Determination of Aflatoxin Levels in Raw Groundnuts from Markets in Lusaka District, Zambia

By

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A dissertation submitted to the University of Zambia in partial fulfillment of the requirements of the degree of Masters of Science in One Health Analytical Epidemiology

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THE UNIVERSITY OF ZAMBIA

DECLARATION

I, BUMBANGI NSONI FLAVIEN, do hereby state that the content of this dissertation is my own work and has not been submitted to another university or institution for any award or degree.

Student name: ……

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CERTIFICATE OF APPROVAL

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3. Name: ……..... Signature: …… Date: ……

In loving memory of my father, Dr. Nsoni-za-Manko Jacques Elaston (1948 – 1997)

DEDICATION

This dissertation is dedicated to my loving mother, Josephine Bakabukila, my sister Laurette Kidimbu and my little brother Giresse Mpanzu, for their endless love, support and encouragement.

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ABSTRACT

Groundnuts, one of the most susceptible crops to aflatoxin (AF) contamination, are widely produced and consumed in Zambia. Previous studies have reported high levels of AFs in groundnuts produced in Zambia. However, there has been no study on the levels of AFs in groundnuts sold in different market types. This cross-sectional study was designed to determine the levels of AFs in raw groundnuts sold in Lusaka District's markets as well as identify factors associated with increased AF presence. Raw groundnut samples were collected from open markets and supermarkets in Lusaka District and analyzed for AF presence using high performance liquid chromatography (HPLC). A questionnaire was also administered to the groundnut's venders to investigate factors contributing to increased levels of AFs in groundnuts. Of the 92 groundnut samples, 51 (55.4%; 95% CI: 44.9 – 65.4) tested positive for presence of AF. The overall median and geometric mean ± standard deviation (SD) concentration for AF were 0.23 parts per billion (ppb) (range: 0.014 to 48.67 ppb) and 0.43 ± 9.77 ppb, respectively. The presence of AF was almost the same in both types of markets. However, the highest concentration was recorded in a sample collected from a supermarket (48.67 ppb). Despite this, the association between market type and presence of AFs was not statistically significant (Pearson $X^2 = 0.0587$, $p = 0.809$). Of 51 samples that tested positive to AF, 6.5% and 12% were above the maximum permissible limits (MPLs) set by the Codex Alimentarius Commission and European Union standards, respectively. There was a significant difference in the levels of AF between *Chalimbana* and *Kadononga* ($p<0.0001$), and also *Chalimbana* and *Makulu red* ($p<0.0001$). *Chalimbana* was the most at risk of AF contaminations, when compared to other groundnut varieties. The high level of AFs in raw groundnuts from both supermarket and open market samples constitutes a health hazard for the population of Lusaka district. Therefore, intervention strategies that reduce the level of AFs contamination in groundnuts should be given priority.

Keywords: Aflatoxin; Groundnut; Risk factors; Zambia

LIST OF ABBREVIATIONS

AF : Aflatoxin

AFB1 : Aflatoxin B1

AFB2 : Aflatoxin B2

AFG1 : Aflatoxin G1

AFG2 : Aflatoxin G2

AFM1 : Aflatoxin M1

AFM2 : Aflatoxin M2

AF : Total Aflatoxin

AOAC : Association of Official Analytical Chemists

CAC : Codex Alimentarius Commission

CSO : Central Statistical Office

DNA : Deoxyribonucleic Acid

ELISAs : Enzyme-Linked Immunosorbent Assays

EU : European Union

FAO : Food and Agriculture Organization

FDA : Food and Drug Administration

FPIA : Fluorescence Polarization Immunoassay

HPLC : High Performance Liquid Chromatography

IAC : Immunoaffinity Column

IARC : International Agency for Research on Cancer

ICRISAT : International Crops Research Institute for the Semi-Arid Tropics

IITA : International Institute of Tropical Agriculture

JECFA : Joint FAO/WHO Expert Committee on Food Additives

LC/MS : Liquid Chromatography/Mass Spectrometry

LCC : Lusaka City Council

LFDs : Lateral Flow Devices

MAL : Ministry of Agriculture and Livestock

MPLs : Maximum Permissible Limits

PCD : Post Column Derivatization

ppb : Parts Per Billion

ROC : Receiver Operating Characteristic

TLC : Thin-Layer Chromatography

USA : United States of America

USDA : United States Department of Agriculture

WHO : World Health Organization

ZARI : Zambia Agriculture Research Institute

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CHAPTER ONE: INTRODUCTION

1.1 Background

Food safety is a fundamental public health concern. A collection of food-borne hazards that are microbiological, chemical or physical in nature, pose risks to health and significant economic losses in both developed and developing countries (FAO/WHO, 2010).

Among chemical hazards, contamination of food by aflatoxins (AFs) has been recently characterized as an important source of food-borne illnesses (Unnevehr L. and Grace D., 2013).

AFs are a group of highly toxic, carcinogenic chemicals produced by several members of the fungal genus *Aspergillus*. This fungus, as well as the toxins it produces, normally resides in soil and plant materials, including grains of cereals, and other plants (Cotty *et al*., 2008).

Humans and animals are affected through contaminated dietary intake. There are several reports worldwide on the harmful effects of AFs in both humans and animals. Indeed, the consumption of these mycotoxins by humans through foods can cause acute and chronic health effects (aflatoxicosis) including immune-system suppression, growth retardation, cancer, and death (Azziz-Baumgartner *et al*., 2005; Gong *et al*., 2004; Wild and Turner, 2002; Williams *et al*., 2004). Aflatoxins are carcinogens and genotoxin agents that directly influence the structure of deoxyribonucleic acid (DNA) (Williams *et al*., 2004).

A chronic exposure to AF may result in liver cancer in both humans and animals (IARC, 1993). This has led the International Agency for Research on Cancer (IARC) to classify aflatoxin B1 (AFB1) as a group 1 carcinogenic agent to humans. The agent or mixture has shown sufficient evidence of carcinogenicity in humans (IARC, 1993). The risk of hepatocellular carcinoma is particularly elevated in individuals with chronic hepatitis B virus infection who also are

exposed to AFs (IARC, 2002). Further, the IARC classifies the aflatoxin M as Group 2B (possibly carcinogenic to humans).

In general, four potential impacts of AF have been identified: (i) deterioration of food and nutritional quality of agricultural products with an accompanying reduction in sensory characteristics, e.g., taste, odor, texture and color, (ii) health-related productivity losses due to mutagenic and carcinogenic effects on humans who consume aflatoxin-contaminated food over an extended period of time, (iii) loss of income from livestock resulting from feeding aflatoxin-contaminated feedstuffs, e.g., higher mortality rates and lower feed to weight conversion ratios for chickens, ducks, egg-layers, and pigs, and (iv) loss of export market and related economic losses due to regulations that restrict international trade of aflatoxin-contaminated grain (Lubulwa and Davis, 1994). Such losses may cost African export up to 400-450 million U.S. dollars annually (Otsuki *et al*., 2001b).

Thus, AF increases morbidity and mortality in developing countries and particularly in Africa (Wu and Khlangwiset, 2010) where it represents an enormous economic and social burden.

1.2 Statement of the Problem and Study Justification

Food security remains an important unachieved Millennium Development Goal in many developing countries. Agriculture production is essential for achieving food security. However, food poisoning, including contamination of agricultural products by AF is a real threat in the fulfillment of this goal. Maize and groundnuts, the most susceptible crops to AF contamination, are staple foods in most African communities (Wu and Khlangwiset, 2010). Any hazard occurring in these food products is likely to affect a large population and consequently contribute to increasing poverty and food insecurity.

Indeed, post-harvest contamination of various agricultural products causes enormous losses to both farmers (loss of livelihood) and the country through export bans, which brings additional cost in the treatment or rejection of these products (Wu *et al*., 2011). In addition, the existing food insecurity in most African countries, compounded by economic losses exposes the population to the consumption of contaminated products (Wild and Gong, 2010). This has been recognized to be the root cause of different outbreaks reported in African countries with high mortality rates (Azziz-Baumgartner *et al*., 2004; Strosnider *et al*., 2006). Furthermore, this situation subjects the population to chronic exposure with even greater consequences (hepatocellular carcinoma, growth retardation in children, decreased immunity).

Despite the magnitude of the problem and the high agricultural production and consumption of groundnuts in Africa (Liu and Wu, 2010), few studies have provided estimates of daily exposure to AFs during non-outbreak periods (Wild *et al*., 1992; Wang *et al*., 2001; Jiang *et al*., 2005). In Zambia, groundnuts production is high (Figure 1) and contributes to the national economy (Sitko *et al*., 2011).

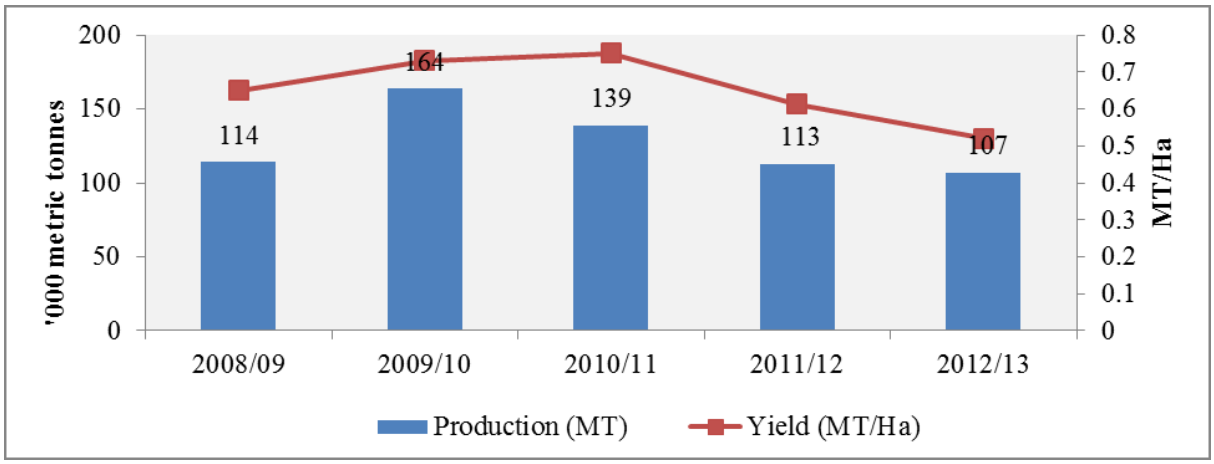


Figure 1: Groundnuts Production and Yields in Zambia, 2008-2011 (MT: Metric Tonnes; Ha: Hectare). Source: CSO/MAL 2008/09-2011/12.

However, Mukuka and Shipekesa (2013) reported a high level, up to 100 ppb, of AF in groundnuts produced in Eastern province of Zambia compared to the maximum permissible limits (MPLs) (15 ppb) set by Codex Alimentarius Commission (CAC) standards. Despite this, little is known about the magnitude of AF contamination levels in groundnuts. Furthermore, there is currently no study in Zambia estimating the levels of AF in groundnuts sold in different market channels; hence the need for this study.

1.3 Objectives

1.3.1 General objective

The purpose of this study was to describe the AFs presence in raw groundnuts sold in Lusaka district's markets.

1.3.2 Specific objectives

(a) To determine the levels of AFs in raw groundnuts sold in different markets of Lusaka district.

(b) To identify factors associated with increased AF presence in raw groundnuts in Lusaka's markets.

1.3.3 Hypothesis

We hypothesized that AF levels in raw groundnuts traded in markets in Lusaka District are higher compared to the MPLs set by the CAC.

CHAPTER TWO: LITERATURE REVIEW

2.1 Historic Background

The first report of AFs goes back to the early 1960s, when they were initially isolated and identified as the causative toxins for the deaths of more than 100, 000 turkeys in the United Kingdom (Filazi and Sireli, 2013). Since then, several cases have been reported worldwide. Currently, it has been estimated that more than 4.5 billion people in developing countries worldwide are at risk of chronic exposure to AFs through contaminated foods (USAID, 2012).

While in developed countries the problem of AF seems to be under control, it remains a big challenge to food safety in several developing countries (DANYA and USAID, 2012).

Indeed, AF poisoning in Eastern Africa has almost become epidemic, especially in arid and semi-arid areas. The most obvious case is that of Kenya where several outbreaks of aflatoxicosis were reported (Strosnider *et al*., 2006). The first outbreak of aflatoxicosis reported in Kenya took place in 1978; other outbreaks occurred in 1981, 2001, 2004 – 2008 which resulted in disease and deaths of humans, and destruction of contaminated maize stocks (Bennett and Klich, 2003; Lewis *et al*., 2005). The largest outbreak reported in the world over the past 20 years occurred in the same aforementioned country, from January to June 2004, affecting 317 cases of whom 125 died (Azziz-Baumgartner *et al*., 2005; Lewis *et al*., 2005; Strosnider *et al*., 2006).

Moreover, contamination of products by AF is also encountered in Western and Central Africa. Ghana, Nigeria, Senegal, Togo, Burkina Faso and Cameroon have recorded AF contamination in sorghum, maize, cottonseed, groundnuts and groundnut products, yam and cassava at different levels with contamination levels generally exceeding the European Union (EU) and

the United States Department of Agriculture (USDA) standards (Bankole and Adebanjo, 2003; Kpodo *et al*., 2000).

Contamination of food products by AFs was also reported in some Southern African countries. In Botswana, AFs were found in maize meal (DANYA and USAID, 2012). Furthermore, a study in Malawi revealed a high concentration of AF of up to 1,020 ppb in grain consumption (Glaston *et al*., 2000).

2.2 Aetiology

Aflatoxins are mycotoxins produced by fungi of the genus *Aspergillus* that essentially belongs to grains storage flora and soil. *Aspergillus* grows optimally at 25 °C with a minimum necessary water activity of 0.75. It starts to produce secondary metabolites at 10-12 ºC, but the most toxic ones are produced at 25°C with a water activity of 0.95 (Gimeno and Martins, 2003).

There is a large number of *Aspergillus* species, basically grouped in three phylogenetically distinct sections. The main producers of AFs are *Aspergillus flavus*, and *Aspergillus parasiticus* (Wu *et al*., 2013). But it has been demonstrated that *Aspergillus nomius*, *Aspergillus pseudotamarii*, *Aspergillus parvisclerotigenus*, and *Aspergillus bombycis* of section *Flavi*; *Aspergillus ochraceoroseus* and *Aspergillus rambellii* from section *Ochraceorosei*; and *Emericella astellata* and *Emericella venezuelensis* from *Nidulatans* section also generate AFs (IARC, 2002). However, the presence of *Aspergillus flavus* does not always indicate harmful levels of AF; it does mean that the potential for AF production is present (USDA, 2012).

There are about 20 types of AFs, but the naturally occurring and well-known ones are aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2) (Wu *et al*.,

2013). These names were given due to their blue (B) or green (G) fluorescence properties under ultraviolet light and their migration patterns during chromatography (Saleemullah *et al*., 2006).

There are also aflatoxin M1 (AFM1) and aflatoxin M2 (AFM2) that are hydroxylated derivatives of AFB1 and AFB2, respectively. These may be found in milk, milk products or meat (hence the designation M1 and M2). They are formed by the metabolism of B1 and B2 in the body of the animals following absorption of contaminated feeds (Wild and Gong, 2010). AFB1 is the most prevalent AF found in cases of aflatoxicosis, and is responsible for acute as well as chronic toxicity, carcinogenicity, teratogenicity, genotoxicity and immunotoxicity (Liu and Wu 2010; Kensler *et al*., 2011; Lizárraga-Paulín *et al*., 2011).

2.3 Epidemiology

2.3.1 Global distribution

The geographical distribution of AF follows that of its producer, which is the fungus Aspergillus. This mold is common and widespread in nature. It occurs in soil, decaying vegetation, hay, and grains undergoing microbiological deterioration and it invades all types of organic substrates whenever and wherever the conditions (high moisture content and high temperature) are favorable for its growth (Udoh *et al*., 2000).

However, AFs are most prevalent in latitudes between 40° N and 40° S of the equator, and the greatest health risk lies within developing countries in tropical regions (Figure 2), which rely on commodities susceptible to contamination by these toxins as their staple food source (IITA, 2011).

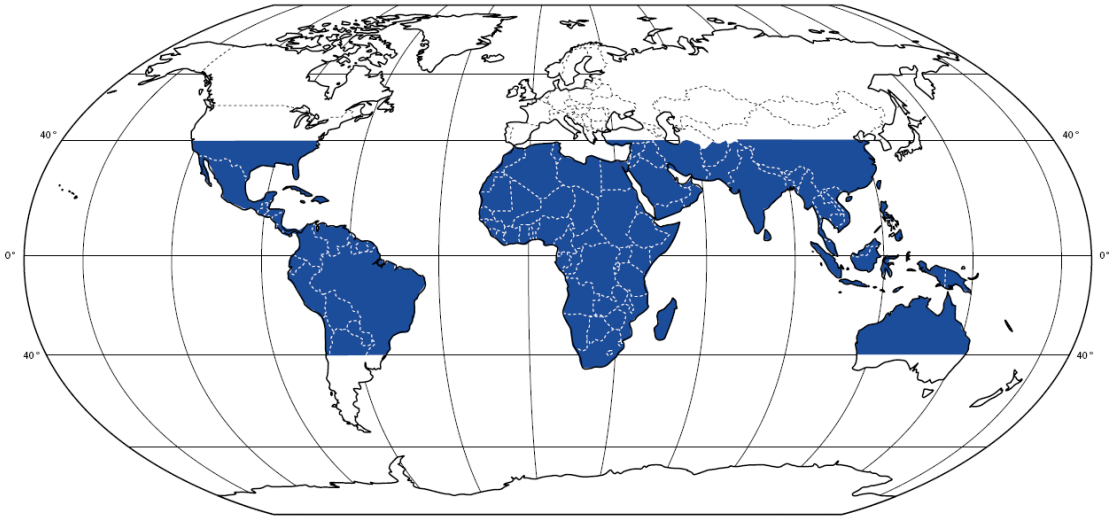


Figure 2: Regional and populations at risk of chronic exposure to uncontrolled contamination of AF (Source: Williams *et al*., 2004).

The Food and Agriculture Organization (FAO) estimates that the fungus affects 25% of the world's crops (USAID, 2012). The Centers for Diseases Control (CDC) estimate that more than 4.5 billion people are chronically exposed to AF through consumption of contaminated foods such as maize and groundnuts (USAID, 2012).

Aspergillus flavus is the most widely reported food-borne fungus outside north-temperate areas. It is especially abundant in the tropics. *Aspergillus flavus* was isolated from 97% of nearly 500 peanut samples examined from South-East Asian sources over the years 1989–91. It reached an average infection rate of more than 40% of all surface-disinfected kernels examined from Thailand and the Philippines, and more than 60% of those from Indonesia. For maize, the figures were 89% of 380 samples, at an average of 38% of all grains infected from Indonesia and the Philippines, and 17% of those from Thailand (Williams *et al*., 2004).

Levels in food commodities from more temperate climates, such as Australia or the United States of America (USA), are much lower (Williams *et al*., 2004).

It seems probable that although *A. parasiticus* has the same geographical range as *A. flavus*, it is less widely distributed. In particular, it has been found in the USA, Latin America, South Africa, India and Australia and rarely in South-East Asia (Williams *et al*., 2004).

Aflatoxins are a far greater problem in the tropics than in temperate zones of the world. However, because of the movement of agricultural commodities around the globe, no region of the world is free of AFs. Therefore, the risk of exposure may not be limited to tropical zones since products from these zones are consumed globally.

However, acute and chronic AF exposures are more likely to occur in developing countries where regulatory limits, poor agricultural practices in food handling and storage, malnutrition, and disease are problems. Aflatoxicosis in humans has been reported in many countries, including India, China, Thailand, Ghana, Kenya, Nigeria, Sierra Leone, and Sudan (FDA, 2012). Because of the alarming numbers, AFs have been considered as an important public health issue (Williams *et al*., 2004).

With regard to AF contamination in foods imported into Japan, relatively low incidences and low levels of AFs have been found in various commodities. Aflatoxin inspection of imported peanuts in Japan (1999–2000) indicated that 355 (6.9%) of 5108 samples were contaminated with AFB1 at levels ranging from 0.2 to 760 ppb, and 145 samples (2.8%) contained over 10 ppb, the maximum permissible limits (MPLs) (IARC, 2002). In commercial nuts and nut products in markets, AFB1 was found in 23 (3.4%) of 673 samples at levels of 0.3–128 ppb in the same country. Imported spices (white and red pepper, paprika and nutmeg) contained AFB1 in 106 (19.4%) of 546 samples at levels of 0.2–27.7 ppb (IARC, 2002).

Data on the occurrence of AFs in imported spices in the European Union (EU) reveal that, among the total of 3098 spice samples including nutmeg, pepper, chili and paprika, 183 samples (5.9%) contained more than 10 ppb AFs (IARC, 2002). In the United Kingdom, 7 of

139 maize samples (5.0%) imported in 1998–99 contained AF in the range of 4.9–29.1 ppb (3.7–16.4 ppb AFB1) (MAFF, 1999).

The French "Direction Générale de la Concurrence, de la Consommation et de la Répression des Fraudes" (DGCCRF) surveyed 635 imported foods between 1992 and 1996, of which 227 (35.7%) had AFB1 levels above 0.05 ppb. The highest levels were found in spices (up to 75 ppb) and dried fruits (up to 77 ppb) (Castegnaro and Pfohl-Leszkowicz, 1999).

Dietary intake of AFB1 was monitored for one week in a number of households in a Chinese village. Aflatoxin B1 was detected in 76.7% (23/30) of ground maize samples (range, 0.4–128.1 ppb); 66.7% (20/30) of cooking peanut oil samples (range, 0.1–52.5 μg/L) and 23.3% (7/30) of rice samples (range: 0.3–20 ppb) (Wang *et al.*, 2001).

Aflatoxin exposure in parts of East Africa is ubiquitous and at high levels throughout the life of the people living there (Mutegi *et al*., 2013).

2.3.2 Factors increasing aflatoxin contamination of crops

The contamination of foods and feeds by AFs depends on biological (biotic) and environmental (abiotic) factors that promote mould growth and toxin production. This can occur both pre- and post-harvest (Okello *et al*., 2010).

In pre-harvest, the important factors are mechanical damage, insect and bird damage, the genotype of the crop planted, drought stress and excessive rainfall (Hell and Mutegi, 2011). The role of these factors in crops contamination by AFs depends substantially on the number of spores of *Aspergillus spp* or propagules (thousands per gram of soil) in the soil (IARC, 2002). Other factors include strain variation in the fungus, interference by other microorganisms, moisture, soil temperatures (around 30 °C) (warm and humid climates), soil pH, the gaseous environment and preservatives (Jaime-García and Cotty, 2010; Okello *et al*., 2010). However,

the incidence and levels of fungal infection and AF contamination vary markedly from one geographical area to another based on the above factors (Kaaya *et al*., 2006; Cotty and Jaime-García, 2007).

Aflatoxin contamination of crops in post-harvest comprises the period from crop maturation to consumption. Factors influencing fungi growth and AFs production during this period are warm, moist conditions (i.e. on the feedlot floor), during transportation and storage (Paterson and Lima, 2010), in the field (heavy rains or floods conditions when harvesting), and insufficiently dried agricultural commodities (Okello *et al*., 2010).

However, during pre-harvest and/or post-harvest, the following factors have been singled out as those that mainly encourage mould growth and AF production in grains and kernels:

a) Moisture content: Soil moisture stress has been reported to enhance pre-harvest AF contamination of produce. Groundnuts exposed to drought stress in the field have been reported to have more *A. flavus* infected kernels than in irrigated plots (Paterson and Lima, 2010). Excessive drought causes strains on pods and testas thus providing entry points for infection by fungi while excessive moisture weakens the pods and testas causing the same effect (Abdullahi, 2004). The amount of moisture in a grain affects both grade and storability and has a critical effect on mould growth and mycotoxin production. It is one of the most important considerations in determining whether AF will develop in crops after harvest. Storage fungi grow at moisture contents in equilibrium with relative humidity ranging from 65-70 to 85-90 percent. *A. flavus* will only grow when the moisture content exceeds 15%, at 80-85% relative humidity and above (Kaaya and Kyamuhangire, 2006).
b) Temperature: The effect of temperature is strongly associated with the effect of moisture. In fact, under favourable temperature and relative humidity conditions,

aflatoxigenic fungi grow on most common grain like cereals and nuts. Production of AFs is optimal at relatively high temperatures, so contamination is most acute and widespread in warm, humid climates (Paterson and Lima, 2010).

c) Mechanical damage to kernels: Cracks and breaks are caused mainly during harvesting and shelling, but also by insect and rodent feeding (Hell and Mutegi, 2011). This makes the kernels more vulnerable to invasion by storage moulds, including *A. flavus*. Under any given environmental conditions, fungal growth is several times faster in damaged than in intact kernels.

d) Storage conditions: Incorrect storage of commodities, including improper dried state of commodities or storage under high humidity with inadequate protection promotes fungal growth (Van der Fels-Klerx *et al*., 2009). Furthermore, the longer the retention under poor storage condition the greater will be the possibility of building up environmental conditions conducive to aflatoxigenic mould proliferation in groundnuts (Kaaya and Kyamuhangire, 2006).

e) Insect infestation or damage: Insects may damage stored grain, but they also interact with fungal colonisation in many different ways. Fungal spores can be carried by insects. Toxin-producing fungi can infect growing crops and may produce toxins prior to harvest or during harvesting and storage (Paterson and Lima, 2010). During storage, insects, due to their metabolic heat and water, can increase the water activity and temperature of grain to levels suitable for fungal growth (Cotty and Jaime-Garcia, 2007).

2.4 Host Range

Aflatoxins contaminate a large fraction of the world's food and feed commodities (Strosnider *et al*., 2006). The major hosts of AFs include maize, groundnut and cottonseed (Cotty *et al*., 2008; Kpodo *et al*., 2000). From time to time, AFs occur in tree nuts, including pecans, hazelnuts and

walnuts, copra and kola nuts (IARC, 2002). Aflatoxins contaminated food were also reported in rice, sorghum, barley, rye, wheat, soya, milk, meat and other derivative products made from these primary feedstuffs (IARC, 2002; Saleemullah *et al*., 2006).

2.5 Human Exposure

Humans are primarily exposed to AF through dietary intake (IARC, 2002). Two pathways of the dietary exposure have been identified:

(a) Direct ingestion of AFs (mainly B1) in contaminated foods of plant origin such as maize, groundnuts and their products.

(b) Ingestion of AFs carried over from feed into milk and milk products including cheese and powdered milk, where they appear mainly as AFM1. In addition to the carryover into milk, residues of AFs may be present in the tissues of animals that consume contaminated feed.

However, exposure to AF is a result of both the level of contamination in a given commodity and the quantity of the commodity that is consumed. Thus, in some areas of the world AF levels in foods might be relatively high but with modest exposures because of a varied diet. While, in Sub-Saharan Africa, similar levels of food contamination will translate to a much higher exposure because, dietary staples in this region (peanuts and maize) are highly susceptible to AFs contamination due to poor grain storage conditions. In many countries and regions, these staples are consumed daily for the majority of the year and may constitute more than 50% of the diet (Wild and Turner, 2002). In addition, these toxins present an elevated thermal stability enabling them to remain in some cooked foods as well as freezing has very little effect on their presence in foods (Sáez *et al*., 2011).

Human exposure to AF may secondarily result from exposure to air and dust containing toxins during the handling of contaminated products (Sorenson *et al*., 1984).

2.6 Aflatoxin Standards for Humans and Animals

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has evaluated aflatoxins B and G on several occasions since 1987 (JECFA, 2008) and has recommended that, due to their carcinogenic potential, dietary exposure to AFs should be minimized as much as possible.

Many studies have tried to determine the toxicity of AF dose necessary to trigger adverse effects in humans and in animals (Gimeno and Martins, 2003; Gong *et al*., 2004; Azziz-Baumgartner *et al*., 2005; Dhanasekaran *et al*., 2011). However, several factors must be taken into account to determine if AF will be lethal to humans and animals. These include the amount of AF consumed daily, the frequency of consumption and nutritional status of the individual (FDA, 2012).

In northwest India, the AF levels found in corn during the outbreak of 1974 were in the range of 0.25 to 15 ppb (Reddy and Raghavender, 2007). In the 1982 Kenyan outbreak, the level of AF intake was at 38 ppb of body weight. While in 2004 and 2005, aflatoxin-contaminated homegrown maize with an average concentration of 354 ppb was the source of the outbreak in Kenya (Strosnider *et al*., 2006).

Thus, due to its high toxicity and carcinogenic properties, legal tolerance levels in different countries have been taken for AF in foods that are destined for human consumption. These limits vary between 4 - 20 parts per billion (ppb) (Wu *et al*., 2013). Information is extremely limited concerning health effects associated with AF concentration between 20 ppb and 300 ppb.

The Codex Alimentarius Commission (CAC), Joint FAO/WHO Food Standards Program adopted levels of 15 ppb as maximum permissible limits (MPLs) for AF in unprocessed peanuts and tree nuts, and 10 ppb for ready-to-eat tree nuts (CAC, 2014). The European Union has the most stringent standards of AFs in the world. The limit is 4 ppb (Wagacha and Muthomi, 2008). The United States adopted 20 ppb as the MPLs for AF in food (Wu *et al*., 2013) and 0.5 ppb in milk (Kensler *et al*., 2010). In China, the levels of AFB1 in peanut butter and sesame paste are set not to exceed 20 ppb (DANYA and USAID, 2012), and 40 ppb in unprocessed maize and groundnuts (Wu *et al*., 2013). However, for most African countries AF is largely unregulated. By 2013, ten African countries, including Kenya (20 ppb), Tanzania (10 ppb), Malawi (10 ppb), Zimbabwe (10 ppb) and South Africa (10 ppb) had set regulations for both unprocessed maize and groundnuts (Wu *et al*., 2013).

Therefore, toxic level of AF in humans is largely unknown as evidenced by the different thresholds that were found in outbreaks reported in different countries (FDA, 2012).

2.7 Diagnosis

2.7.1 Clinical signs

Adult humans usually have a high tolerance of AF, and, in the reported acute poisonings, mortality is usually reported in children (Williams *et al*., 2004).

The adverse effects of AFs in humans and animals have been categorized in two general forms:

2.7.1.1 Acute aflatoxicosis

It occurs when moderate to high levels of AFs are consumed (Lewis *et al*., 2005). Specific, acute episodes of disease is characterized by hemorrhage, acute liver damage which manifests as severe hepatotoxicity with a case fatality rate of approximately 25% (Strosnider *et al*., 2006),

edema of feet, absorption and/or metabolism of nutrients and alteration in digestion. The early symptoms of hepatotoxicity from aflatoxicosis can include anorexia, malaise, and low-grade fever. Acute high-level exposure can progress to potentially lethal hepatitis with a rapid development of ascitis, portal hypertension, vomiting, abdominal pain, jaundice, fulminant hepatic failure and death (Walderhaug, 1992; Strosnider *et al*., 2006).

2.7.1.2 Chronic aflatoxicosis

Chronic effects result from ingestion of low to moderate levels of AFs. These effects are usually subclinical and difficult to recognize at the beginning of exposure (Jolly *et al*., 2009; Ndung'u *et al*., 2013).

It has been well documented that chronic AF exposure, particularly AFB1 is associated with an increased risk of developing hepatocellular carcinoma as well as disorders of immune function and nutritional problems such as growth retardation in children (Wu and Khlangwiset, 2010; Wu *et al*., 2011). Aflatoxin exposure can also exacerbate problems of pre-existing health conditions. People infected with hepatitis B who are exposed to AF, are 30 times more likely to develop liver cancer than those who do not (Liu and Wu 2010). At the global level, estimated AF exposure contributes to between 4.6 percent and 28.2 percent of all cases of liver cancer. It should be noted that most of these cases have been observed in Sub-Saharan Africa, South-East Asia and China. It should also be noted that these regions have the highest exposure to AF levels (Kirk *et al*., 2006; Liu and Wu 2010).

Aflatoxin B1 also chemically binds to DNA and causes structural DNA alterations with the result of genomic mutation (Groopman *et al*, 1985). Ingestion of AF, viral diseases and hereditary factors, have been suggested as possible etiological agents of childhood cirrhosis (Dhanasekaran *et al*., 2011). There is evidence to indicate that children exposed to AF through breast milk and dietary items such as unrefined groundnut oil, may develop cirrhosis.

Malnourished children are also prone to childhood cirrhosis on consumption of contaminated food (Dhanasekaran *et al*., 2011). Several investigators have suggested AF as an etiological agent of Reye's syndrome in children in Thailand and New Zealand, though there is no conclusive evidence as of yet (Dhanasekaran *et al.*, 2011).

2.7.2 Laboratory analytical methods

Analytical methods for mycotoxins in feeds and foodstuffs generally require toxin extraction from the matrix with an adequate extraction solvent, a clean-up step intended to eliminate interference from the extract, and, finally, detection/determination of the toxin by suitable analytical instruments/technologies (Figure 3). Clean-up is essential for the analysis of mycotoxins at trace levels, and involves the use of solid phase extraction, and multifunctional or immunoaffinity columns (Giniani *et al*., 2011).

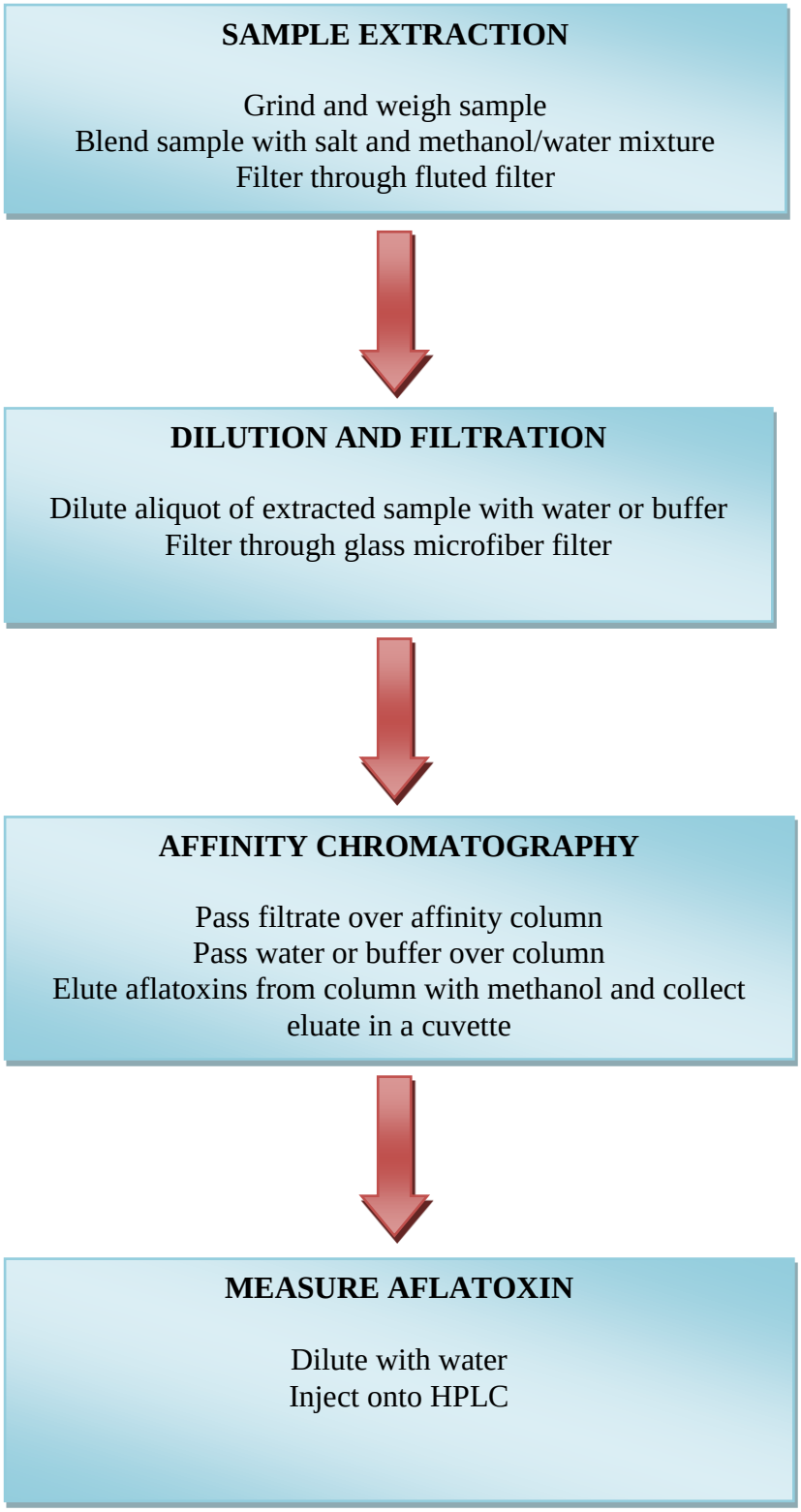


Figure 3: Laboratory process involved in AF analysis, case of HPLC. (Source: AflaTest® HPLC VICAM, 2015)

2.7.2.1 Detection and determination of aflatoxin level

The different analytical methods of AFs can be divided into two groups:

2.7.2.1.1 Classical analytical technologies for aflatoxins analyses

This group includes:

2.7.2.1.1.1 Thin-layer chromatography (TLC)

Thin-layer chromatography (TLC) is among the most widely-used analytical methods. This is simple and cost-effective technique especially used for AFs analysis in developing countries. It is used for screening purposes, multi-mycotoxin analysis and when low detection limits are not required (Pascale and Visconti, 2008).

Although TLC is a powerful tool for the simultaneous analysis of multiple samples for multiple mycotoxins, it cannot be used for sensitive or precise measurements unless densitometric analyses are performed (Pascale and Visconti, 2008). Highly reproducible, reliable results can be obtained if an autospotter is applied to the samples. TLC can be used without cleaning up the extract, but extract purification prior to spotting increases sensitivity (Stroka *et al*., 2000). However, it is labour intensive, and limited in the number of samples that can be tested in a day (Stroka *et al*., 2000).

2.7.2.1.1.2 High performance liquid chromatography (HPLC)

HPLC coupled with ultra-violet, a diode array detector (DAD) or a fluorescence detector (FD) currently is the most widely used technique for the identification of the major mycotoxins in food commodities (Giniani *et al*., 2011). HPLC/FD is highly sensitive, selective, accurate and repeatable. Specific labeling reagents have been developed, and are commercially available, for the derivatization of non-fluorescent mycotoxins to form fluorescent derivatives. Either pre-

column derivatization, with trifluoroacetic acid (TFA), or post-column derivatization, with Bromide or Iodine, can be used to identify aflatoxins B1, B2, G1 and G2 (Pascale and Visconti, 2008).

These methods have been adopted as official or standard methods by the International Association of Official Analytical Chemists (AOAC) or the European Standardization Committee. In particular, methods for measuring AFs in maize, raw peanuts and peanut butter; AFB1 and total aflatoxins in pistachios, figs and paprika; AFM1 in milk; AFB1 in baby food (Fu *et al*., 2008). In addition, HPLC/immune-affinity column methods have been validated for the measurement of AFs in hazelnut paste (Ibáñez-Vea *et al*., 2011).

2.7.2.1.1.3 Liquid chromatography/mass spectrometry (LC/MS)

Liquid chromatography coupled with mass spectrometry has been used for many years mainly as technique for AFs quantification and confirmation of identities. In the past decade, LC/MS was the most promising technique for simultaneously screening, identifying and measuring a large number of mycotoxins (Sorensen and Elbaek, 2005).

Accuracy, precision, and sensitivity of LC/MS methods may vary depending on the mycotoxin, matrix and instrument with the sensitivity of the method depending on the ionization technique used. Quantitative measurement of mycotoxins by LC/MS often is unsatisfactory due to matrix effects and ion suppression. Purification of extracts by MycoSep® or immunoaffinity columns generally is needed prior to mass spectrometry detection (Pascale and Visconti, 2008).

The coupling of HPLC to mass spectrometry is the more commonly employed detection technique in the last years (Giniani *et al*., 2011).

2.7.2.1.1.4 Enzyme-linked immunosorbent assays (ELISAs)

Since the late 1970s, AF-specific antibodies have been developed. The antibody development has led to the development of enzyme-linked immunosorbent assays (ELISAs) for AFs (Li *et al*., 2009). The ELISAs are mainly used for qualitative, semi-quantitative and quantitative analysis of AF and other mycotoxins in a number of food matrices. In general, ELISAs do not require clean-up procedures and the extract containing the mycotoxin is analyzed directly (Li *et al*., 2009). It provides fast and inexpensive screening assays; but lack accuracy at very low concentrations (competitive assays) and is limited in the range of matrices examined. Flow-through enzyme immunoassays for field use have been developed for the rapid detection of AFB1, AFM1 and provide consistent results with those obtained by HPLC. This assay was both accurate and reliable giving no false compliant and only a few false noncompliant results (Li *et al*., 2009).

2.7.2.1.2 Emerging technologies for aflatoxins analyses

Recently, a variety of emerging methods based on novel technologies have been proposed for AFs analyses. They include:

2.7.2.1.2.1 Lateral flow devices (LFDs)

A lateral-flow device, also called an immunochromatographic test, is a rapid immunoassay based on the interaction between specific antibodies, immobilized on a membrane strip, and antibody-coated dyed receptors, *e.g.*, latex or colloidal gold, that react with the analyte to form an analyte-receptor complex (Tang *et al*., 2009).

Competitive LFDs rely on the competition of the analyte, *e.g.*, AF, in solution for the binding sites of the labeled receptor. The test line contains an analyte-conjugate attached to the membrane that binds unbound receptor to form a colored analyte-conjugate receptor complex.

The control line includes a specific antibody attached to the membrane that binds with the labeled receptor.

LFDs are user-friendly format, cost-effective and provide a rapid response. These features make strip tests ideal for applications such as "on-site" detection of environmental and agricultural analytes (Tang *et al*., 2009). LFDs are often used for the determination of AFB1 in maize and peanuts, AFM1 in milk and other mycotoxins (Van der Spiegel *et al*., 2013).

2.7.2.1.2.2 Fluorescence polarization immunoassay (FPIA)

FPIA is a simple technique that measures interactions between a fluorescently labeled antigen and a specific antibody. FPIAs have been used for the rapid determination of AFs in solution though low accuracy and sensitivity were problems when these assays were used with cereal samples (Saha *et al*., 2007).

2.7.2.1.2.3 Capillary electrophoresis

Capillary electrophoresis is an analytical technique that allows good separation of mycotoxins from potential interfering species present in the extract on the basis of electrical charge. These methods have been developed for various mycotoxins, including AFs. Capillary zone electrophoresis with laser-induced fluorescence has been used for the analysis of AFB1 in maize (Pascale and Visconti, 2008). These methods are comparable in sensitivity, precision and accuracy to HPLC methods. The use of less expensive capillaries, the absence of organic solvents during the detection step, and shorter analysis times, all make capillary-zone electrophoresis methods viable alternatives to those requiring HPLC (Dong Yiyang, 1999).

2.7.2.1.2.4 Fiber-optic immunosensors

Evanescent wave-based fiber-optic immunosensors have been developed for the detection of fumonisin B1 and AFB1 in maize. A non-competitive assay was used for AFB1 that takes

advantage of the native fluorescence of this mycotoxin. The sensor could detect 2 ng/ml of AFB1 in phosphate buffered saline solution. Problems due to refractive index-related effects were observed in the presence of organic solvents, which reduced the specificity of the assay (Maragos and Thompson, 1999).

2.7.2.1.2.5 Biosensors

A biosensor is an analytical device that incorporates a specific biological element, *e.g.*, an antibody, which creates a recognition event and a physical element that transduces the recognition event into acoustic, electrical or optical signal. Immunochemical biosensors that use surface plasmon resonance, quartz crystal microbalance and screen-printed carbon electrodes have been described for the detection of mycotoxins.

Competitive surface plasmon resonance-based immunoassays have been used for rapid screening of AFB1 and other mycotoxins in naturally contaminated matrices (Van der Gaag *et al*., 2003). These methods are able to detect AFs at very low levels with good accuracy and precision.

The use of surface plasmon resonance equipment with four flow cells enables the detection of four mycotoxins in a single measurement (Van der Gaag *et al*., 2003).

2.8 Prevention and Control

The AF contamination is a serious health concern rooted in the entire food chain, hence the need for a multidisciplinary approach in the search for solutions. Interventions to reduce aflatoxin-induced illness can be roughly grouped into three categories: agricultural, dietary, and clinical.

2.8.1 Agricultural

Agricultural interventions are methods or technologies that can be applied either in the field (pre-harvest) or in drying, storage and transportation (post-harvest) to reduce AF levels in food. Agricultural interventions can thus be considered "primary" interventions, because they directly reduce AF levels in food.

2.8.1.1 Pre-harvest handling methods

These include the use of bio-control agents that establish a process of competitive displacement using non-toxigenic strains by different agricultural techniques, and developing breeds stronger or more resistant crop strains. These methods are:

2.8.1.1.1 Biological control

Biological strategies employed instead of traditional chemical pesticides, are environmentally friendly and come from natural resources. These strategies include beneficial insects, plant extracts (Reddy *et al*., 2009), or the introduction of other natural organisms such as Methyleugenol (Sudhakar *et al*., 2009).

In the prevention of AF contamination, biocontrol methods can be applied before harvest or in the fields when the plants grow and become mature. However, these methods are not as effective as those using chemical methods.

2.8.1.1.2 Competitive displacement

The application of non-toxigenic strains of *A. flavus* on crops promotes competition with those that are toxic, resulting in a significant reduction or elimination of toxigenic strains that produce AF (Probst *et al*., 2011).

This method has proven to be very effective in the fight against AF contamination in various cultures (Probst *et al*., 2011).

The application of this method can be done in different ways and this influences the effectiveness of results. It may involve: 1) inoculating the soil with a non-toxigenic strain, 2) spraying of crops with conidia or spores of non-toxigenic strain and 3) spraying the plants with a product which comprises hydrosoluble non-toxigenic strain. The most effective means of delivering non-toxigenic strains to replace competitively toxigenic *A. flavus* is through aerial spray of formulated non-toxigenic strains (Lyn *et al*., 2009).

2.8.1.1.3 Agricultural techniques

The use of agricultural techniques, such as crop rotation (Jaime-Garcia and Cotty, 2010) and interventions to reduce exposure to environmental stress (Cotty and Jaime-Garcia, 2007) may also reduce AF contamination.

2.8.1.1.4 The genetic improvement of plants to provide resistance

Inserting the DNA of *Bacillus thuringiensis* (Bt) in maize directly enables the insecticidal toxins production by the plant itself. Therefore, Bt maize is less susceptible to the penetration by insects (Mwangi and Ely, 2001).

2.8.1.2 Post-harvest handling methods

These methods provide a set of tools that eliminate or limit the spread of AFs in agricultural products throughout the harvest. These include food processing, storage strategies such as drying and improved conditions and measures that are appropriate and well adapted to the agro-ecological zone.

2.8.1.2.1 Food processing

Food processing does not completely eliminate AFs contained in harvested crops but it significantly reduces AF levels in the final product (Scudamore, 2008).

This procedure can be done using various techniques including those that involve removing the shell of certain foods such as oats and pistachios (Scudamore, 2008); and torrefaction of foodstuffs such as groundnuts (Siwela *et al*., 2011). It should be noted, however, that cooking and canning have little effect on mycotoxins in general. Scudamore (2008) showed that only 23 percent of mycotoxins are lost when preparing meals at home, especially typical maize porridge in Africa and that canning contaminated food results in a loss of 15 percent of mycotoxins.

2.8.1.2.2 Storage strategies

These include:

(a) Drying

The heat does not eliminate exposure to AFs, but the gradual elimination of humidity in the crop is an effective method to prevent fungal growth and mold. The method often used is the heated drying. It is effective in limiting the spread of *A. flavus* but also AFs (Magan and Aldred, 2007).

(b) Storage conditions

Measures should be taken to minimize fluctuations in temperature and humidity in silos and other storage buildings to prevent the increased risk of AF contamination of harvested crops. Furthermore, the presence of insects in warehouses must be avoided since their activity increases the temperature and level of humidity in the cultures. This can lead to fungal growth, which in turn may result in the production of mycotoxins (Magan and Aldred, 2007). The use

of short-term silo bags can help guard against infiltration of insects and moisture. However, for long-term storage, the impermeability may result in fungal growth (Udoh *et al*., 2000).

The storage of certain products such as maize near or above the fireplace is correlated to lower levels of AF. Indeed, Udoh *et al*., (2000) reported that the dry smoke makes the stored crops less susceptible to fungal growth and insect infestation (Udoh *et al*., 2000).

2.8.1.2.3 Handling contaminated products

The AF contaminated foods can be detoxified by the use of inorganic salts and organic acids such as sodium carbonate, sodium bicarbonate, potassium carbonate, ammonium carbonate, acetic acid, the preopinante sodium and ammonia (Shekhar *et al*., 2009); and AFB1 binding agents (Oluwafemi and Da-Silva, 2009).

2.8.2 Dietary and Clinical

Dietary and clinical interventions can be considered as secondary interventions. They cannot reduce actual AF levels in food, but they can reduce aflatoxin-related illness.

2.8.2.1 Vaccination against Hepatitis B

Vaccination against hepatitis B is not officially considered as a control intervention against AFs, because the vaccine has no effect on AF levels in diets. However, knowing that AF is involved in the occurrence of hepatocellular carcinoma, together with hepatitis B and hepatitis C as major risk factors, vaccination therefore reduces the synergistic impact of hepatitis B and the AF in inducing liver cancer (Wild and Hall, 2000; Wu and Khlangwiset, 2010). Vaccination may also play a role in reducing liver cirrhosis caused by AF (Kuniholm *et al*., 2008).

2.8.2.2 Awareness campaigns

Awareness campaigns aimed at improving the knowledge in order to change attitudes and practices of both agricultural and food production methods in the community are a major pillar in the fight and prevention against AFs. This method proved to be effective during aflatoxicosis outbreak in Kenya in 2005 (Strosnider *et al*., 2006).

2.8.2.3 The use of biomarker technology

Studies of methods in which animals and humans metabolize AF provided opportunities to develop approaches of chemoprevention (bio-score) in human populations. These methods cannot reduce the levels of AFs in foods but may prevent occurrence of diseases related to AFs by reducing the bioavailability of AFs or reactive oxygen species that binds to DNA to initiate cancer (Wild and Turner, 2002).

2.8.2.4 Epidemiological monitoring systems

Recent AF exposure is reflected by the yellow colour of urine excreted, but only a small fraction of the dose is excreted in this way (Williams *et al*., 2004). Given that confirmatory tests using biomarkers is limited, an active and organized reporting system of suspected AF cases may allow earlier detection of potential outbreaks and a more rapid response (Strosnider *et al*., 2006).

In view of all these methods elucidated above, it appears that the contamination of agricultural products and human by AFs can be prevented and controlled. However, there is no single practice that can reduce aflatoxin-contamination risk to zero. The extent to which effective management modifications can be implemented is determined by agro-ecology and economic scale of the operation.

2.9 Public Health Impact of Aflatoxins

Aflatoxin has been recently considered as an important public health issue (Williams *et al*., 2004). It is a major contributor to the disease burden in both humans and animals when present in grains consumed and other staple foods and feedstuffs (Wu *et al*., 2011).

Exposure to contaminated products may lead to acute or chronic toxicities ranging from deleterious effect of several systems such as the central nervous, cardiovascular, pulmonary and digestive systems to death (Liu and Wu, 2010). AFs are also carcinogenic, mutagenic (IARC, 2002), teratogenic (Gimeno and Maria, 2003) and immunosuppressive (Turner *et al*., 2003). Furthermore, epidemiological studies have shown a strong correlation between exposure to AFs and primary liver cancer (Wild and Gong 2010). Thus, diseases caused by AF lead to reduced life expectancy, especially in developing countries where the burden is huge.

Aflatoxin exposure in children is also associated with child stunting and child neurological impairment (Gong *et al*., 2004), which are symptoms usually associated with malnutrition (Golden, 2010). A cross-sectional study in Benin and Togo investigated AF exposure in children and showed that exposure to this toxic contaminant increased markedly following weaning and was associated with reduced growth (Gong *et al*., 2004). It was further reported that the presence of AF in dairy milk **(**Van der Spiegel *et al*., 2013) increased the exposure of children since dairy milk is a major component of their diet.

It should also be noted that women consuming AF contaminated food can pass AF to their breast milk; a direct threat to the health of infants. The amount of AFM1 excreted in milk is in direct proportion to the intake of AFB1 (Van der Spiegel *et al*., 2013). Several studies presented evidence to indicate that children exposed to AF through breast milk (Van der Spiegel *et al*., 2013) and dietary exposure from unrefined groundnut oil and parboiled rice may develop liver cirrhosis (Dhanasekaran *et al*., 2011).

Besides the chronic exposure, outbreaks due to acute exposure to AF represent a great public health burden. In fact, several human mortalities have been reported in many countries as a result of aflatoxicosis outbreaks (Bennett and Klich, 2003; Lewis *et al*., 2005; Azziz-Baumgartner *et al*., 2005; Strosnider *et al*., 2006).

2.10 Economic Impact of Aflatoxins

Aflatoxins have a considerable negative impact on the national economies particularly in developing countries. Indeed, for most developing countries, the export of agricultural products including cereals, oilseeds, tubers, dried fruits and coffee grains to Europe and America constitute the basis of the economy in the agricultural sector. However, stringent measures imposed either by importing countries or by international guidelines relating to the contaminated products by AFs are a real barrier to the cross-border or transcontinental trade (Otsuki *et al*., 2001a, b). Many countries maintain non-tariff barriers which are not based on laws, treaties, or official regulations, and are therefore barriers to trade.

The main non-tariff barriers include technical barriers, such as safety standards, electrical standards, environmental standards, sanitary and phytosanitary standards and other codes of protection. Costs associated with compliance to these standards and rejection of food products can be substantial. The case of the EU, a major market for African agricultural products (Otsuki *et al*., 2001a), has however the strictest regulations on AFs. Certainly, the implementation of an European Union (EU) AF standard which is lower (4 ppb AFB1) than the internationally accepted Codex Alimentarius standard might reduce health risks by 2.3 deaths per billion people per year, but with a reduction of 64% in the export of cereals and peanuts from Africa to Europe at a cost of US$ 670 million (Otsuki *et al*., 2001*b*).

2.11 Aflatoxin in Zambia

Mycotoxin contamination of food systems has been reported to be a major problem in Zambia (Kankolongo *et al*, 2009). Most of the staple diets in Zambia (maize, peanuts, and their products) (JAICAF, 2008; Sitko *et al*., 2011) are susceptible to AFs contamination. Previous studies have reported high levels of AF exceeding the MPLs for Codex Alimentarius standards in maize (Kankolongo *et al*., 2009) and in groundnuts (Njapau *et al*., 1998; ICRISAT/ZARI, 2013). A maximum level of 4,980 ppb was reported in groundnuts in Nyimba, Eastern province (ICRISAT/ZARI, 2013).

As a consequence, Zambia which was once a net exporter of groundnuts to Europe, now cannot export groundnuts to Europe or even to South Africa because of concerns of high levels of AF (Mukuka and Shipekesa, 2013). Overall, AF contamination has cut across the value chain, affecting farmers, traders, processors, markets, and finally, consumers (IITA, 2011).

Currently, to mitigate pre-harvest AF contamination, Zambia Agriculture Research Institute (ZARI) in partnership with the International Institute of Tropical Agriculture (IITA) is conducting research focused on a biological control of AFs in groundnuts and maize. Based on a comprehensive survey carried out in Eastern and Central Provinces, isolates of aflatoxin-producing and closely related fungi from maize and groundnuts have been created by ZARI. The isolates have been used to develop a bio-control product called *Aflasafe* made up of locally isolated atoxigenic strains (ICRISAT/ZARI, 2013). This will safeguard the health of the population as well as promote groundnut export market development.

CHAPTER THREE: MATERIALS AND METHOD

3.1 Study Area

The study was conducted in Lusaka District of Lusaka province, one of the ten provinces in Zambia (Figure 4). Lusaka is the capital city of Zambia.

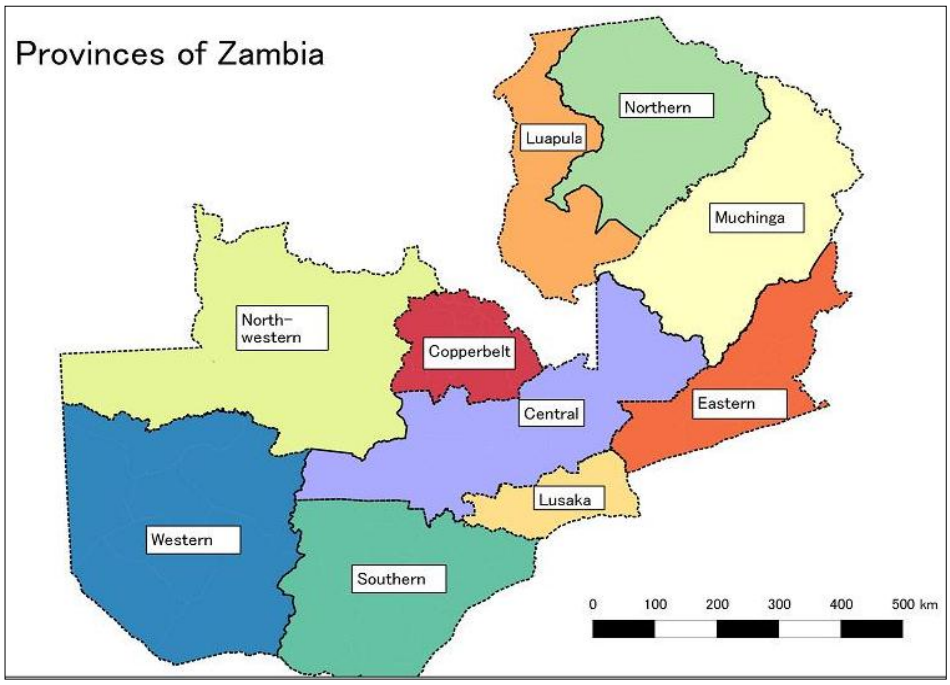


Figure 4: Map of Zambia showing the 10 provinces (Source: www.mapsofworld.com)

Lusaka province is situated in south central Zambia, located at 15º25' Latitude South and 28º17' Longitude East, the city sits on a plateau at 1280 meters in altitude (www.mapsofworld.com). The capital, Lusaka, is the centre of both commerce and government in Zambia and connects to the country's four main highways heading north, south, east and west. Lusaka province has eight districts namely: Chilanga, Chirundu, Chongwe, Kafue, Luangwa, Lusaka, Rufunsa and Sibuyunji, districts (MLGH, 2015) (Figure 5). Its population is estimated at 2,191,225 (CSO, 2012). However, about 80% of this population (1,747,152) as well as major commercial

activities are concentrated in Lusaka District (CSO, 2012), hence its preferential selection as our study area.

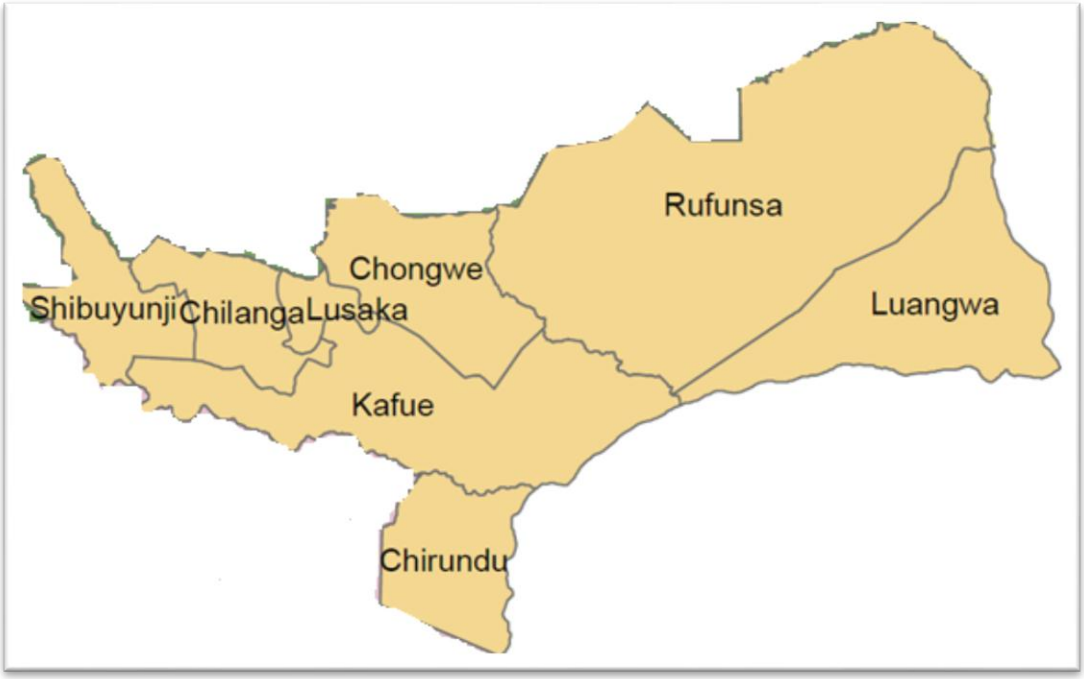


Figure 5: Map of Lusaka province showing the 8 districts (Source: Anon, 2015)

The markets frames in Zambia are still relatively underdeveloped (Munguzwe, 2012). Originally, all markets in Zambia were owned by the local authorities like the Lusaka City Council (LCC), which fall under the Ministry of Local Government and Housing (MLGH). However, on one side, due to the sprawling informal sector and demand pressure for more trading places, markets with rudimentary structures without title to land have sprung up through traders coming together. Some of these traders continue trading informally while others get registered as market cooperatives under the Ministry of Agriculture and Livestock (MAL). On the other hand, due to the openness to foreign direct investment, the private supermarket with good and more hygienic infrastructures have been built more recently (Munguzwe, 2012).

Most commercial activities are concentrated in the Central Business District (CBD) along Cairo road, Cha Cha Cha road and Freedom way. However, the pattern is slowly changing as more

business houses open up in various parts of Lusaka. Other facilities can be found in the second class shopping area of Kamwala. Lusaka District has also a number of small shopping facilities in the residential suburbs such as Northmead, Woodlands, Kabulonga, Olympia, Rhodes Park, Avondale, etc. At the time of writing this report, Manda Hill, Arcades and East Park Mall along the Great East Road, Crossroads Shopping complex along Leopard Hill road, Makeni mall along Kafue road and Down Town on the Southern edge of the city were among the shopping malls that provided quality shopping accommodation outside the traditional CBD of Lusaka (LCC).

Thus, two market systems are found in Lusaka district: the open market system and the supermarket system. The open market system regroups sector of open air markets, dispersed informal vendors and traditional shops characterized by poor handling of commodities (Munguzwe, 2012). Most of the markets under LCC as well as the cooperative markets fall under the open market system. This is the predominant type of market system found within Lusaka district. The supermarket system regroups sector of markets housed in closed buildings, e.g. chain of shopping malls. Most of the private markets fall under this type of market system. Although the supermarket system is growing, this rate of growth is much slower than once thought and too slow to transform open market systems over any acceptable time frame (Munguzwe, 2012).

3.2 Study Design

A cross-sectional study design was conducted in Lusaka District for a period of one (1) month, March 2015. Lusaka District was purposively selected because its markets receive agricultural products from all provinces of the country, other African countries and the world. The heterogeneity regarding the origin of these groundnuts made markets of Lusaka District a better framework for the study. This allowed us not only to determine the level of AF in groundnuts

sold in these markets, but also to try to estimate the areas of origin that provided peanuts highly contaminated with AFs (i.e. province and district within Zambia or other countries where peanuts were imported).

3.3 Operational Definitions

(a) Raw groundnut

Raw groundnuts were defined as groundnuts, dried, shelled and sold unprocessed in the target markets at the time of the study.

(b) Open market

Open markets were defined as markets not housed in a building, where foodstuffs are sold exposed in the open air and spread on shelves or the ground (Figure 6).

Figure 6: Example of an open market

(c) Supermarket

Supermarkets were defined as markets housed in a closed building with modernized facilities, i.e. mall, arcades (Figure 7).

Figure 7: Example of a supermarket

3.4 Sample Size Estimation

Open market and supermarket of Lusaka District formed the sampling frame. A list of 28 markets were obtained from LCC, 29 cooperatives markets and 12 supermarkets were identified giving a total of 69 markets. The markets under LCC as well as the cooperative markets were categorized as open markets.

The sample size was determined as follows:

$$n = Z^2_{1-\alpha/2} * \frac{P(1-P)}{d^2}$$ (WHO, 1991)

Where:

- n is the sample size estimate

- $Z_{1-\alpha/2}$ is the Z critical value for alpha (α)
- P is the anticipated proportion of samples positive to AF
- d is the absolute precision required on either side of the proportion

The following assumptions were considered:

- The level of significance was set at 95% and α = 5%, therefore $Z_{1-\alpha/2}$ = 1.96
- The absolute precision required on either side of the proportion was assumed to be 10 percentage points of the true value.
- The anticipated proportion of samples positive to AF was assumed to be 80% (Kamika and Takoy, 2011). In their study conducted during the rainy season, Kamika and Takoy, (2011) reported the presence of AF in a proportion of 90%, 100%, and 80% in October, November and December, respectively. WHO, (1991) suggested that if the anticipated proportion is given as a range, the value closest to 0.5 should be used, hence 80% was used.

Then, $n = (1.96)^2 * \frac{0.8(1-0.8)}{(0.1)^2}$

n = 61 Markets

Given that there were limited funding to cover all the markets (in Lusaka district), the estimate sample size was adjusted for fine population according to the formula below:

$$n' = \frac{1}{\frac{1}{n} + \frac{1}{N}}$$

Thus, a sample size of 32 markets was required.

3.5 Sampling Technique

Using a proportional stratified random sampling, a total of 26 open markets and 6 supermarkets were included in the study. Within each stratum (type of market), simple randon sampling was done to obtain the required number of markets ensuring that all the constituencies were represented.

From each open market, all raw groundnuts vendors were identified and a number were assigned to each of them. Then the simple random sampling technique was applied to select at least 3 vendors. For the market having a large number of raw groundnuts vendors, at least 10% of them were selected. From the selected vendors, 500 g of raw groundnuts samples were purchased and a questionnaire was administered to those vendors in order to collect information on factors suspected to explain the levels of AF in these products.

From each supermarket, at least 500 g of raw groundnuts of each variety was purchased. The same questionnaire that was administered to the open market vendors was also administered to the supermarket vendors.

3.6 Laboratory analysis

The samples analysis was conducted in the chemistry laboratory at Zambian Agriculture Research Institute (ZARI).

Samples were analyzed using AflaTest® commercial kit with HPLC method certified by the AOAC® Official Methods Program, as official method 991.31 applicable for the determination of aflatoxin B1, B2, G1 and G2 both by fluorometry and HPLC analysis in corn, peanuts and peanut butter (Figure 8).

Figure 8: Aflatoxin analysis using AflaTest® with HPLC at ZARI

3.6.1 Chemicals and reagents

Acetonitrile and methanol were purchased from Sigma-Aldrich® (Germany). For high-performance liquid chromatography (HPLC), HPLC-grade reagents were used. Aflatoxin B1, B2, G1 and G2 for standard test were purchased from Trilogy Analytical Laboratory (USA) (Lot 120316 – 090, Total concentration AF: 5.0 µg/ml, Total aflatoxin B1, B2, G1, G2: 4/1/4/1). The concentration was determined according to AOAC International Official Methods of Analysis. An immunoaffinity column (IAC), the AflaTest® column (Vicam, Watertown, MA, USA), was used.

3.6.2 Sample preparation

For minimizing the sub-sampling error in AFs analysis, all the samples were ground using a domestic grinder (Jura-CAPRESSO INC, Model N°503, China). Then 25 g of ground sample with 5 g NaCl were weighed and mixed for analysis.

3.6.3 Extraction and clean up

The mixture was placed in a blender jar for extraction using 125 ml of methanol: water (70:30). The solution was blended at high speed for 2 minutes and then filtered using fluted filter paper. After filtering, the extract was diluted with 30 ml of purified water before being filtered through a glass microfiber filter into a clean vessel.

AflaTest® immune-affinity columns (IACs) were used to clean up the samples. Fifteen milliliters of the filtrate diluted extract was passed through the AflaTest® IAC at a rate of about 1-2 drops/second until air came through column. Then, the column was washed twice with 10 ml of purified water at a rate of about 2 drops/second; and the glass cuvette (VICAM part # 34000) was placed under AflaTest® IAC and 1.0 ml HPLC grade methanol was added into glass syringe barrel. Finally, AflaTest® IAC was eluted at a rate of 1 drop/second by passing the methanol through the column and all of the sample eluate (1.0 ml) was collected in a glass cuvette. An additional 1.0 ml of purified water was poured in the eluate then, analyzed by HPLC.

3.6.4 HPLC procedure

Reverse-phase HPLC was mainly applied to quantify AF along with fluorescence detector followed by post column derivatization (PCD) involving bromination using a water HPLC system (pump 1525; fluorescence detector 2475; analytical column Nova-pack-C18 250×4.6 mm: 5 μm). Kobra cell was used and bromide was added to the mobile phase to achieve PCD. Fifty microliter (50 μl) of diluted AF eluate was then injected into HPLC. Mobile phase included water, methanol, and acetonitrile mixture with the 600:300:200 (V/V/V) ratio. The recovery was greater than 70% of aflatoxin B1, B2, G1, G2.

3.7 Data Analysis

Data were summarized and analyzed using Excel 2007® and Stata® version 13.0 softwares, respectively. Since the data were skewed, a log transformation was done to facilitate estimation of parameters compliant to the normal distribution. A sample was considered as positive to AF if at least one of the four types was positive on HPLC chromatogram reading. The median and the geometric mean were used to estimate the central tendency while the Chi-square test was used to test for association between hypothesized categorical risk factor and the outcome (AF presence). A logistic regression was used to determine multiple effects of predictor variables on the outcome (AF presence).

CHAPTER FOUR: RESULTS

4.1 Distribution of groundnut varieties and production sources

A total of 92 groundnuts samples were analyzed from the open markets (n= 73) and supermarkets (n= 19). Three varieties of raw groundnuts mostly sold in Lusaka district's markets were *Chalimbana* (Virginia runner type, large and tan kernels), *Kadononga*, and *Makulu red* (Virginia bunch type, small and red kernels). It was observed that 75% (95% CI: 65.0 – 82.9) of raw groundnut varieties traded on Lusaka district's markets comprised the C*halimbana* variety (Figure 9). In both open and supermarkets, *Chalimbana* variety was observed in high proportion of 76.7% and 68.4%, respectively (Table 1). It was further observed that 92.4% (95% CI: 84.7 – 96.4) of raw groundnuts sold in these markets were produced in Zambia (Figure 10).

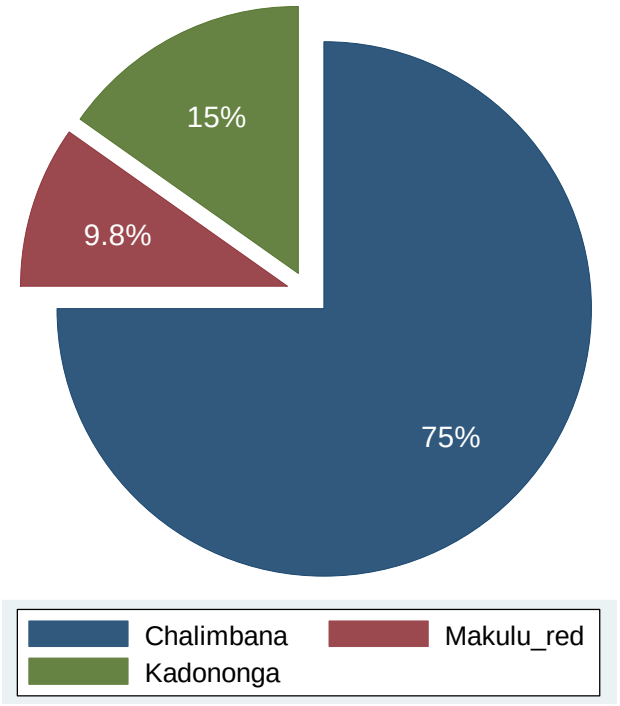


Figure 9: Distribution of groundnuts varieties in Lusaka's district markets (2015)

Table 1: Distribution of groundnut variety according to the types of markets in Lusaka District (2015).

Type of Market	Groundnut Varieties			Total
	Chalimbana (%)	Makulu red (%)	Kadononga (%)	
Supermarkets	13 (68.4)	2 (10.5)	4 (21.1)	19 (100)
Open markets	56 (76.7)	7 (9.6)	10 (13.7)	73 (100)
Total	69 (75.0)	9 (9.8)	14 (15.2)	92 (100)

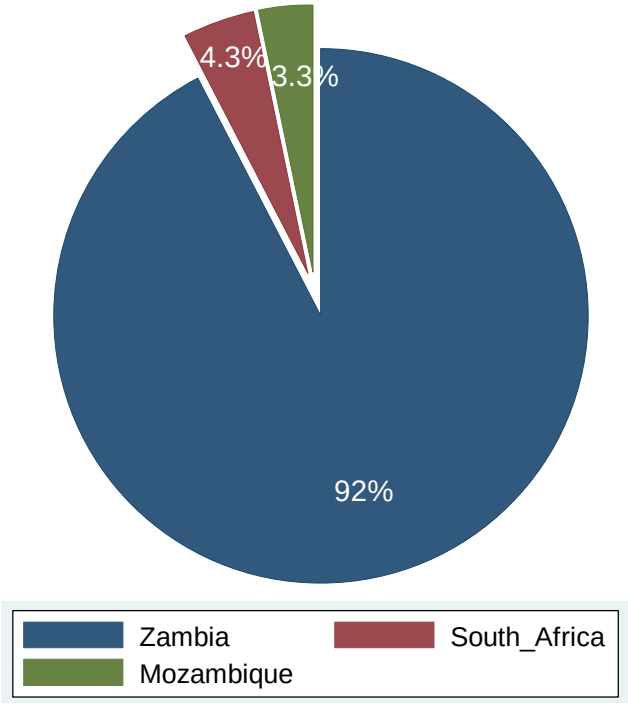


Figure 10: Origin of groundnuts sold in Lusaka district's markets (2015)

4.2 Distribution of aflatoxin levels in groundnuts and market types

Overall, 55.4% (95% C.I: 44.9 – 65.4) of the samples tested positive for AF presence. It was observed that AFB1 and AFB2 were the most common types occurring at the same prevalence (Table 2).

Table 2: Proportion estimation of AFB1, AFB2, AFG1, AFG2 and Total AF

Variables	Proportion (%)		[95% Conf. Interval]	
AFB1	Positive	**44.6**	34.6	55.0
	Negative	55.4	44.9	65.4
AFB2	Positive	**44.6**	34.6	55.0
	Negative	55.4	44.9	65.4
AFG1	Positive	**22.8**	15.3	32.7
	Negative	77.2	67.3	84.7
AFG2	Positive	**7.6**	3.6	15.3
	Negative	92.4	84.7	96.4
AF	Positive	**55.4**	44.9	65.4
	Negative	44.6	34.6	55.0

Number of observation = 92

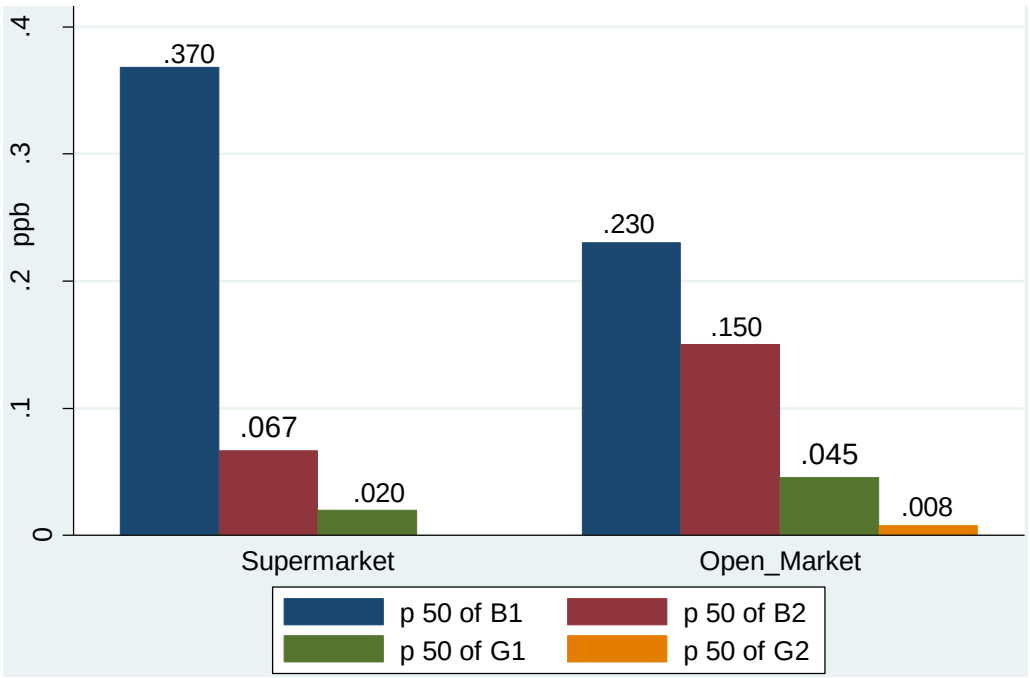
The overall median concentration for AF in groundnuts traded on Lusaka markets from both types of markets was 0.23 ppb ranging from 0.014 to 48.67 ppb. Since the data were skewed, a log transformation into normal distribution was done, which gave a geometric mean ± standard deviation of 0.43 ± 9.77 ppb (Table 3). AFB1 had the highest overall mean ± standard deviation concentration (0.45 ± 9.41 ppb) and the highest median concentration (0.23 ppb) (Table 3). The distribution of median concentration for AFB1 by market type was 0.37 ppb and 0.23 ppb in

supermarkets and open markets, respectively (Figure 11). The highest recorded single observation of AF concentration level was observed for AFB1 (46.6 ppb) while the lowest was recorded for AFG2 (0.04 ppb) (Table 3).

Table 3: Summary of the mean, median and range concentration (ppb) of aflatoxins

Variables	Positive Observations	Median (ppb)	*Mean ± SD (ppb)	Min (ppb)	Max (ppb)
AFB1	n = 41	0.23	0.45 ± 9.41	0.015	46.60
AFB2	n = 41	0.132	0.15 ± 7.87	0.006	13.17
AFG1	n = 21	0.028	0.04 ± 3.76	0.005	0.51
AFG2	n = 7	0.008	0.012 ± 2.34	0.006	0.04
AF	n = 51	0.23	0.43 ± 9.77	0.014	48.67

***Geometric mean**



p 50: Percentile 50

Figure 11: Distribution of median concentration of aflatoxins (ppb) per type of markets in Lusaka District (2015).

The distribution of all the positive samples to AF was not the same throughout the Lusaka district's markets. Overall, 78.4% (95% 64.7 – 87.8) of all raw groundnuts samples tested positive for AF were sold in the open markets. However, considering the two types of markets individually, the proportion of positive samples (57.9% and 54.8% for the supermarkets and open markets, respectively) were almost the same. This is supported by the non-significant of Chi square Test of association between the type of markets and the positivity of the samples to AF (Pearson $X^2 = 0.0587$, $p = 0.809$) (Table 4).

Considering the distribution of positive samples to AF, it was observed that *Chalimbana* groundnut variety was the most susceptible to AF contamination with 84.3% of positive samples (95% CI: 71.1 – 92.1), as compare to the other varieties. There was an apparent difference in the levels of AFs among the three varieties, although this difference was marginally not significant at 95% confidence level (Pearson $X^2 = 5.8531$, p-value = 0.054) (Table 4). However, the difference in the levels of AFs between *Chalimbana* and *Kadononga* ($p<0.0001$), and *Chalimbana* and *Makulu red* ($p<0.0001$) were significant. But there was not between *Kadononga* and *Makulu red* ($p = 0.543$).

Table 4: Distribution of positive samples (%) to AF according to the types of markets and groundnut varieties in Lusaka District (2015)

AF test	Types of Markets		Groundnut Varieties			Total
	Supermarkets	Open Markets	Chalimbana	Makulu red	Kadononga	
Positive (%)	11 (21.6)	40 (78.4)	43 (84.3)	4 (7.8)	4 (7.8)	51 (100)
Negative (%)	8 (19.5)	33 (80.5)	26 (63.4)	5 (12.2)	10 (24.4)	41 (100)
Total (%)	19 (20.6)	73 (79.4)	69 (75.0)	9 (9.8)	14 (15.2)	92 (100)

Out of all (n = 92) of positive samples to AF, 6.5% (n = 6) (95% CI: 2.9 – 13.9) and 12% (n = 11) (95% CI: 6.7 – 20.5) had AF levels above the MPLs set by the CAC and EU standards, respectively (Figures 12 and 13).

Furthermore, of all (n = 40) the groundnut samples from the open markets that tested positive for total aflatoxins, 12.5% (n = 5) (95% CI: 5.1 – 27.4) and 25% (n = 10) (95% CI: 13.7 – 41.2) were above the MPLs for CAC and EU standards, respectively (Table 5). On the other hand, 9.1% (n = 1) (95% CI: 1.1 – 47.7) of all (n = 11) the positive groundnuts samples from the supermarkets had AF levels above the MPLs for both standards (Table 5). However, there was no difference between the types of markets and the concentration levels of AF based on either the MPLs standards for CAC (p = 0.756) or for the EU (p = 0.256).

It was also observed that out of all (n = 43) the *Chalimbana* groundnut variety that tested positive for AF, 13.9% (95% CI: 6.2 – 28.4) (n = 6) and 25.6% (95% CI: 14.5 – 41.2) (n = 11) were above the MPLs for CAC and EU standards, respectively (Table 5). On the other hand,

for all *Makulu red* and *Kadononga* groundnut varieties samples that tested positives for AF, the levels were within the acceptable limits for both standards (Table 5).

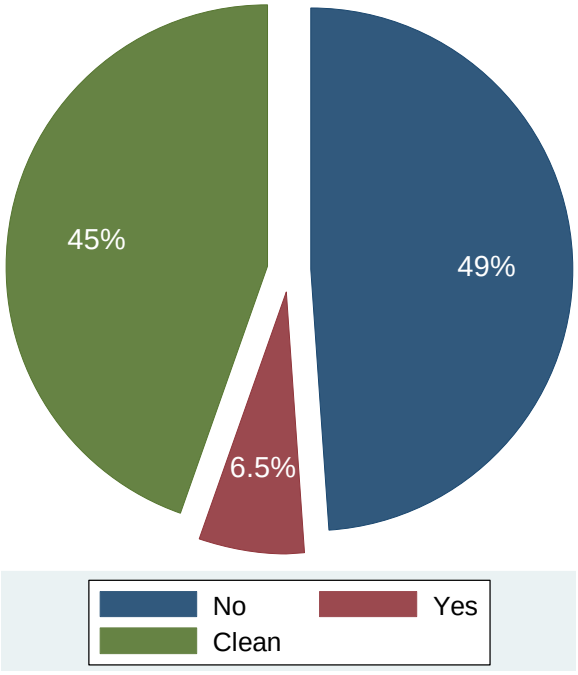


Figure 12: Proportion of AF levels above the Codex Alimentarius Standard (in red)

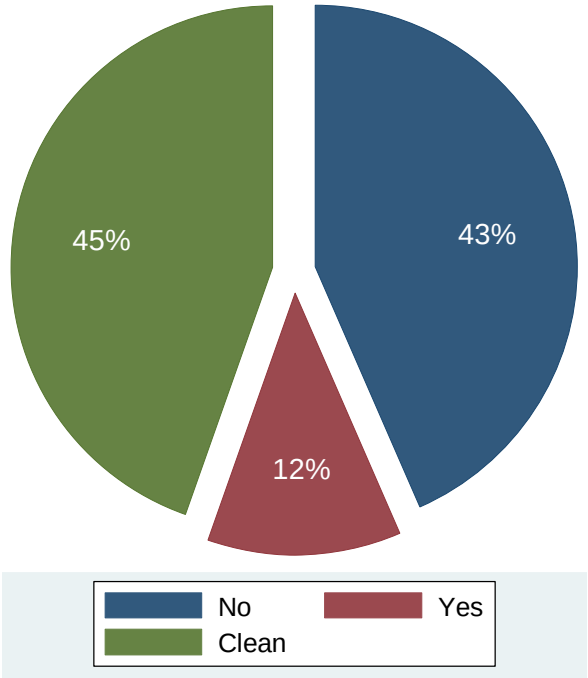


Figure 13: Proportion of AF levels above EU Standards (in red)

Table 5: Distribution of positive samples to AF according to the MPLs per types of markets and groundnuts varieties

AF Above MPLs		Types of Markets		Groundnut Varieties			Total
		Supermarkets	Open Markets	Chalimbana	Makulu red	Kadononga	
Codex std	Yes (%)	1 (9.1)	5 (12.5)	6 (13.9)	0 (0.0)	0 (0.0)	6 (11.8)
	No (%)	10 (90.9)	35 (87.5)	37 (86.1)	4 (100.0)	4 (100.0)	45 (88.2)
EU std	Yes (%)	1 (9.1)	10 (25.0)	11 (25.6)	0 (0.0)	0 (0.0)	11 (21.6)
	No (%)	19 (20.6)	73 (79.4)	32 (74.4)	4 (100.0)	4 (100.0)	40 (78.4)
Total (%)		11 (100.0)	40 (100.0)	43 (100.0)	4 (100.0)	4 (100.0)	51 (100.0)

4.3 Relationship between factors associated with aflatoxin presence in raw groundnuts samples

Among all factors included in the study, the univariate analysis indicated that only groundnut variety was marginally associated with the presence of AF in raw groundnuts at 95% confidence level (p-value = 0.054) (Table 6).

The logistic regression model revealed that *Chalimbana* groundnut variety is a useful variable in the prediction of AF positivity. In fact, after adjusting for the effect of storage place and the type of package, the estimated odds of being positive for AF for *Chalimbana* groundnut variety were 3.58 times (95% CI: 1.29 – 9.91) compared to the odds of being AF positive for *Kadononga* and *Makulu red* groundnut varieties. This CI does not overlap 1.0 confirming that the effect of groundnut variety on the AF positivity was significant (Table 7).

The Hosmer and Lemeshow test (X^2 = 0.67; Prob> X^2 = 0.9549) gives a non-significant result indicating that the model fits adequately to the data and the area under the ROC indicates that the model can correctly predict up to 66.6% of the outcome (Figure 14).

Table 6: Factors associated with aflatoxin presence in raw groundnuts samples.

Variables		**Proportion AF test positive (%)**	**P-value**
Type of markets	Supermarkets	57.9	0.809
	Open Markets	54.8	
Country of origin	Zambia	56.5	0.713
	Mozambique	33.3	
	South Africa	50.0	
Peanut variety	Chalimbana	62.3	0.054
	Makuru red	44.4	
	Kadononga	28.6	
Type of packaging	Opened permeable packaging	58.5	0.788
	Opened Impermeable packaging	50.0	
	Closed impermeable packaging	51.5	
Storage place after the daily selling	Store room	50.9	0.433
	Under the raised concrete surface	68.8	
	On the selling shelves	57.9	
Duration of raw peanut on the market	Less than 15 days	53.9	0.634
	More than 15 days	59.3	

Table 7: Results of the logistic regression model

	Odds Ratio	Z	P>|z|	[95% Conf.	Interval]
Chalimbana	3.58	2.45	0.014	1.29	9.91
Storage place1	0.38	-1.79	0.073	0.13	1.09
Type of Package1	2.36	1.65	0.099	0.84	6.58
Constant	0.54	-1.12	0.263	0.18	1.59

Storage place1: Store room; Type of Package1: Permeable package and in open air

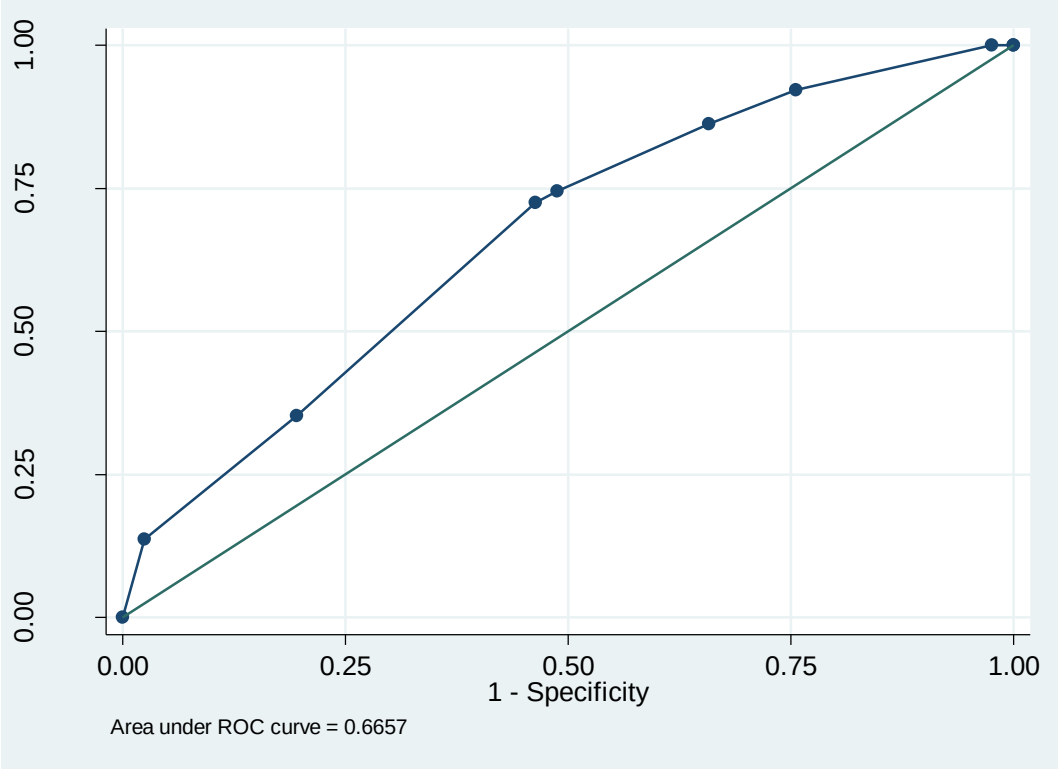


Figure 14: ROC curve analysis for aflatoxin positivity in groundnuts in Lusaka district

CHAPTER FIVE: DISCUSSION

The aim of this study was to determine the levels of AF in raw groundnuts sold in Lusaka district's markets and identify factors associated with increased AF presence in raw groundnuts in Lusaka's markets.

5.1 Determination of aflatoxins levels in raw groundnuts

It was observed that most groundnuts traded on Lusaka District's markets were contaminated with AF (55.4%), but the concentration levels were generally low. These results are comparable to those obtained by Kaaya *et al*. (2000) in Uganda who reported a proportion of 50% of positives samples to AF. However, studies conducted in Western Kenya (Mutegi *et al*., 2009) revealed levels of AF ranging from 0 to 2687.6 ppb and 0 to 7525.0 ppb from the households in two different districts. The high levels of AF could be explained by the fact that groundnut samples from the Kenyan study originated from humid agro-ecological zones which is a risk factor for the development of fungi that produce AFs (Cotty and Jaime-Garcia, 2007), and also poor storage conditions of groundnuts which characterize most of African villages (Hell and Mutegi, 2011).

The levels of AFB1, the most carcinogenic type, were detected in the range of 0.015 to 46.6 ppb in forty-one raw groundnuts samples (44.6% of prevalence) and had the highest concentration levels (46.6 ppb). This observation corroborated what was observed in similar studies conducted in D.R.C. (Kamika and Takoy, 2011), in Sudan (Shami Elhaj and Ahmed Altayeb, 2011), and in Malaysia (Suliman *et al*., 2007) where proportions of 53%, 53.3%, and 50%, respectively, of AFB1 in groundnuts samples were reported. In contrast, the aforementioned studies differ by their range of concentration levels which are higher (1.5 – 390, 0.8 – 547.5, 13.47 – 404.00 ppb, respectively) compared to our study. Nevertheless,

several studies, including those in Botswana (Siame *et al*., 1998), in Hong Kong (Lund *et al*., 2000) showed relatively low levels of contamination (0.8 to 16.00, 3.2 to 16.00 ppb, respectively) of AFB1 in groundnuts, supporting the findings in the current study.

Although the concentration level of AF detected was relatively low in most samples, the population of Lusaka District may not be safe from the adverse effects of AFs considering the fact that groundnut is among the staple food in Lusaka District (Sitko *et al*., 2011). Previous studies demonstrated that high intake of aflatoxin-contaminated foodstuff, even at relatively low level, is harmful for human health (Shephard, 2008; Wu *et al*., 2013). The situation is more critical when we consider that groundnuts powder is often added to maize porridge for infants because of its high protein content. Maize, another susceptible crop to AFs contamination, is consumed almost every day in Lusaka district, thus increasing the risk of double exposure to AF from both the groundnut and maize source.

The geometric mean ± standard deviation concentration levels of 0.43 ± 9.77 ppb was observed for AF. These results are supported by Ostadrahimi *et al*. (2014) in Iran who reported a mean ± standard deviation concentration of 3.03 ± 8.6 ppb. In a similar study conducted in D.R.C., Kamika and Takoy, (2011) reported a high mean concentration of 205.7 and 23.37 ppb during the rainy and dry seasons, respectively. This could be attributed to the fact that the samples in that study were drawn exclusively from markets in rural area characterized by poor handling of food commodities, which has been described as a factor associated with AF contamination of products (Kaaya *et al*., 2006, Huang *et al*., 2010).

In the current study, we observed low concentration levels of AFB2, AFG1 and AFG2 detected in the range of 0.006 to 13.17, 0.005 to 0.51, 0.006 to 0.04 ppb, respectively. This corroborates what has been reported in other studies where the presence of the three types of AFs in

groundnuts samples were observed in low levels compared to the B1 (Huang *et al*., 2010, Siwela *et al*., 2011).

5.2 Distribution of positive raw groundnuts samples to AFs by market types

The difference in the distribution of positive raw groundnuts samples to AFs between the two types of markets was not statistically significant in our study (Pearson chi2 = 0.0587, p = 0.809). This might be explained by the fact that most of raw groundnuts sold in both supermarkets and open markets were bought from the same source (Soweto market), the main wholesaling market in Lusaka District (Munguzwe, 2012).

However, the highest level of AFs concentration was detected in samples from the supermarkets (48.67 ppb for AF, 46.6 ppb for AFB1 with a median of 0.37 ppb). One would have expected raw groundnuts sold in the open markets to have the highest levels of AFs compared to the ones sold in supermarkets. This might be the result of the sorting practices in most of the open markets whereby discolored, broken or shriveled nuts are continuously discarded. While in the supermarkets, once the raw groundnuts are packaged, they are more likely to be kept in such condition until they are sold off the shelves. Previous studies reported that the grounding sorting significantly reduces the levels of AFs in groundnuts (Galvez *et al*., 2003; Ndung'u *et al*., 2013). Furthermore, the groundnuts in open markets are exposed "in the open air" so, they are continuously drying; while in supermarkets, they are packed in plastics that limit loss of moisture.

In contrast, 12.5% (n = 5) and 25% (n = 10) of all (n = 40) the positive raw groundnut samples to AF from the open markets were above the MPLs for Codex Alimentarius Standards (15 ppb) (CAC, 2014) and EU standards (4 ppb) (Wu *et al*., 2013), respectively. While, from the supermarkets 9.1% (n = 1) of all (n = 11) the positive raw groundnuts samples to AF exceeded

the MPLs for both standards. In a study conducted from markets in Kenya, Mutegi *et al.*, (2013) reported a high proportion of AF contamination exceeding the CAC and the EU standards in the open markets compared to the supermarkets. These results corroborate the findings of the current study.

The high proportion of AF contamination exceeding the standards set by the CAC and the EU observed in the open markets, despite the sorting that is practiced and the continuous drying effect, might be explained by several factors among them poor handling and storage conditions. In most open markets, groundnuts are stored crowded with other foodstuff and exposed to insect activities. These are among factors that could increase the likelihood of crop contamination by AF (Cotty and Jaime-Garcia, 2007). Groundnuts sold in the open markets are also exposed to rainwater by the fact that they are usually packed in open permeable bags. Thus, on one side, the sorting practical can reduce the number of crops apparently highly contaminated whilst the continuous drying can slow the growth of fungus with effect on the levels of AFs produced. On the other side, these two factors cannot eliminate the presence of AF nor their accumulation over time at a level exceeding the MPLs, given that there is this vicious circle.

Therefore, the human exposure to AF seems to be high in groundnuts sourced from the open markets. Our statement is supported by Shephard (2008) who used a risk assessment paradigm to show how MPLs for AFs in some African countries where maize and peanuts consumption is high may not adequately protect human health.

5.3 Factors associated with increased levels of aflatoxins in raw groundnuts

Of all factors studied, only the variety of groundnuts was significantly associated with the levels of AFs in raw groundnuts samples. It was observed that *Chalimbana* variety was the

most susceptible to AFs contamination compared to the other varieties. Previous studies have described *Chalimbana* variety (Virginia runner type) possessing factors that facilitate its contamination to AFs. These include the long duration it takes to maturity, 150 – 160 days, increasing its exposure to the rainfall (Hell and Mutegi, 2011; Mukuka and Shipekesa, 2013); the extremely labour intensive it takes during harvesting by the fact that the uprooting process require intensive digging as pod formation takes place all along the creeping branches of the plant and the pegs are thinner and weaker which means that pods often become separated from the plant at harvest (Ross and Matthew de Klerk, 2012). This extends the harvest period and the field exposure of the nuts to AFs after the physiological maturity (Guo *et al*., 2008).

Furthermore, the runner type (*Chalimbana*) does not resist drought and disease as compared to the bunch type (Ross and de Klerk, 2012). Okello *et al*. (2010) reported that excessive drought causes strains on pods and testas thus providing entry points for infection by fungi.

It is also hypothesized that big size of *Chalimbana* compared to other varieties investigated might play also a role in the vulnerability of this variety to AF contamination. Being relatively large in size implies that it is likely not to dry fast after harvest, thus prolonging the availability water activity (α_w) required for fungal growth.

CHAPTER SIX: CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The present study has demonstrated the wide contamination frequency of raw groundnuts to AF in Lusaka district's markets. Although the supermarkets have better infrastructures compared to the open markets, the current study did not find a significant difference in the proportion of contaminated raw groundnuts with AFs between the two market types. However, open markets had high proportion of samples with AF levels above the CAC and the EU standards; while, the highest concentration of AFs recorded in a single raw groundnuts sample from a supermarket.

The incidence of positive raw groundnuts to AF exceeding the MPLs for CAC and the EU standards was high in both supermarkets and open air markets. This constitutes a health hazard for the population of Lusaka District considering that groundnuts are among the staple foods in Lusaka District. The situation is more critical when we consider that groundnuts powder is often added to maize porridge for infants because of its high protein content. Maize, another susceptible crop to AFs contamination, is consumed almost every day in Lusaka.

Further, the variety of groundnut plays a significant role in the contamination of groundnuts to AFs. In this case, *Chalimbana* variety was found to be the most at risk of contamination with AFs.

However, the current study did not explore all key factors that could promote mold growth and AFs production in groundnuts such as the moisture content, the levels of humidity, and crops damage due to time and funding limitations.

6.2 Recommendation

Considering the high incidence of AF exceeding the MPLs of both CAC and EU standards observed in raw groundnuts, and the fact that this crop is a staple food in Zambia, the following recommendations are made:

a. Public awareness on practices that reduce the AFs contamination in groundnuts should be conducted;

b. Improvement of the facilities where the nuts are sold and stored within Soweto market need to be done since this is the source of most of the groundnut sold in Lusaka district;

c. A similar study based on maize need to be carried out in order to have an insight on the levels of AFs contained in the two staple food highly consumed in Lusaka district.

d. A human exposure assessment to AFs through consumption of groundnut and maize need to be carried out in order to determine the public health impact caused by AFs to the Zambian population.

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