33	65.59
	Colour	0.00	16.67	0.00	0.00	2.15
	No flies seen	0.00	0.00	8.11	26.67	7.53
	Not wet	6.90	0.00	13.51	0.00	7.53
	No smell	0.00	0.00	16.22	0.00	6.45
	Less weight	6.90	0.00	0.00	0.00	2.15
	Eating	13.79	0.00	0.00	0.00	4.30
Omena drying done on	Sack	0.00	0.00	16.22	0.00	6.45
	bare ground	0.00	0.00	0.00	0.00	0.00
	Nets	93.10	75.00	0.00	0.00	38.71
	Papyrus mats	0.00	0.00	0.00	33.33	5.38
	Polythene /Kapera	0.00	25.00	83.78	66.67	47.31
	Slab	6.90	0.00	0.00	0.00	2.15
Treatment with anti-fungal	Yes	0.00	0.00	29.73	0.00	11.83
	No	100.00	100.00	70.27	100.00	88.17

Omena fish after harvesting were dried near the shores on nets on bare ground beside heaps of refuse and gutter of draining trenches and open sewage. This practice constitutes a major avenue for fungal infestation leading to production of secondary metabolites including aflatoxin. This confirms findings of Eyo (1992) who has contended that fishes are vulnerable to attack by microbes because of poor unhygienic methods of storage, processing, transportation and

handling. Sun drying was the common method of fish preservation in this study areas. The materials for sun drying and the fishes overloaded on troughs during transportation could lead to improper drying as well as temperature increase encourages fungal attack. These findings agree with Abila (2003) who asserted that the dirty clothes worn by the fishermen, the use of unhygienic fishing materials by fish handlers encourage fish fungal attack.

4.1.3 Knowledge and perception of aflatoxin contamination in Omena samples

The total respondents 69.89 % had never noticed moulds in Omena and a substantial 65.59 % are not aware of aflatoxins contamination in fish. Respondents who indicated that fish which does not dry enough are re-dried were 16.13 % and these were done on nets inside the store or mixed with *ochong'a* (fish Feed-Lake shrimps) to be ground and used as chicken feed (26.88%) or pig feed (3.23%). Still a large number of respondents, 38.71 %, answered that Omena does not refuse to dry but always dries (Table 4.3).

Table 4.3 Aflatoxin knowledge/awareness and utilization methods of mouldy

Variable		Sampling sites (Frequency %)				
		Migori N=29	Kisumu N=12	Nairobi N=37	Siaya N=15	Mean frequency % N=93
Where fish not dry enough taken	House/Store	13.79	0.00	0.00	0.00	4.30
	Net	6.90	0.00	0.00	0.00	2.15
	Re-dry	31.03	0.00	0.00	40.00	16.13
	Cattle feed	6.90	0.00	0.00	0.00	2.15
	Chicken feed	20.69	0.00	35.14	40.00	26.88
	Pig feed	0.00	0.00	0.00	20.00	3.23
	Buy less & sell all	0.00	50.00	0.00	0.00	6.45
	Always dries	20.69	50.00	64.86	0.00	38.71
Have you ever noticed moulds on *Omena*	Yes	37.93	0	29.73	26.67	26.88
	No	62.07	58.33	70.27	73.33	69.89
	No answer	0	41.67	0.00	0.00	3.23
If Yes are you aware of aflatoxins in Kenya?	Yes	37.93	0	0	0	11.83
	No	62.07	58.33	70.27	66.67	65.59
	No answer	0	41.67	29.73	33.33	22.58

The result of this study showed a wide gap in knowledge on aflatoxin within among those respondents in the study areas. Only 11.83 % could link fungi to mycotoxin contamination and

perceived related health risks. These findings agree with those of other previous studies by Ezekiel *et al*. (2013) who reported a significant number of people Nigeria were poorly informed of virulent *Aspergillus* section Flavi and AFB1 in the peanut cake. Low awareness of mycotoxins contamination in fish could be attributed to high illiteracy levels among the fish processors with only 16.13 % who had attained secondary level. This agrees with the results of Dosman *et al*. (2001) which underscored that persons endowed with higher education levels are possibly better knowledgeable and aware most food contaminants than individuals with less education as they have more access and are likely to seek for more information on food safety and related issues. Findings in Nigeria found that educational achievement is necessary for food safety awareness by the public of. For those with little or no formal education, more strength lies with this as a simply passed on competence as mycotoxin linked issues are not particularly dealt with in the curricular of any fundamental or high school within Nigeria as can apply to other states in Africa and beyond. Similarly, Adekoya *et al.* (2017) emphasized that education level correlates positively with mycotoxins awareness.

4.2 Aflatoxin and moisture content levels in Omena samples

4.2.1 Aflatoxins strains and quantities in Omena sample

The four major groups of aflatoxins namely G1, B1, B2 and G2, were determined and found to be present in the Omena samples in various quantities (Table 4.4). The maximum allowable limit is 10 μg/kg for total aflatoxin and 5 μg/kg aflatoxin B1 (Kenya Bureau of Standards KS 1470:2011).

Over the wet season, the highest G2 aflatoxins levels were in CBD (1.29 μg/kg) followed by Gikomba (1.06 μg/kg) the least was from Dunga (0.16 μg/kg). In the dry season, the highest G2 aflatoxin levels was recorded in Gikomba (1.79 μg/kg) and the lowest was in Usenge (0.56 μg/kg) (Table 4.4). Over the wet season, the highest G1 aflatoxins levels was in Usenge (0.89 μg/kg) and the highest B2 occurred at Sori (0.41 μg/kg). On the other hand, dry season, the highest G1 was in Gikomba (0.27 μg/kg). In the wet season, the highest B1aflatoxin levels was recorded in Dunga (1.20 μg/kg) with the dry seasons' highest being at Sori (0.51 μg/kg).

There was no major difference in the concentration of total aflatoxins in both the selling points and landing sites in both wet and dry seasons (Table 4.4). During the wet season, highest total aflatoxins levels were recorded in Dunga (2.48 μg/kg) closely followed by Usenge (2.10 μg/kg) and Karen Shopping Centre (1.64 μg/kg) while the lowest was recorded in Toi (0.97 μg/kg). Similarly, in the dry season, the highest total aflatoxin levels were recorded in Gikomba (2.23 μg/kg), closely followed by CBD (1.61 μg/kg), Sori (1.41 μg/kg) while the lowest were in Karen Shopping Centre (0.61 μg/kg).

Dunga, Usenge, Karen Shopping Centre and CBD had higher aflatoxin levels during the wet season than the dry season (Table 5). Sampling sites with higher aflatoxin levels during the dry season than the wet season included Sori, Gikomba and Toi. However, there was no significant difference in the total aflatoxin in wet season in the study sites (Table 4.4). During the dry season, Gikomba samples recorded significantly higher aflatoxin levels compared to other sampling sites (Table 4.4).

Table 4. 4: Total aflatoxin levels in Lake Victoria Shores & Nairobi selling points

Study sites	**Wet season**	**Dry Season**
	Aflatoxin (μg/kg)	**Aflatoxin (μg/kg)**
Dunga	2.48 ±1.04	1.28±0.40
Sori	1.16 ±0.14	1.41 ±0.47
Usenge	2.10 ±0.73	0.80±0.45
Gikomba	1.06 ±0.61	2.23±1.28
Karen Shopping Centre	1.84 ±0.70	0.61±0.06
Toi	0.97 ±0.14	1.29±0.23
CBD	2.05±1.12	1.61±0.24
P value	0.685	0.551
LSD	2.23	1.74
CV	0.357	0.403

Values aflatoxin content ± S.E respectively

Results obtained from this study showed that aflatoxins were found to be linked with *Rastrineobola argentea* sold in various markets in Nairobi County and the main *Omena* landing beaches in Lake Victoria though not in significant levels ($P<0.05$). Aflatoxin contamination of Omena samples could be attributed to the unhygienic drying and storage methods adopted by the *Omena* processors. The total aflatoxin levels varied from 0.61 µg/kg – 2.48 µg/kg in wet and dry season. The research finding concurs with earlier studies by Adebayo-Tayo *et al.* (2006) reported varying levels of aflatoxin ranging from 1.5 µg/kg – 8.1 µg/kg in smoked-dried fish stored for sale in Uyo, Akwa-Ibom State.

Although, total aflatoxins levels obtained in this study are within the accepted Kenyans permissible limits of 10 µg/kg, prolonged intake of these metabolites may constitute health hazard. Several studies have reported chronic exposure to low levels of aflatoxins are linked with immunosuppression, micronutrient deficiencies, liver cancer and malnutrition including and stunting, neurological impairment, and child mortality (Bbosa *et al.,* 2013). Chronic exposure to aflatoxin may also interfere with metabolism of micronutrients, resulting in malnutrition deficiencies in children under 5 years of age (Mupunga *et al.*, 2017). Stunting rate among children below 5 years in the Nyanza region was 26.9 percent KNBS (2010). Consumption of 0.25 ng/kg of aflatoxin contaminated food have been related with a frequency of within 3.2- 20 cancer cases/year/10^6 (Henry *et al.*, 2001).

Similarly, Bbosa *et al.* (2013) observed that the chronic effects are usually subclinical and difficult to recognize such as impaired food conversion and slower rates of growth with or without the production of an overt aflatoxin syndrome. Previous studies have shown chronic exposure to aflatoxicosis could be linked to, teratogenic effects associated with congenital malformations, mutagenic effects causing changes alteration in the genetic code of the DNA and the carcinogenic effect such as the genotoxic effect in which electrophilic carcinogens alter genes through interaction with DNA (Bbosa *et al.*, 2013). Therefore, even low levels of aflatoxin exposure as are in this study may have negative health effects on consumers.

4.2.2 Percentage moisture content in Omena samples

Percentage moisture content varied across the sampling sites over wet and dry seasons. The percentage moisture content was highest in Gikomba during the wet season at 13.33% and the highest percentage moisture content during the dry season was at Usenge at 12.89%. The lowest percentage moisture content during the wet season was in Karen Shopping Centre at 10.24% and the lowest during the dry season was in CBD at 11.48%. The percentage moisture content in all the sites were above the acceptable limits in *Omena* fish in Kenya of 5-7% as per the Kenyan standard (Kenya Bureau of Standards KS 1470:2011). Additionally, moisture content varied at the sampling sites in both wet (P=0.00151) and dry season (P<0.0001) (Table 4.5).

Table 4.5: Percentage moisture content around Lake Victoria Shores and Nairobi selling points

Sampling sites	**Wet season**	**Dry Season**
	% Moisture	**% Moisture**
Dunga	11.10 ±0.04	11.97 ± 0.40
Sori	11.19 ±0.06	11.53±0.14
Usenge	11.30 ±0.06	12.89 ±0.19
Gikomba	13.33 ±0.09	11.85 ±0.20
Karen Shopping Centre	10.24 ±0.07	11.95±0.08
Toi	13.20 ±0.60	11.93±0.25
CBD	11.60 ±0.06	11.48±0.21
LSD	0.714	0.538
CV	0.098	0.0389
P value	<0.0001	0.00151

Values percentage moisture content ± S.E respectively

The percentage moisture content in the study sites varied from 10.24 to 13.33% which is approximately two times more than the acceptable limits value in Omena fish in Kenya. This high moisture content could be attributed to insufficient drying of the samples which relies

mainly on sun drying for approximately 1-2 days. Additionally, it could be due to poor storage in sacks that do not absorb moisture.

4.3 Proximate calcium content in Omena samples

Calcium levels varied across the sampling sites in both wet and dry season. During the wet season, highest calcium levels were recorded in Usenge at 2939.75 mg/kg while the least was in CBD at 2253.40 mg/kg. During the dry season, Dunga recorded the highest calcium levels of 2663.53 mg/kg, while Sori had the least calcium values of 1873.00 mg/kg (Table 4.6).

Table 4.6: Calcium levels in Omena around Lake Victoria Shores and Nairobi selling points

Sampling sites	Wet season	Dry season
	Calcium(mg/kg)	Calcium(mg/kg)
Dunga	2582.42 ± 0.88	2663.53 ± 0.85
Sori	2844.93 ± 1.65	1874.00 ± 1.40
Usenge	2939.75 ± 0.57	2430.88 ± 0.77
Gikomba	2413.84 ± 1.73	2432 .37 ± 1.72
Karen Shopping Centre	2476.38 ± 1.17	2611.48b ± 1.30
Toi	2465.27 ± 1.28	2463.54 ± 0.88
CBD	2253.40 ± 1.17	2026.85 ± 1.57
LSD	3.84	3.832
CV	0.095	0.125
P value	<0.0001	<0.0001

Values are calcium content ± S.E respectively.

Calcium is important mineral required for bone formation while fish is known for being a good source of this mineral, especially small fish (Kawarazuka and Bene, 2011; Roos *et al.,* 2007). In this study calcium ranged from 1873 to 2939.75 mg/100 g; figures within FAO mean values of 19-881 mg/100g and comparable to Mohamed *et al.* (2010) study who reported 107- 588 mg/100g of calcium in fish and Luczynska *et al*. (2009) who obtained 53-103 mg/100 g. The

recommended daily intake of calcium for adults is 1000 - 1300 mg (Joint FAO/WHO Expert Committee on Food Additives, 2001). The findings of the current study present *Omena* fish around Lake Victoria as excellent sources of calcium. Based on these results, it can be concluded that regular consumption of Omena will provide good bone formation and maintain skeletal integrity. The high calcium content in comparison with other fresh water species is as a direct result of inclusion of skeletal muscles in the edible portion that contains calcium and iron in high amounts. This concurs with previous study by Ghelichpour and Shabanpour (2011) and Owaga *et al*. (2010) who reported high calcium contents in fish.

4.4. Proximate Iron content in Omena samples

Iron content in *Omena* fish varied across the sampling sites over the wet season and dry season (Table 4.7).

Table 4.7: Iron levels in the Omena samples for Lake Victoria and Nairobi outlets

Sampling sites	**Wet season**	**Dry season**
	Iron(mg/kg)	**Iron(mg/kg)**
Dunga	10.65 ± 0.45	15.43 ± 0.14
Sori	9.97 ± 0.01	8.05 ± 0.03
Usenge	11.55 ± 0.33	7.09± 0.03
Gikomba	7.80 ± 0.07	6.05± 0.03
Karen Shopping Centre	5.96 ± 0.21	6.55± 0.12
Toi	12.14 ±0.03	10.42± 0.3
CBD	7.32 ± 0.11	6.41± 0.22
LSD	0.697	0.480
CV	0.250	0.393
P- value	<0.0001	<0.0001

Values are iron content ± SE respectively.

During wet season the highest iron content was recorded in Toi at 12.14 mg/kg with Karen Shopping Centre recording the lowest level at 5.96 mg/kg. Similarly, during dry season, Dunga

recorded the highest iron levels at 15.43 mg/kg while Gikomba recorded the least iron content at 6.05 mg/kg (Table 4.7).

Iron is essential for a several physiological activities in the body which include circulating oxygen through the body. It prevents anaemia prevalence from all causes. Iron deficiency (with or without anemia) is widespread in African except in South Africa who consistently record the lowest figures. Elsewhere in Africa, anaemia is widespread affecting more than 45% of women and 70% of young children in Gambia, Malawi and Côte d'Ivoire, Deficiency in iron often troubles more than half of young children except in Kenya, but less so in women, except in Egypt, as prevalence remains below 18% (Abbaspour, 2014). In this study the iron concentration in Omena fish was 5.96 – 15.43 mg/100 g. The proposed intake of iron as a nutrient for adult females is between the ages of 19-50 years is 24 mg/day. Based on this work, *Omena* fish can provide up to 64.29 % of daily iron requirement for women if 100 g of fish is consumed. Most of the iron content in this study area are below the iron content reported in Bangladesh whose values were 13 mg/100 g. Study by Guerin *et al.* (2011) carried out with fish from a French market reported much lower concentration levels of Fe (0.13 - 1.9 mg/100 g). The differences in iron content in fish could be due to environmental conditions in which fish are harvested, fish diet, water quality and species studied. Hence, Omena should be major source of iron content required in the body.

4.5 Correlation between moisture, aflatoxin, calcium and iron levels

During the dry season, aflatoxin had a very weak negative correlation with calcium ($r = -0.167$; $P = 0.464$) and iron ($r = -0.053$; $P = 0.819$) and a negative weak correlation with moisture ($r = -0.229$; $P= 0.305$). This indicates that in this study when aflatoxin was high the iron, calcium, and moisture contents were low in Omena. Further, correlation analysis shows that moisture had a very weak positive correlation with iron ($r = 0.018$, $P=0.937$) and a moderate positive correlation with calcium ($r = 0.443$, $P= 0.027$). Meaning samples with high percentage moisture content levels tended to have high iron and calcium. Additionally, calcium and iron had a moderate positive correlation in both dry ($r = 0.410$, $P= 0.0446$) and wet season ($r = 0.553$; $P= 0.00254$). On the other hand, during the wet season, similar results were obtained in which aflatoxin had a very weak negative correlation with iron (-0.038; $P= 0.871$) and a weak negative correlation with

moisture (r = -0.312; P = 0.149) while in this study aflatoxin showed a very weak positive correlation with calcium (r = 0.035; P= 0.881) in addition, iron registered a weak positive correlation with moisture (r = 0.283, P= 0.196). Further, analysis showed a weak negative correlation between moisture and calcium (r = -0.309, P=0.153) (Table 4.8).

Table 4.8: Correlations matrices between variables; moisture, aflatoxin, calcium and iron content

	Dry season				Wet season			
Correlation	Aflatoxin	Moisture	Calcium	Iron	Aflatoxin	Moisture	Calcium	Iron
Aflatoxin	1	-0.229	-0.167	-0.053*	1	-0.312	0.035*	-0.037
Moisture	-0.229	1	0.443*	0.018*	-0.312	1	-0.309	0.283*
Calcium	-0.167	0.443*	1	0.410*	0.035*	-0.309	1	0.553*
Iron	-0.053	0.018*	0.410*	1	-0.037	0.283*	0.553*	1

Values are coefficients at 95% significance level; * positive correlation

The findings in this study showed a weak negative correlation association between aflatoxin and moisture and a very weak correlation between aflatoxin and iron in both dry and wet season. This concurs with the findings of Tiwari *et al*. (1986) who found out that iron decreased the aflatoxin production to different levels. The weak negative correlation between aflatoxin and percentage moisture content in both wet and dry seasons also agrees with Pesavento *et al*. (2016) who reported a negative correlation between moisture and mould content in chilli samples. However, all the Omena samples had percentage moisture content above the Kenyan national standard (Kenya Bureau of Standards KS 1470:2011) regulatory limit of 5 to 7% making them susceptible to aflatoxin attack as is shown by the aflatoxin presence in the samples proving the fact that high moisture content promotes fungal attack. Furthermore, other contributing factors to aflatoxin contamination such as temperature, humidity or storage may be a third confounding variable resulting in the negative aflatoxin moisture correlation. The negative correlation of aflatoxin and moisture in Omena merits a further research study. Figures 4.4 and 4.5 show the relationship between aflatoxin and moisture content.

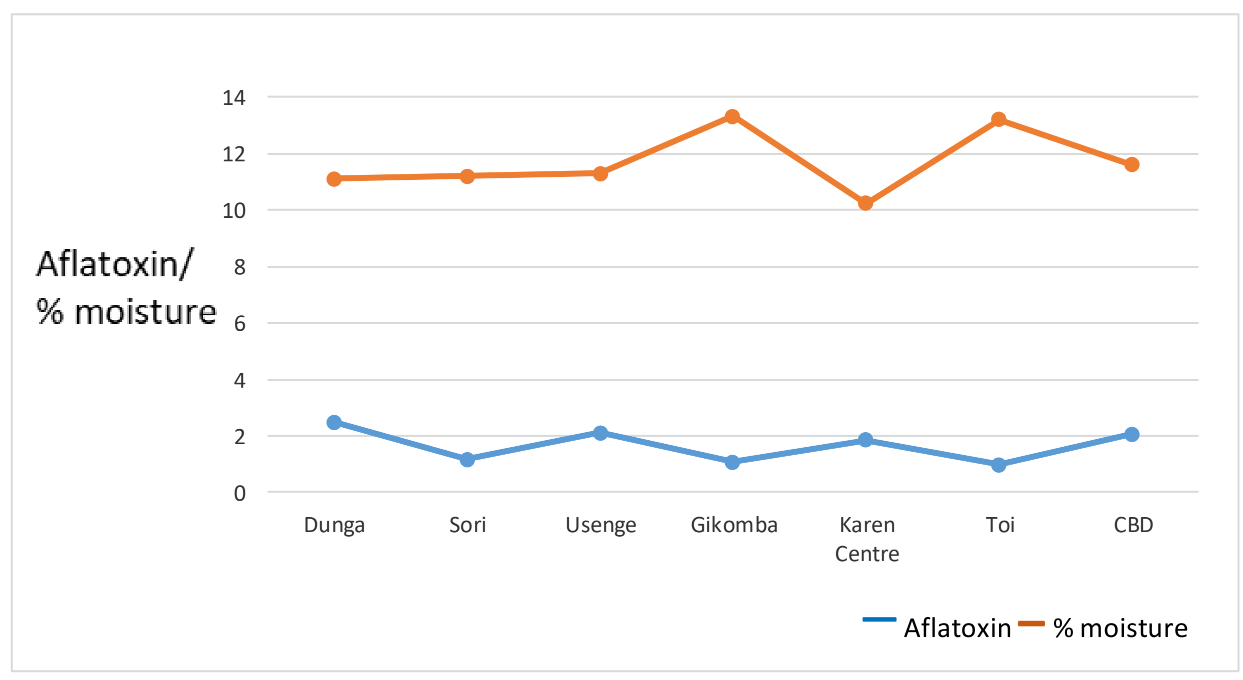

Figure 4.4 The correlation between aflatoxin and moisture content during the wet season

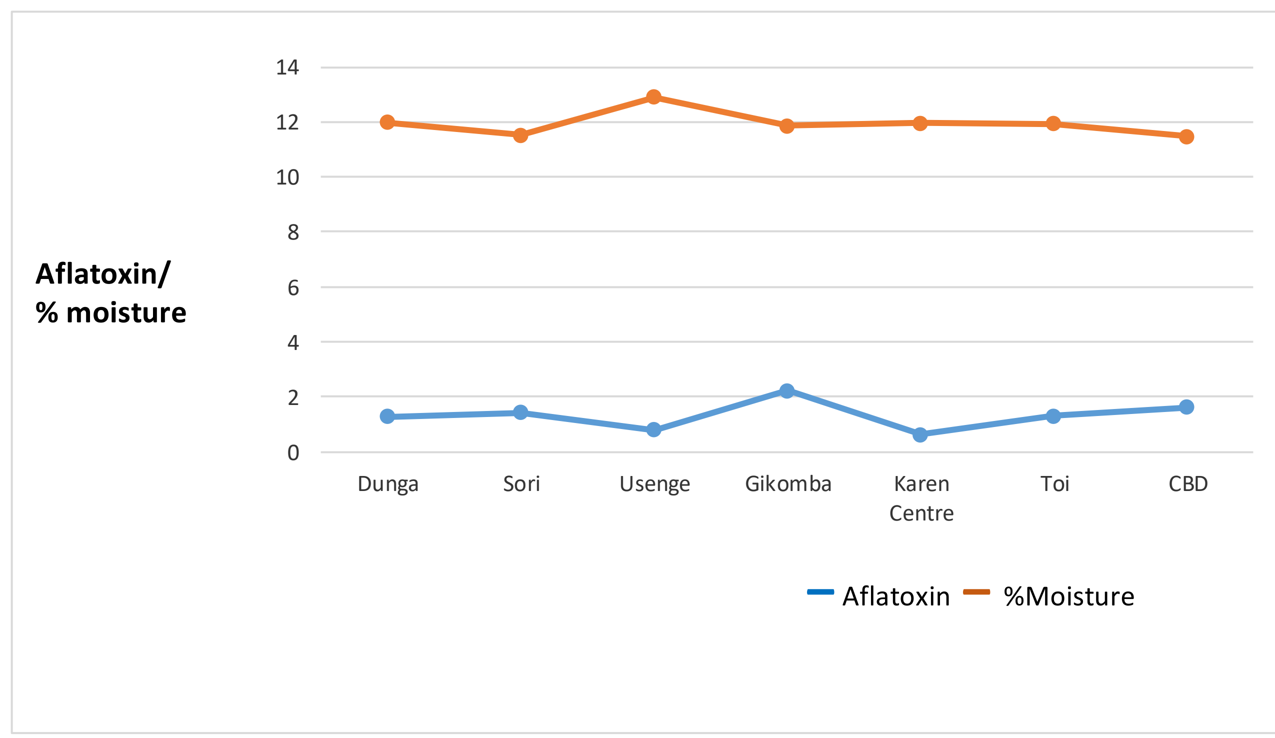

Figure 4.5 The correlation between aflatoxin and moisture content during the dry season

The findings in this study in which calcium and iron were positively correlated in both dry and wet seasons are in agreement with Reddy and Cook (1997) whose long term studies to investigate composite and varied complete diets showed that calcium does not have a significant effect on iron absorption.

CHAPTER FIVE

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The findings provide indepth analysis of the public's understanding, practices and perception regarding aflatoxins contamoination in *Omena*. The study revealed that participants along the *omena* value addition chain have not been sensitized on harmful effects linked to aflatoxin contamination and its relation with high moisture content.

The consumers of *Omena* in the selected counties of Nairobi, Kisumu, Siaya and Migori are potentially exposed to low and high levels of aflatoxin contamination. This renders the public and livestock vulnerable to aflatoxin related risks and morbidities.

Omena is rich in iron and calcium and is relatively cheap for inclusion in human dietary. The findings have contributed to science of analytical chemistry since the concerned counties will be able to mitigate aflatoxin challenges while putting into use the high calcium and iron content in omena for improved diet.

5.2 Recommendations

Taking into account the need to achieve both food safety and food security for endangered persons in the study areas and in Kenya, we make the following recommendations:

Research Recommendations

1) A study on determination of other mycotoxins in omena.
2) The correlation coefficient of aflatoxin and moisture in Omena

Policy Recommendations

1) The national and county Governments as well as non-governmental organizations should play a prominent role in raising awareness of the public health impacts of aflatoxin as well as aflatoxin identification.

2) Good hygiene practices and HACCP ought to be utilized during the processing harvesting, and distribution chain of Omena fish. Processing to be done in sanitary circumstances to take care of probable hazards linked aflatoxin contamination.
3) Traders should employ better preservation methods and better sun-drying technology and stored Omena to be ventilated well to reduce aflatoxin occurrence in these samples.
4) Routine sampling and testing of omena for aflatoxin contamination should be done.

REFERENCES

Abbaspour, N., Hurrell, R., Kelishadi, R. (2014). Review on iron and its importance for human health. *J. Res. Med. Sci.* **19** (2), 164–174.

Abila, O.R., (2003) Food Safety in Food Security and Food Trade. Case Study in Kenya Fish Exports 2010 *Vision for Food Agriculture and Environmental Focus* **10**, Brief 8 of 17. International Food Policy Research Institute (IFPRI).

Adebayo-Tayo, B.C., A.A. Onilude, C. Bukola, A. Abiodun and Ukpe, G.P. (2006) Mycoflora of Smoke Dried Fishes Sold in Uyo, Eastern Nigeria. *World Journal of Agricultural Sciences*, **4** (3):346-350.

Adejola Q.A., (2006) Presence of aflatoxins in smoked-dried fish sold in Abeokuta, Ogun State, and Southwestern Nigeria *African Journal of Food Sciences*. **4** (4): 127 – 135.

Adekoya I, Njobeh P, Obadina A et al (2017) Awareness and prevalence of mycotoxin contamination in selected nigerian fermented foods. *Toxins* (Basel) **9**:363. https://doi.org/10.3390/toxins9110363

Agag B.I. (2004) Mycotoxins in foods and feeds *Ass. Univ Bull Environ Res* **7**:173–206

Aguilo, P.L., Esperence A., Mbau, E., Palmer., Patel Amanda Sparkman, T., (2007) *Attracting Investment to Kisumu: Opportunities and Challenges*. Report prepared for Earth Institute, Millennium Cities Initiative, Columbia University.

Akande, G.R. and J.G. Tobor, (1992) Improved utilization and increased availability of fishing products as an effective control of aggravated animal protein deficiency induced malnutrition in Nigeria. *Proceedings of the 10th annual conference of the Fisheries Society of Nigeria,* pp: 18-31.

Akande G.R., Diei-Ouadi Y. (2010). Post-harvest losses in small-scale fisheries. Case studies in five sub-Saharan African countries. *FAO Fisheries and Aquaculture Technical Paper* No. **550**. Rome, Italy.

Alshannaq, A.; Yu, J.H. (2017) Occurrence, toxicity, and analysis of major mycotoxins in food. *Int. J. Environ. Res. Public Health* 2017, **14**, 632.

AQUASTAT - FAO's Global Information System on Water and Agriculture (2020) http://www.fao.org/aquastat/en/

Association of Official Analytical Chemists (AOAC), (2000). Official Methods of Analysis of

AOAC International.17th Edn, Vol. **2**, Chapter 39, 40, 44 and 47. O.M.A., Gaithersburg, Maryland, Washington, DC. USA.

Azziz-Baumgartner, E., Lindblade, K., Gieseker, K., Schurz Rogers, H., Kieszak, S., Njapau, H., Schleicher, R., McCoy, L. F., Misore, A., DeCock, K., Rubin, C., Slutsker, L. and Aflatoxin Investigation Group (2005): Case-control study of an acute aflatoxicosis outbreak, Kenya, 2004. *Environmental Health Perspectives*, **113** 1779–1783.

Atalla MM, Hassanein NM, El-Beih AA, Youssef YA (2003). Mycotoxin production in wheat grains by different *Aspergilli* in relation to different relative humidities and storage periods. *Nahrung*. 2003 Feb;**47**(1):6-10. doi: 10.1002/food.200390017. PMID: 12653428.

Bankole, S.A. and Adebanjo, T., (2003). Mycotoxins in the West Africa, current and possibilities of controlling it. *African Journal of Biotechnology,* **2**(9): 254-263.

Barrett, J. 2000. Mycotoxins: of molds and maladies. *Environ. Health Perspect*. **108**: A20-A23

Bbosa, G. S., Kitya, D., Lubega, A., Ogwal-Okeng, J., Anokbonggo, W. W., and Kyegombe, D. B. (2013). "Review of the biological and health effects of aflatoxins on body organs and body systems," in *Aflatoxins – Recent Advances and Future Prospects*, ed. M. Razzaghi-Abyaneh (Rijeka: InTech), 239–265.

Bennett J. W and Klich, M. (2003). Mycotoxins. *Clinical Microbiology Reviews*, vol. **16**, no. 3, pp. 497–516. Washington DC.

Bianchini A., Bullerman L.B., (2014) Mycotoxins Classification In Encyclopedia Of Food Microbiology (Second Edition) Science Direct.

Bille P.G. and Shemkai R.H. (2006) Process development, nutrition and sensory characteristics of spiced-smoked and sun-dried Dagaa (Rastrineobola argentea) from Lake Victoria, Tanzania *African Journal of Food, Agriculture, Nutrition and Development* Vol. **6** No. 2 (2006) / Articles. https://www.journalquality.info/jpps-criteria/one-star (assessed: 2019-03-01).

Buere CR (2005). Fish processing technologies http:www.geocities.com/fish processing/page 11 Modified23/0412005 Retrieved 25/03/2009.

Buluma M. E. (2012) A Study on the Supply, Market and challenges of Fish Selling Business in Nairobi's City Market and Gikomba Market. Research by: Buluma M. E. Presented to: Mr. Boniface Okada Buluma. Duration: 2012..

Cardwell K. F. and S.H Henry, (2004) Risk of Exposure to and Mitigation of Effect of Aflatoxin on Human Health: A West African Example. *Toxin Reviews,* **23** (2-3): 217-247.

Centre for Disease Control (CDC) (2004) "Outbreak of aflatoxin poisoning--Eastern and Central Provinces, Kenya, January-July 2004." *MMWR Morb Mortal Wkly Rep* **53** (34): 790-3

Chamberlain, T. and Titili, G. (2001). Sea Food Processing. Community Fisheries Training's marine Studies Programme, *Pacific Series* **6**. University of the South Pacific, 2001.

Chukwuka O.K., Okoli I.C., Opara M.N. Omede A.A., Ogbuewu I.P. and Iheshiulor O.O.M., (2010). The Growing Problems of Mycotoxins in Animal Feed Industry in West Africa: A Review: *Asian Journal of Poultry Science***, 4**:122-134

Cimbalo. A, Alonso-Garrido M., Font, L. Manyes G. (2020). Toxicity of Mycotoxins in Vivo on Vertebrate Organisms: A Review. *Food and Chemical Toxicology* Volume **137** 2020 Article 111161

Codex Standard 193-1995 Adopted (1995); Revised 1997, 2006, 2008, 2009; Amended 2009, 2010 Codex General Standard for Contaminants and Toxins in food and feed Codex STAN 193-1995 CXS 193e

Council for Agricultural Science and Technology (CAST) (2003). Mycotoxins – Risks in Plants, animals and human systems. *Task Force Report*, No. **139**, 1-191. Ames, Iowa.

Cressey Peter & Dr Thomson Barbara (ESR) and John Reeve (MPI) (2006) The New Zealand Mycotoxin Surveillance Program 06-14 Report Series FW0617 *Risk Profile Mycotoxin in the New Zealand Food Supply MPI Technical Report* – Paper No: **2016/21**

Daniel J.H., Lewis L.W., Redwood Y.A., Kieszak S., Breiman R.F., Flanders W.D., Bell C., Mwihia J., Ogana G., Likimani S., Straetemans M., McGeehin M.A. (2011) Comprehensive assessment of maize aflatoxin levels in Eastern Kenya, 2005-2007. *Environ Health Perspect*. 2011 Dec; **119**(12):1794-9. Doe: 10.1289/ehp.1003044. Epub 2011 Aug 15. PMID: 21843999; PMCID: PMC3261970.

Dosman Donna M., Adamowicz Wiktor and Hrudey Steve E. (2001) Socioeconomic Determinants of Health and Food Safety-Related Risk Perceptions May 2001 *Risk Analysis* **21**(2):307-17 Source PubMed

Dvoráčková, I., & Pichova, V. (1986). Pulmonary interstitial fibrosis with evidence of aflatoxin

B1 in lung tissue. *J Toxicol Environ Health*.1986; **18** (1):153-7: 10.1080/15287398609530856.PMID:3084802

Eaton D.L. and Groopman J. D. (1994). The Toxicology of Aflatoxins Human health, veterinary health and agricultural significance. *Elsevier* 1994

Essien J.P. Ekpo M.A. Akan B. (2006) Mycotoxigenic and proteolytic potential of moulds associated with smoked shark fish (*Chlamydoselachus anguincus*) November 2006 *Journal of Applied Sciences and Environmental Management* **9**(3)
Follow journal DOI: 10.4314/jasem.v9i3.17352

Eyo, A.A., (1992) Traditional and improved fish handling, preservation and processing techniques, NAERLS/NIFER national workshop on fish processing, storage, marketing and utilization, pp: 15

Ezekiel N. Chibundu, Sulyok Michael, Babalola D.A. Warth,Benedikt Ezekiel,V Krska,Rudolf (2013) Incidence and consumer awareness of toxigenic Aspergillus section Flavi and aflatoxin B1 in peanut cake from Nigeria Article in *Food Control* **30** (2).596-601.

FAO, (2016) *the State of World Fisheries and Aquaculture*, Springer, Rome, Italy.

FAO. (2018). The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals. Rome. Licence: CC BY-NC-SA 3.0 IGO.

FAO. (2020). The State of World Fisheries and Aquaculture 2020. Sustainability in action. Rome. ISBN 978-92-5-132692-3 https://doi.org/10.4060/ca9229en

Farm Africa (2016) Market Study of the Aquaculture Market in Kenya: Kenya Market-Led Aquaculture Programme, *Springer*, Nairobi, Kenya 2016.

FDA (Food and Drug Administration) (2000) Guidance for Industry: Action Levels for Poisonous or Deleterious Substances in Human Food and Animal Feed. 2000. <http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/ChemicalContaminantsandPesticides/ucm077969.htm#afla> Last updated 11.09.11.

Fisheries Annual Report, (2010) Department of Fisheries, Ministry of Livestock and Fisheries. Kenya 2010.

Food and Agricultural Organization (FAO) (2004) Drought in Kenya: Food Situation Is Deteriorating Rapidly - First Starvation-Related Deaths. 2004.

Food and Agriculture Organization (FAO) (2015). Fisheries Division, Fishery and Aquaculture country profile: The Republic of Kenya 2015. *Country brief Prepared*: February 2015 Content provided by FAO.

Ghelichpour M. and Shabanpour B. (2011). The investigation of proximate composition and protein solubility in processed mullet fillets October 2011 *International Food Research Journal* **18**(4):1343

Gitu K. W. (2004) Agricultural Development and Food Security in Kenya: Building a Case for More Support.2004.

Godwin, H.T., (2006) Determination of Moisture and Total Solids. USDA National Nutrient Data Bases for Standard Foods. (www.people. umass.edu/ mcclemen/581 moisturehtml). Accessed on 13th October, 2012.

Gong Y.Y, Turner P.C., Hall A.J. Wild C.P., Leslie J.F., Bandyopadhyay R., Visconti A. (2008) Aflatoxin exposure and impaired child growth in West Africa: an unexplored international public health burden, Mycotoxins. Detection Methods, Management, Public Health and Agricultural Trade, 2008 Wallingford, Oxfordshire, UK CAB *International* (pg. 53-65).

Guerin T., Chekri R., Vastel C., Sirot V., Volatier J.L., Leblanc J.C., Noel L. (2011). Determination of 20 trace elements in fish and other seafood from the French market. *Food Chem.* **127**:934-942

Hassan E. M. Farag, Azza A. El-Tabiy and Heba M. Hassan, (2011) Assessment of Ochratoxin A and Aflatoxin B1 Levels in the Smoked Fish with Special Reference to the Moisture and Sodium Chloride Content. *Research Journal of Microbiology*, **6**: 813-825.

Hendrickse, R. G., (1997) of sick turkeys, kwashiorkor, malaria, perinatal mortality, heroin Addicts and food poisoning: research on the influence of aflatoxins on child health in the Tropics. *Ann. Trop. Med. Parasitol*. **91**:787-793.

Henry S.H, Whitaker T, Rabbani I, Bowers J, Park D, Price W, Bosch FX, Pennington J, Verger P, Yoshizawa T, van Egmond H, Jonker MA and R Coker, (2001) Aflatoxin M1. *JECFA* **47**. Food and Drug Administration, Washington DC, USA.

Hernandez O.M., Fraga J.M.G., Jimenez A.I., Jimenez F. & Arias J.J. (2004). Characterization of

Honey from the Canary Islands: determination of the mineral content by atomic absorption spectrophotometer. *Food Chemistry* **93** (3): 449–458. Universidad de LaLaguna, E-38071 La Laguna, Tenerife, Spain.

Herzallah S. M., (2009) "Determination of aflatoxins in eggs, milk, meat and meat products using HPLC fluorescent and UV detectors," *Food Chemistry*, *vol*. **114**, no. 3, pp. 1141–1146, 2009.

Heshmati, A Sadati, R. Ghavami, M. and Mousavi Khaneghah, A. (2019). The concentration of potentially toxic elements (PTEs) in muscle tissue of farmed Iranian rainbow trout (*Oncorhynchus mykiss*), feed, and water samples collected from the west of Iran: a risk assessment study. *Environmental Science and Pollution Research*, vol. **26**, no. 33, pp. 34584–34593.

Horn, B.W. and J.W. Domer, (2002). Effect of competition and adverse culture conditions on aflatoxin-production by Aspergillus flavus through successive generations. *Mycologia*, **94:** 741-751.

International Agency for Research on Cancer (IARC) (1993). Ochratoxin A. In: IARC *Monographs on the Evaluation of Carcinogenic Risks to Humans*, Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins. Vol. **56.** Lyon: IARC Press; 1993. p. 489-521.

International Agency for Research on Cancer (IARC) (2002). Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. IARC *Monogr Eval Carcinog Risks to Hum* **82**:171–300. Lyon: IARC Press; 2002.

ICRI. (2000). History and occurrence of aflatoxin. In: Naguib, K., Naguib, M.M. Park, D.L. and Pohland, A.E. (Eds), *Proceedings of International symposium on mycotoxins*, pp: 33-46 Iowa State University Extension and outreach Grain Storage Team 2016.

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) (2007): Financial Statements for December 31, 2007 with Independent Auditor's Report CGSpace A Repository of Agricultural Research Outputs.

IUCN (2014) International Union for Conservation of Nature (IUCN). Published: January 06, 2012 O.M.A., Gaithersburg, Maryland, Washington, DC., USA.

Isabel, M.T., Alegres, R., Antonio, O. J., Ramus, N and Vilente, S., (2007) Effects of Mycotoxins in Foods. *European Journal of Plant Pathology*, **88**:112-114.

ISO 1998-1:1998 Petroleum industry — Terminology — Part 1: Raw materials and products Status: Published Publication date: 1998-11 Technical Committee: ISO/TC 28 Petroleum and related products, fuels and lubricants from natural or synthetic sources ICS > 75 > 75.080.

Jay, M.J, Martin, J.L. and David, A.G., (2005) Modern Food Microbiology. 7th edn. *Springer, New York,* pp. 443-454.

Joint FAO/WHO Expert Committee on Food Additives (JECFA) (2001). Meeting, Safety Evaluation of Certain Mycotoxins in Food by Food and Agriculture Organization of the United Nations. Joint FAO/WHO Food Standards Program, (2001) CODEX Alimentarius Commission, 24th Session, Geneva, Switzerland, July 2-7.

Jonathan H. W., Timothy D. P., Pauline E. J., Jonathan K. S., Curtis M. J., Deepak A.l (2004) Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions *The American Journal of Clinical Nutrition*, Volume **80**, Issue 5, 2004, Pages 1106–1122.

Jumbe, J. Kibas, P. Kakongoro, D. and. Tumwebaze, R. (2010). Current state of handling, processing and quality of Omena (Rastrionebola argentea) in mfangano and rusinga islands, Kenya," *Fisheries & Aquaculture Cluster Proceedings*, vol**. 7**, pp. 57–68.

Kaiming T., (2020) Aflatoxin and its Toxic Tragedies in Kenya,*Journal of Young Investigators* Vol **38** Issue 2 Carlifonia- Davis USA, 2020.

Kang'ethe E.K., Mibui G.M, Randolf T.F. and Lang'at K. A. (2007) Prevalence of Aflatoxin M1 and B1 in Milk and Animal Feeds from Urban Smallholder Dairy Production in Dagoretti Division, Nairobi, Kenya. *East Afr. Med. J.,* **84**: 83-86. 92.

Karan D. D., Vukojevic J. B. ORCID, M. Dragan R. ORCID, L. M., Jankovic V. (2005) Control of the presence of mold and mycotoxins and certain spices used in the meat industry *Meat technology* 2005, vol. 46, no. 5-6, p. 306-310 Institute for Hygiene and Meat Technology, Belgrade "JEVREMOVAC".

Kariuki, M.B.J. (2011). Analysis of Market Performance: A Case of 'OMENA' Fish in Selected outlets in Kenya. (M.Sc. Thesis), Egerton University.

Kasomo, D. (2006) Research Methods. Egerton University Press, Egerton

Kathyryn, A.W., (2007) Water Relations of Xerophilic Fungi Isolated from Indonesian Dried

Fish. *International Journal of Food Microbiology.***7**: 73.

Kawarazuka N, Bene C., (2011) The potential role of small fish species in improving Micronutrient deficiencies in developing countries: Building evidence. *Public Health Nutr.***14**:1927-1938.Source PubMed.

Kenya Bureau of Standards (1988) Dry-milled maize products. Kenyan Standard No 05158. Nairobi: Kenya Bureau of Standards.

KNBS, M. I. (2010). Kenya Demographic and Health Survey (KDHS) 2008-09. Nairobi: Kenya National Bureau of Statistics.

Kenya Marine and Fisheries Research Institute (KMFRI) (2017) Kenya's Aquaculture Brief Status, Trends, Challenges and Future Outlook.Kenya Marine and Fisheries Research Institute, Mombasa, Kenya. 12pgs.*Compiled by: KMFRI Aquaculture Division Researchers,* Kenya.

Kenya Marine And Fisheries Research Institute (KMFRI) (2019) Fresh Water Systems Fact Sheet Mapping of small-fish foods (Omena & Caridina) along the value chain in L. Victoria to identify critical points for intervention 2019 KMF/GOK/2019-20/C1.13 (iii).

Kenya National Bureau of Statistics KNBS (2019) Kenya Population and Housing Census- Population by County and Sub County.Published by source: 15 November 2019.

Kibugu, J. K., Ngeranwa, J. J., Makumi, J. N., Gathumbi, J. K., Kagira, J. M., Mwangi, J. N., Muchiri, M. W.and Mdachi, R. E. (2009). Aggravation of pathogenesis mediated by Ochratoxin A in mice with Trypanosoma brucei rhodesiense. *Parasitology*. **136**: 278-281.

Kenya Bureau of Standards KS 1470: (2011) Dried R*astrineobola Argentea Omena/dagaa/mukene* — specification 2nd edition.

Kumar P., Mahato D.K., Kamle M., Mohanta T.K., Kang S.G. (2016) Aflatoxins: A global concern for food safety, human health and their management.Front. *Microbiol.* 2017; 7:2170. Doe: 10.3389/fmicb.2016.02170.

Lalah J. O., Omwona S. and Orony D. A.O. (2019) Aflatoxin B1: Chemistry, Environmental and Diet Sources and Potential Exposure in Human in Kenya Submitted: April 19th 2019 Reviewed: July 23rd 2019 Published: October 5th 2019 DOI: 10.5772/intechopen.88773.

Lawley, R. (2013). Aflatoxins: food safety watch, the science of safe food. Retrieved from www.foodsafetywatch.org/factsheets/aflatoxins (Accessed on August 14, 2015).

Luczynska J., Tonska E., Luczynski J.,(2009).Essential mineral components in muscles of six Freshwater fish from the Mazurian Great Lakes (Northern Poland).*Arch.Pol.Fish.***17**:171-178.

Lampel K., Lampel A., Al-Khaldi S. & Cahill, S.M. (Eds) (2012). *Bad Bug Book; food borne pathogenic microorganisms and natural toxins handbook*, **2nd** edn, U.S. Food and Drug Administration, Silver Spring, MD, USA.

Lewis L., Onsongo M., Njapau H., Schurz-Rogers H., Luber G., Kieszak S., Nyamongo J., Backer L., Dahiye M.A., Misore A., DeCock K., Rubin C., and the Kenya Aflatoxicosis Investigation Group (2005) Aflatoxin Contamination of Commercial Maize Products during an Outbreak of Acute Aflatoxicosis in Eastern and Central Kenya. *Environmental Health Perspectives* Vol. **113**, No. 12 Published:1 December 2005. https://doi.org/10.1289/ehp.7998

Liu Y, Wu F. (2010) Global burden of aflatoxin-induced hepatocellular carcinoma: a risk assessment. *Environ Health Perspect* 2010; 118: 818–24.

Makun, H.A, Anjorin, S.T., Moronfoye, B., Adejo, F.O., Afolabi, O.A., Fagbayibo, G., Balogun, B.O. and Surajudeen, A.A. (2010). Fungal and aflatoxin contaminations of some Human food commodities in Nigeria. *African Journal of Food Sciences.* **4** (4): 127 – 135.

Opiyo M. A., Marijani E., Muendo P., Odede R., Leschen W.,& Charo-Karisa H., (2018) A review of aquaculture production and health management practices of farmed fish in Kenya, International *Journal of Veterinary Science and Medicine***, 6**:2, 141-148.

Medard M., Sobo F., Ngatunga T., Chirwa S., (2001) Women and Gender Participation in the fisheries sector in Lake Victoria *Researchgate* 2001.

Merkel, Alexander CLIMATE-DATA.ORG https://en.climate-ata.org AM Online Projects – Degerforserstr. 4774229 Oedheim.

Mohamed E.A.H., Al-Maqbaly R., Mansour M.H. (2010).Proximate Composition, amino acid And mineral contents of five commercial Nile fishes in Sudan. *Afr. J. Food Sci.***10**:650-654.

Moss, M.O., (2002). Mycotoxin review-2. Fusarium. *Mycologist.* Volume **16** Issue 4 pp. 158-161.

Mozola, M.A., (2001). Detection of Microorganisms in Food. Marcel Dekker. New York. Pp

156.

Mugenda, O.M. and Mugenda, A.G. (2003) Research Methods, Quantitative and Qualitative Approaches. ACT, Nairobi.

Mupunga, I., Mngqawa, P., & Katerere, D. R. (2017). Peanuts, Aflatoxins and Undernutrition in Children in Sub-Saharan Africa. *Nutrients*, ***9***(12), 1287.

Murjani, G., (2003) Chronic Aflatoxicosis in fish and its relevance to human health. *Central Institute of Freshwater Aquaculture,* India.

Mycotoxins in Grain (1997) Gasga-Group for Assistance on Systems Relating To Grain Harvest CTA-Technical Centre for Agricultural and Rural Cooperation ACP-EU *Technical Leaflet* No.**3** Published by: CTA, Postbus 380 6700 AJ Wageningen The Netherlands.

Ngwenya, B. N., Mosepele, K. K., & Magole, L. (2012). A case for gender equity in governance of the Okavango Delta fisheries in Botswana. *In Natural Resources Forum* (Vol. **36**, No. 2, pp. 109-122). Oxford, UK: Blackwell Publishing Ltd.

Nielsen S. Suzanne (2010) Food Analysis Laboratory Manual Second Edition Purdue University West Lafayette, IN, USA *Springer*.

Njapau H, Trujillo S, Pohland AE, Park DL (Eds) (2008) Mycotoxin Contamination and Control. Bloomington, In*: Authorhouse;* **113**: 132.

Nyamwaka, I. Nyamache, A. and Maingi, J. (2017). Microfungi associated with sun dried Rastrineobola argentea sold in Gucha South District in Kenya. *Microbiology Research Journal International*, vol. **19**, no. 3, pp. 1–8 *DOI: 10.9734/MRJI/2017/32067 Published: 10 April 2017.*

Nyamwaka Irene Seila (2014). Determination of fungi and factors associated with their growth on sun dried Rastrine obolaargentea in Gucha South, Kisii County, Kenya. Thesis. Page 54-68 Kenyatta University.

Nyangaga, D. K. (2014). Traders' awareness and level of aflatoxins in human foods and cattle Feeds in selected markets and stores in Nairobi County, Kenya. Kenyatta University. Https: //doi.org/REG. NO: I56/CE/11988/07.

Ocloo, F. C. K., Otoo, G., Nkansah, R. M., Mahami, T., Odonkor, S., & Quayson, E. T. (2011). Functional and physicochemical characteristics of starch obtained from gamma-irradiated

sweet potato (Ipomea batatas L.). *Journal of Agriculture and Food Technology,* **1**(7), 116-122.

Obiero K., Meulenbroek P., Drexler S., Dagne A., Akoll P.,Odong R., Arara B. K. and Waidbacher H. (2019) The Contribution of Fish to Food and Nutrition Security in Eastern Africa: Emerging Trends and Future Outlooks *Sustainability* **11**:15.

Oduor, O., Peter, M., Shitanda, D., Odiero, M. and Kituu, G. (2010) Drying Characteristics and Some Quality Attributes of *Rastrineobola argentea* (Omena) and *Stolophorus delicatulus* (Kimarawali). *Journal of Food*, *Agriculture*, *Nutrition and Developmment*, **10**(8):2998-3014.

Ofulla, A.V.O., Jondiko, J.O., Gichuki, J. and Masai, M.D., (2007). Reduction of post-harvest losses in fish for enhanced food security in the Lake Victoria basin baseline survey Report. *Commission for Higher Education (CHE), KMFRI and Maseno University:* 1-68.

Okoth S., Nyongesa B., Ayug V., Kang'ethe E., Hannu and Vesa Joutsjoki (2012). Toxigenic Potential of Aspergillus Species Occurring on Maize Kernels from Two Agro-Ecological Zones in Kenya *Toxins 2012*, **4**, 991-1007.

Olufemi J. Olapade and Daniella F.Sesay (2018) Women involvement in the fishery activities of two coastal communities in Sierra Leone. *African Journal of Agricultural Researc*h Vol.**14** (5) pp 279-286, 2019 doi: 10.5897/AJAR2018.13574.

Omojowo, F.S., P.F. Omojasola, M.O. Kolawole, E.O. Ngwu, G.B. Oluborode and C.O. Adetunji, (2010). Effect of brining on the microbial quality and safety of smoked catfish. *N. Y. Sci. J*., **3**: 20-26.

Onyango A.W., Receveur O. and S.A. Esrey (2002) The contribution of breastmilk to toddlers' diet in Western Kenya. *Bulletin of the World Health Organization;* **80**: 292-299.

Onyango D.M., Sifuna A.W., Otuya P., Owigar R., Kowenje C., Lung H.B.O. & Oduor A.O. (2017). Evaluation of fish processing and preservation systems along the shores of Lake Victoria towards enhancement of sun drying technology. *Int. J. Food Sci. and Nutr. Engr*., **7**(5): 111–118.

Owaga E.E. Onyango C.A. Njoroge C.K. (2010) Influence of Selected Washing Treatments and Drying Temperatures on Proximate Composition of Dagaa (Rastrineobola argentea), A Small Pelagic Fish Species *African Journal of Food Agriculture Nutrition and Development* **10**(7).

Owaga, C.K Onyango, E.E. and Njoroge, C.A. (2009) Investigation of mycoflora on dagaa Rastrineobola argentea) as affected by washing and drying methods. *Journal of Applied Biosciences,* vol. **19**, pp. 1074–1081.

Pascale, M., & Visconti, A. (2008) Overview of detection methods for mycotoxins. In J. F. Leslie & A. Visconti (Eds.), *Mycotoxins: Detection Methods, Management, Public Health and Agricultural Trade* (pp. 171–183). Trowbridge: Cromwell Press.

Peraica M., Radiĉ A., Luciĉ A., and Pavloviĉ M. (1999) Toxic effects of mycotoxins in humans Bulletin of the World Health Organization, *Bull World Health Organ.* 1999; **77**(9):754–766.

Pesavento, G., Ostuni, M., Calonico, C., Rossi, S., Capei, R., & Lo Nostro, A. (2016) Mycotic and aflatoxin contamination in Myristica fragrans seeds (nutmeg) and Capsicum annum (chilli), packaged in Italy and commercialized worldwide. *Journal of preventive medicine and hygiene*, **57**(2), E102–E109.

Pinotti, L.; Ottoboni, M.; Giromini, C.; DellOrto, V.; Cheli, F. (2016) Mycotoxin contamination in the EU feed supply chain: A focus on cereal byproducts. *Toxins*, **8**, 45.

Prasanna V. Dharani A. (2018) Introduction to Microbiology Volume **Two**: *Virology, Mycology & Environmenta*l. Idea Publishing 2018

Reddy M.B and Cook J.D. (1997) Effect of calcium intake on non-heme iron absorption from a complete diet. *Am J Clin Nutr* 1997;**65**(6):1820-5.

Report on Public Health, (2006) The Mycological Examination of Foods. Edn.14, Report of the Public Health Laboratory Services Standing Committee on the Mycological Examination of Food Supplies. *Reports on Public Health and Medical Subjects.* No. **126**.London: HMSO.

Rosner, F., (1998) Genetic susceptibility testing. *Cancer*, **82**(1), 232-234.

Roos N, Wahab AM, Chamnan C, Thilsted SH (2007). The role of fish in Food-based strategies to combat vitamin A and Mineral Deficiecies in developing countries. *J. Nutr.* **137**:1106-1109.

Sablani, S.S., Rahman, M.S., Haffar, F., Mahgoub, I.O., Al-Marzouki, A.S., Ruzeiqi, M.H., Al-Habsi, A. and Al-Belushi, R.H., (2009) Drying rates and quality parameters of sardine processed using solar dryers. *Agricultural and Marine Sciences,* **8**(2): 79-86

Sage, L., Krivolobok, S., Delbos, E., Seigle-Murandi,F.,Creppy, E.E., (2002) Fungal flora and

ochratoxin A production in grapes and musts from France.*Journal of Agricultural and Food Chemistry*.**50**: 1306-1311.

Sarma, U. P., Bhetaria, P. J., Devi, P., & Varma, A. (2017) Aflatoxins: Implications on Health. *Indian journal of clinical biochemistry : IJCB*, ***32***(2), 124–133.

Scott P.M, Trucksess M.W. (1997) Application of immunoaffinity columns to mycotoxin analysis. *J AOAC Int*. 1997 Sep-Oct;**80**(5):941-9. PMID: 9325571.

Singh, R. P. and Desrosier, N. W. (2018) "Food preservation". Encyclopedia Britannica, 28 Sep. 2018, https://www.britannica.com/topic/food-preservation. Accessed 18 May 2021.

Shimadzu Corporation (2016) Application Handbook Liquid Chromatography International Marketing Division No.L428

SPSS Inc. (2007) SPSS 14.0 for Windows Evaluation Version SPSS Inc. SPSS for Windows, Version **16.0**. Chicago, SPSS Inc

Sorensen, L.M., J. Mogensen and K.F. Nielsen, (2010) Simultaneous determination of ochratoxin A, mycophenolic acid and fumonisin B2 in meat products. *Anal. Bioanal. Chem.*, **398**: 1535-1542.

Steel, K.J., (2009) The effects of water, pH and Nutrients on the Growth of *Aspergillus fumigatus* on foods. *Journal of General Microbiology*, **33**:103-108.

Stone, R. (2002) Peering into the shadows: Iraq's bioweapons program. *Science* **297***:1110-1112. Sustainable Aquaculture.*

Strosnider, H., E. Azziz-Baumgartner, M. Banziger, R.V. Bhat and R. Breiman *et al*., (2006) Workgroup report: Public health strategies for reducing aflatoxin exposure in developing countries. *Environ. Health Perspect*., **114**: 1898-1903.

Thomas, M., (2008) The Growth Conditions for Fungi in Foods. *Monthly Bulletin of the Ministry of Public Health Laboratory Services*, **60**: 37-40.

Tee E. Siong, Khor Swan Choo and Siti Mizura Shahid (1989) Determination of Calcium in Foods by the Atomic Absorption Spectrophotometric and Titrimetric Methods Pertanika **12**(3), 303-311 (1989) *Division of Human Nutrition Institute for Medical Research* 50588 Kuala Lumpur, Malaysia.

Tiwari R. P. Mittal V., Bhalla T. C., Saini S. S., Singh G., and D. V. Vadehra, (1986) “Effect of metal ions on aflatoxin production by *Aspergillus parasiticus*,” *Folia Microbiologica*, vol**. 31**, no. 2, pp. 124–128.

Turner P.C., Collinson A.C., Cheung Y.B., Gong Y.Y., Hall A.J. and A.M. Prentice (2007) Aflatoxin exposure in utero causes growth faltering in Gambian infants. *Int J Epidemiol;* **36**:1119-1125.

Turner, P.C., Moore, S.E., Hall, A .J., Prentice, A.M. and Wild, C.P., (2009) Modification of immune function through exposure to dietary aflatoxin in Gambian children. *Environmental Health Perspective*, **111**: 217–220.

Vannuccini, S., Kavallari, A., Bellù, L. G., Müller, M., and Wisser, D. (2018) "Understanding the impacts of climate change for fisheries and aquaculture: global and regional supply and demand trends and prospects," in *Impacts of Climate Change on Fisheries and Aquaculture*, ed M. Barange (Rome: FAO), 41–62.

Wallace D.R. (2014) Encyclopedia of Toxicology (Third Edition), 2014 scholars.okstate.edu From Wikipedia, the free encyclopedia.

Wandera S.B. (1991) Seasonal Abundance, Vertical Migration And The Fishery Of Dagaa *Rastrineobola Argentea* In The Ugandan Waters Of Lake Victoria 1991 Uganda Freshwater Fisheries Research Organisation Jinja, Uganda.

WHO, (2005) *Public Health Strategies for Preventing Aflatoxin Exposure,* which was sponsored by the World Health Organization and the U.S. Centers for Disease Control (CDC) and Prevention and held in Geneva in July 2005.

WHO (2002).The World Health Report (2002): Reducing Risks, Promoting Healthy Life By World Health Organization WHO Library Cataloguing in Publication data.

Williams, J. H., Phillips, T. D., Jolly, P. E., Stiles, J. K., Jolly, C. M., & Aggarwal, D., (2004) Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. *The American journal of clinical nutrition*, **80**(5), 1106–22.

Wilson D.M., Mubatanhema W., Jurjevic Z. (2002). Biology and Ecology of Mycotoxigenic *Aspergillus* Species as Related to Economic and Health Concerns. In: DeVries J.W., Trucksess M.W., Jackson L.S. (eds) *Mycotoxins and Food Safety. Advances in Experimental Medicine and Biology*, vol **504**. Springer, Boston, MA

Whitaker T. B., Slate A.B., Doko M. B., Maestroni B. M., Cannavan A. .(2010) Sampling Procedures to Detect Mycotoxins in Agricultural Commodities -*Joint FAO IAEA Programme* Springer Science Business Media B.V. 2010.

World Bank (2019) Africa Program for Fisheries Agriculture And Environmental Services Discussion Paper 03 December 2013 Fish To 2030 *Prospects For Fisheries And Aquaculture World Bank Report Number* **83177**-Glb.

APPENDICES

APPENDIX I QUESTIONNAIRE

QUESTIONNAIRE FOR ASESSMENT OF FISHING AND HANDLING CHALLENGES AFFECTING OMENA BUSINESS

PURPOSE AND BACKGROUND

This questionnaire is part of a study for an award of Masters Degree in Analytical Chemistry of the University of Nairobi. The study seeks to determine the challenges the OMENA business people encounter, with a view to synthesize the data and recommend appropriate development interventions. The findings of the study will be released to the relevant stakeholders for the purpose of addressing the challenges by developing appropriate interventions.

BENEFITS

There is no risk involved and the researcher is confident that the findings and the recommendations of this study will ultimately benefit the OMENA business people and the community at large.

RIGHT TO REFUSE

Participation in the study is entirely voluntary and any one is at liberty to take part in the study. If you consent, please go ahead and provide your valued responses to the questions below.

GENERAL DETAILS

Name of County: ……………………………………………………………………………

Name of Sub-county: ……………………………………………………………………….

<u>PERSONAL DETAILS</u>

Name of the respondent (Optional)………………………………………………

Age……………………………………………………………………………….…

Gender…………………………………………………………………………..

Education level (Highest): College………Secondary………Primary……………None……

Please indicate your area of business (ship-owner, fisherman, importer, exporter, processor, etc.).

How long have you been in *Omena* business? Please tick as appropriate.

<1 year…………b) 1-5 years………….c) 5-10 years…………d) >10 years……..

What is the average amount of OMENA you process/sell per month?....................................

Section B:Fish Processing Methods and knowledge/awareness on mycotoxin

How is fishing of Omena done?.............

In which season do you have the highest catch of OMENA?

During rainy? ……… b) dry season?........

12. Where do you sell most of the OMENA?

a) Locally in county market………b) Markets outside the County?...........

13. What is the targeted end use of OMENA?....

14. How is drying carried out ?...............

15. How long is drying done?....

16 When is fish deemed to be dry enough?...

Is drying on bare ground or on other material?..

How is OMENA stored i) before drying?.... ii) after drying….

18. Is there any treatment done to Omena? (with antifungal?)…

19. Where is fish not dried enough taken? …

20. Have you ever noticed moulds on the Omena …………

If yes, do you know how harmful they are to human beings? ....

If yes, are you aware of mycotoxin in Kenya?..

21. Is there a body looking at drying and processing of Omena at the beach?..

22. How is fish transported from to markets outside?..

23. How is the fish packaged during transportation?..

24 Have you had any training on processing OMENA?...

25. Other issues: Are there any other issues that are not mentioned in this questionnaire that you would like to be addressed.

APPENDIX II Proximate quantities of Aflatoxins B1, B2, G1, and G2

Study sites	Wet season					Dry Season				
	G2 (µg/kg)	G1 (µg/kg)	B2 (µg/kg)	B1 (µg/kg)	Total (µg/kg)	G2 (µg/kg)	G1 (µg/kg)	B2 (µg/kg)	B1 (µg/kg)	Total (µg/kg)
Dunga	0.16 ± 0.12	0.77± 0.77	0.34± 0.34	1.20 ± 0.00	2.48 ± 1.04	1.28± 0.40	0±0	0±0	0±0	1.28±0.40
Sori	0.31 ± 0.29	0 ± 0.00	0.41± 0.41	0.44±0.76	1.16 ± 0.14	0.74±0.12	0.16±0.08	0±0	0.48±0.35	1.41 ±0.47
Usenge	0.78 ± 0.51	0.89± 0.85	0±0.00	0.44± 0.00	2.10 ± 0.73	0.56±0.32	0.16±0.08	0±0	0.09±0.06	0.80±0.45
Gikomba	1.06± 0.61	0 ± 0.00	0±0.00	0±0.00	1.06 ± 0.61	1.79±1.02	0.27±0.16	0±0	0.17±0.11	2.23±1.28
Karen Shopping Centre	1.12 ± 0.35	0 ± 000	0±0.00	0.71± 0.58	1.84 ± 0.70	0.61±0.06	0±0	0±0	0±0.00	0.61±0.06
Toi	0.69 ± 0.04	0.28 ± 0.14	0±0.00	0±0.00	0.97 ± 0.14	1.24±0.21	0±0	0±0	0.05±0.04	1.29±0.23
CBD	1.29 ± 0.72	0.76 ± 0.40	0±0	0±0	2.05 ± 1.12	1.22±0.14	0.19±0.09	0±0	0.2±0.11	1.61±0.24

Values aflatoxin G2. G1, B2, B1, Total ± S.E respectively