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DEPARTMENT OF CHEMISTRY

AFLATOXIN LEVELS IN GROUNDNUT AND MAIZE CROPS AND RISK ASSESSMENT BASED ON CONSUMPTION IN GHANA

BY

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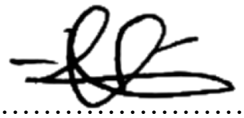
DECLARATION

I, Richard Boadu Opoku, the thesis's author, hereby affirm that I alone was responsible for the work contained in this thesis, which was done at the University of Ghana's Department of Chemistry under the supervision of Dr. Enock Dankyi and Prof. Dorcas Osei Safo.



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DEDICATION

I dedicate this work to my uncle Mr. Nana Yaw Boahene for your love, care and support. God richly bless you and increase you.

ACKNOWLEDGEMENT

I want to sincerely thank my supervisors for their assistance in making this effort a reality. I owe a debt of gratitude to my supervisor, Dr. Enock Dankyi, for his unwavering support and assistance during my time as a student at the University of Ghana. I want to express my gratitude to my co-supervisor, Prof. Dorcas Osei Safo, for all the support she gave me and my research project, as well as for your vast knowledge and timely advise that made it possible to complete this work successfully. I would also like to express my gratitude to the Senior Members of the Chemistry Department at the University of Ghana, whose significant contributions to this study through a number of seminar presentations have made it a success.

I want to express my thanks to my family for their unwavering encouragement and support, especially for taking care of my wellbeing and other eventualities.

Lastly, I praise the All-Powerful God for His mercy and strength, which enabled me to finish this program.

ABSTRACT

Aflatoxins are a type of mycotoxins that contribute to about 25% loss of annual crop production worldwide. Significant human exposure to aflatoxins is associated with detrimental health implications. Thus, the extensive presence of aflatoxins in food and feed poses a huge threat to public health in many countries in Africa including Ghana. Staples foods such as groundnut and maize are highly susceptible to aflatoxin contamination. Despite their broad exposure, research on aflatoxins in food and exposure to the population is limited and mostly restricted to exported foods. This exposes millions of people to potentially acute or chronic amounts of aflatoxins. In this work, the concentrations of aflatoxins AFB1, AFB2, AFG1, and AFG2 level in 303 samples, including 165 samples of maize and 138 samples of groundnut, obtained from homes, markets, and storage centres in eight regions of Ghana was carried out. The samples were analyzed by extracting aflatoxin with methanol/water, cleaned up on an immunoaffinity column and analysed using Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) with fluorescence detection. Based on the data obtained, aflatoxins were quantified in 80.6% of the maize samples with levels ranging from 0.20 to 1129.7 µg/kg. The quantified levels of aflatoxins in groundnut samples ranged from 0.20 to 1242.9 µg/kg, with an occurrence of 73.9% in samples. Total aflatoxins were present in more than 50% of maize and 26% of groundnut samples at concentrations that exceeded the Ghanaian standard of 10 µg/kg. The study suggests that the Ghanaian population may be exposed to aflatoxins at significant concentrations, given that, the values obtained in this study represent some of the highest levels and prevalence recorded in the country. This study provides useful data for policy decision-making on the prioritization of aflatoxin as a significant food safety concern in Ghana.

Table of Contents

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AFB1 – Aflatoxin B1

AFB2 – Aflatoxin B2

AFG1 – Aflatoxin G1

AFG2 – Aflatoxin G2

EDI - Estimated Daily Intake

ELISA - Enzyme-linked immunosorbent assay

EU – European Union

GSA – Ghana Standard Authority

HI – Hazard Index

HPLC - High-Performance Liquid Chromatography

IAC - immunoaffinity columns

IARC - International Agency for Research on Cancer

LOQ – Liquid of Quantification

MOE – Margin of Exposure

TLC - Thin-layer chromatography

CHAPTER ONE

1.0. GENERAL INTRODUCTION

1.1. Background

There have been reports of mycotoxins contamination all over the world, and it is estimated that many individuals in sub-Saharan Africa, Asia, and Latin America are exposed to high amounts of mycotoxins [1]. These toxins greatly increase morbidity and mortality, especially in children, when present in sufficient amounts [2], [3]. Most nations across the world have investigated mycotoxins such as aflatoxins, fumonisin, and ochratoxin A due to their negative impact on crop output and health consequences on humans [4]. However, the most researched mycotoxins among these are aflatoxins [5]. Several common fungi of the *Aspergillus genus including A. flavus, A. parasiticus, A. nomius, and A. pseudotamarii* primarily produce aflatoxins as secondary metabolites [6]. The four primary categories of metabolites generated by these *Aspergillus* species are aflatoxin B1, B2, G1, and G2. According to research, aflatoxin B1 is the most toxic metabolite, with the order of toxicity of remaining metabolites as follows: AFB1 > AFB2 > AFG1 > AFG2 [7][8]. Studies have shown that the high prevalence of *A. flavus* S-strain in food is responsible for the high incidence of aflatoxin B1 in different dietary samples [6].

Literature reports have shown that eating meals contaminated with aflatoxin has caused several deaths, especially in sub-Saharan Africa [1]. In the mid-2000s, there were multiple occurrences of aflatoxicosis in Kenya, where 125 people perished after eating maize contaminated with a high level of aflatoxins [9]. Long-term exposure to aflatoxins may be associated with immune system suppression, malnutrition, poor nutrient absorption, growth retardation, and a decrease in the bioavailability of zinc and vitamin A [10]. Acute exposure to aflatoxins can cause illnesses such as jaundice, liver cancer, and even death [11], [12]. Consequently, aflatoxin B1 is classified as a

class 1 carcinogen by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the International Agency for Research on Cancer (IARC) [13] due to its high propensity to cause cancer.

The primary staple foods in sub-Saharan Africa are maize, nuts, millet, and other cereals [14]. *Aspergillus spp* which produces aflatoxin grow on these crops. In Ghana, maize and groundnuts, which constitute the main staple crops, are also susceptible to these mycotoxins, especially aflatoxins [15]. This has raised much concern about the health of persons who rely on these crops as their main source of food. Besides, groundnut and maize farmers loose income since about 25% of their crops are destroyed by these fungi [16].

Groundnut (*Arachis hypogaea*) is one of the most significant oilseed crops in the global agricultural trade. This leguminous crop meets the protein needs of many households in sub-Saharan Africa who cannot afford animal protein, making it one of the region's main sources of protein. Most farmers in Ghana grow groundnuts as a source of income, particularly in the Northern, North east and Savannah regions, which accounts for around 94% of all groundnut production in the nation [17]. According to a survey conducted in April 2019, 32% of Ghanaians consume groundnuts or products made from them at least three times each week, while 68% do so at least once weekly [18]. Groundnuts are an essential source of protein, lipids, energy, and minerals that contribute to nutrition and are a significant source of income for many subsistence farmers [19]. It can be eaten raw, boiled, roasted, or processed into foods including oil, flakes, sweets, biscuits, groundnut soup, and weanimix, a formula food made from groundnut and maize for the infant [20]. Several conditions, such as drought, diseases like rust, rosette, early and late leafspots, and pests like leaf miners and aphids, all hinder the production of groundnuts [21]. In addition to these constraints, contamination of groundnut seed with aflatoxins can result from

mainly *Aspergillus flavus* and *Aspergillus parasiticus* fungi infections [22], which can make groundnut unsafe for consumption and trading.

Although groundnut is important for nourishment, its susceptibility to aflatoxin infection is worrying. The majority of farmers dry groundnut by heaping them in the field after harvesting which may considerably increase the risk of aflatoxin contamination due to the development of worms and moisture in the heap [22].

One of the most extensively farmed and consumed cereal crops in Ghana is maize (*Zea mays*). Most farmers cultivate common maize varieties such as 'Obaatanpa', 'Mamaba', 'Dadaba', and 'Aburohoma' [23]. With an annual growth rate of 8.06% [24], Ghana's capacity to produce maize is currently 2.76 million metric tons [25]. The grain, leaves, cob, tassel, and stalk are used to make a variety of food and non-food products [26], and almost every part of the maize crop is important in Ghana. Maize serves as both a staple food and a significant component of poultry feed. The middle belt, including Eastern, Ashanti, Ahafo, Bono and the Bono East regions, accounts for the majority of maize produced since these agro-ecological zones provide the rainfall and temperature requirements for growing most crops, including maize [27]. When maize is grown, the maturation period for harvest typically lasts three to four months [28]. Some crucial nutrients that are required for the human body to function properly are found in maize which comprises 72% carbohydrate, 10% protein, 4% fat and 14% fibre content, with about 365 Kcal/100 g energy density [29]. Vitamins B, essential minerals, and fibre are all present in maize [30].

Research has shown that aflatoxin B1 is the most frequently occurring congener, followed by B2, G1, and G2 [31]. According to reports, unfavourable circumstances during the storage and transportation of groundnut and maize could compromise the quality of these food crops [32]. Considering the potential risk to public health from exposure to aflatoxins, a threshold limit of 10

µg/kg for total aflatoxins has been established for raw maize and groundnuts and 5 µg/kg for aflatoxin B1 by the Ghana Standards Authority [8].

Although earlier research has reported concentrations of aflatoxins in a variety of food products, there is generally low knowledge about aflatoxin levels in the raw staples sold in the various marketplaces, homes and storage centres throughout Ghana. As a result, millions of people may be exposed to potentially high amounts of aflatoxins due to the lack of current research data in these staple crops. This study aimed to fill this knowledge gap by providing significant data on aflatoxin levels in raw maize and groundnuts from major growing and selling regions throughout Ghana.

1.2. Problem statement

According to the International Agency for Research on Cancer (IARC), millions of people in Africa are chronically exposed to aflatoxins due to the consumption of contaminated foods. Maize and groundnut-based foods represent some of the most nutritious and affordable dishes in Ghana. However, they pose serious health risk due to the susceptibility of the food crops to aflatoxins, partly due to poor storage and handling. Besides their potential role in carcinogenesis, aflatoxins also cause more than 25% of the yearly global food loss, which is a substantial economic burden.

Despite the widespread exposure, research on aflatoxins levels in food and exposure to the public is low and focused mostly on foods meant for export, leaving millions to potentially harmful amounts of mycotoxins. In light of this, this study sought to assess the levels of aflatoxins in maize and groundnuts sold in various markets across Ghana. The expected outcome is to create awareness among the public and also generate data to help policymakers and stakeholders to put in the requisite mitigation measures aimed at meeting the acceptable limits of aflatoxin in maize and groundnut in the country.

1.3. Research hypothesis

Groundnut and maize in Ghana are susceptible to aflatoxin contamination. Aflatoxin infestation occurs before, during and after the harvesting of crops. Aflatoxin contamination of cereals and legumes after harvest is caused by factors such as temperature, improper storage, and transportation of farm produce. Consequently, it is hypothesized that groundnut and maize from homes, storage centres and markets may be contaminated with aflatoxins.

1.4. General research objectives

1.4.1. Aim

The main goal of this study is to investigate the levels of aflatoxins in raw groundnut and maize in homes, storage centres and markets across Ghana.

1.4.2. Specific Objectives

The following are the specific objectives of the present study:

- Extract and purify aflatoxin congeners from maize and groundnut samples using an immunoaffinity assay.
- Assess the levels of aflatoxin in maize and groundnut using High-Performance Liquid Chromatography coupled to a fluorescence detector.
- Estimate the health risks associated with the average consumption of maize and groundnut.
- Compare the results obtained with recommended standards.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. Mycotoxins

Approximately 400 secondary metabolites with toxigenic potentials produced by about 100 fungi species have been identified to date [33]. These chemicals are known as mycotoxins, a diverse class of compounds that affect the health of both animals and humans [2]. Each year, mycotoxigenic fungi infect various food crops before or after harvest, contaminating about 25% of the world's food supply [34]. Most environmental conditions, such as excessive moisture in the field and under storage conditions, temperature variations, humidity, drought, different harvesting practices, and insect infestation, have an impact on the mycotoxin contamination levels in different crops [35]. Plant products can include many mycotoxins, either from the same or separate fungal species. These mycotoxins which are secondary metabolites appear to serve no purpose in the regular metabolism of fungi. These compounds are non-immunogenic and range in structure from groups with 6-8 typically arranged heterocyclic rings with a total molecular weight of >500 Da to simple heterocyclic rings with molecular weights of up to 50 Da [36]. Mycotoxins are a problem in food and their derivatives worldwide, not just in developing countries. Mycotoxins have an impact on agriculture in many nations, impacting or even hindering export, lowering animal and crop productivity, and endangering human health [37]. They can get into the food chains of both humans and animals either directly or indirectly. Any crops that have previously been infected by a toxigenic fungus still contain the mycotoxins even after the fungus has been removed during processing. This is known as indirect contamination of foodstuffs and animal feed. On the contrary, direct contamination occurs when fungi infect the product or food, leading to the production of mycotoxins. Humans mostly ingest mycotoxins through the intake of infected crops, as well as through the consumption of foods manufactured from animal products including milk, cheese,

meat, and other animal goods [38]. Some of the studied mycotoxins include aflatoxins, fumonisins, trichothecenes, zearalenone, citrinin, and Ochratoxin A *(figure 2.1)* [39].

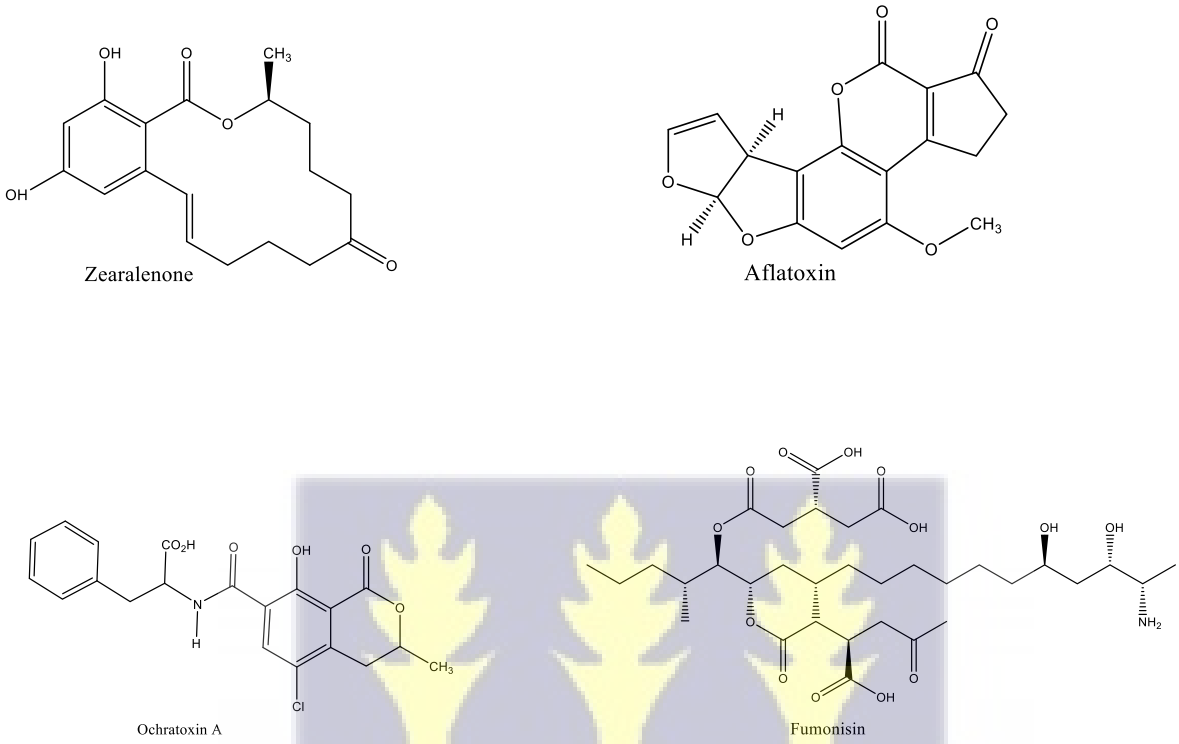


Figure 2.1 Structures of some known mycotoxins

2.2. Aflatoxins

In the late 1950s and early 1960s, researchers in England found a poison known as aflatoxin, which caused a deadly turkey illness [40]. During this period, 100,000 turkeys in London's poultry farm died of the turkey "X" disease after being fed with contaminated Brazilian groundnut meal. The appearance of the mysterious turkey "X" sickness marked the beginning of the discovery of aflatoxins [41].

Aflatoxins, a class of secondary metabolites produced by *Aspergillus*-related fungi, is generated from furanocoumarin polyketides. Despite the discovery of 18 aflatoxins, only four - Aflatoxin

B1, Aflatoxin B2, Aflatoxin G1, and Aflatoxin G2 *(figure 2.2)* - pose a threat to the safety of food [42]. Several species have been linked to the production of aflatoxins, including *A. flavus* (which generates AFB1 and AFB2), *A. parasiticus* (which produces AFB1, AFB2, AFG1 and AFG2), *Aspergillus nomius* (produces AFB1, AFB2, AFG1 and AFG2), *Aspergillus ochraceoroseus* (which generate AFB1, AFB2, AFG1 and AFG2) [43].

In addition to grains and seeds like maize, peanuts, and tree nuts, soil, plant, and animal remains may also contain several *Aspergillus* species. Long-term drought, excessive temperatures, the composition of the substrate, the length of time it is stored, and the environment are said to be the primary determinants of fungal development and the production of aflatoxins in food [34]. Although crop contamination with aflatoxins is a global problem, crops found in the tropical and subtropical [44] locations are more likely to be contaminated than those in temperate regions because these environments have the ideal humidity and temperature levels for *Aspergillus* species' toxin production [45].

Storage under circumstances that encourage fungus growth and toxin generation is a key contributor to aflatoxin contamination [46]. Naturally occurring aflatoxins have been categorized by IARC as human carcinogenic. The most prevalent and potent of the aflatoxins is aflatoxin B1 (AFB1). In the liver, aflatoxin B1 is converted to AFB1-8, 9-exo-epoxide, which produces an AFB1-N7-guanine DNA adduct that is promutagenic and results in G to T transversion mutations [47]. Up to 50% of tumours in human hepatocellular carcinoma (HCC) from high aflatoxin exposure areas have been revealed to contain a particular arginine (AGG) to serine (AGT) point mutation at codon 249 of the TP53 tumour suppressor gene [48].

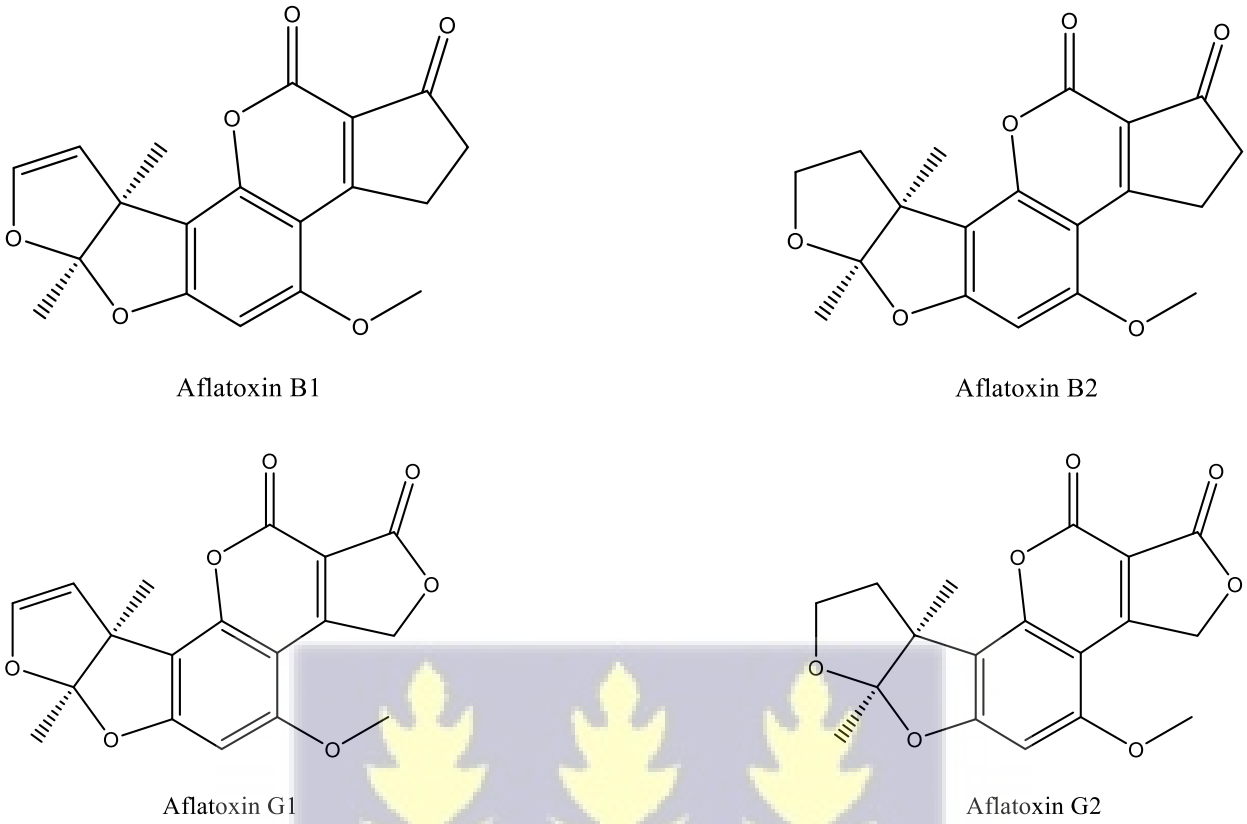


Figure 2.2 Chemical structures of aflatoxins B1, B2, G1 and G2.

2.3. Aflatoxin levels in crops and food products

The majority of Africa's regions are susceptible to fungal growth and aflatoxin contamination [32] which have been reviewed by various authors [49][4][50][20] [47]. Because of this, persons living in many communities in Africa may be exposed to aflatoxins before birth and throughout their lifetimes which has a major negative impact on their health [52]. There has been a persistent issue with aflatoxin contamination in many foods in Africa. Traditional cereals like sorghum and millet have been replaced by maize in many areas as the favoured cereal for food, feed and industrial usage [53]. Studies have, however, shown that compared to sorghum or millet, it is substantially more colonized by *Aspergillus* species that produce aflatoxins [54]. Aflatoxins and other

mycotoxins, which are produced by diverse mycotoxigenic fungi, contaminate a wide range of crops and foods globally, particularly those produced in tropical regions. A variety of factors, such as crop kinds and environmental factors, affect the level of aflatoxin contamination [46]. Many food crops are affected by aflatoxin, although some crops are more vulnerable to it than others. A crop's susceptibility to fungus invasion and subsequent toxin formation [55] is determined by a mix of environmental and intrinsic crop characteristics, including nutritional content, moisture content, and pH, among others [56]. The influence of environmental conditions on agricultural sensitivity to aflatoxin contamination is sufficiently understood, as reviewed and described by Bhatnagar-Mathur et al., 2015 [46].

Maize (*Zea mays*), which is produced and consumed globally, is one of the main sources of aflatoxin exposure to people. Several epidemics of aflatoxicosis have been caused by contaminated maize [57]. Because of this, maize-related dietary information is an essential part of many analyses of the risk and exposure to aflatoxins. Since maize is a staple food in areas where the climate is conducive to fungal development and the formation of aflatoxin, human and animal exposure to aflatoxin through the consumption of maize continues to be a serious food safety concern [56]. Numerous data show that practically all regions of the world's maize and items made from it are contaminated with aflatoxins [58]. Aflatoxin production in maize begins in the field when the kernels are infected with the fungus responsible for producing aflatoxins, and it may increase as the products move up the value chain [59]. In a study, aflatoxin incidence increased as the maize progressed up the value chain, from 32% at preharvest to 100% at the retail level based on 52 maize samples evaluated, according to a study conducted by Kamika and Tekere et al (2016) [56]. They also observed that aflatoxin levels increased from 3.1 to 300 ng/kg, which is more than the 10 ng/kg Codex Alimentarius upper limit for total aflatoxins [56].

Rice (*Oryza sativa*), one of the most consumed grains worldwide, is another food crop susceptible to aflatoxin infection [60]. Most of the environment where rice is grown is conducive to the development of fungus and aflatoxins. Consequently, its aflatoxin contamination begins in the field where grains are contaminated with aflatoxin-producing fungi [61]. Aflatoxin levels in 186 samples of rice from a study conducted in Guyana revealed that 16 of those samples, respectively, had detectable levels that were higher than the US and EU legal limits for aflatoxin [62]. In another study, 208 samples of rice and rice-based products were evaluated using samples gathered from central Punjab, Pakistan [56]. The study revealed that 35% of the samples were contaminated with aflatoxin and that 24% and 19% of those samples, respectively, exceeded the regulatory limits for total aflatoxins and aflatoxin B1 set by the EU [63]. The authors found that the mean levels of aflatoxin B1 and all other aflatoxins are greater in brown rice. Another study by Kortei et al showed that 3.7% (1/27) of rice samples analyzed in Accra, Ghana was above the permitted levels of 5 and 10 µg/kg for aflatoxin B1 and total aflatoxins respectively [64]. Although rice has lower aflatoxin levels than other cereals like maize, it is nonetheless a common diet around the world. Consequently, it can be a significant pathway for exposure to aflatoxins [65].

Aflatoxin contamination of groundnuts in Africa and other regions of the world has been studied, and the results show that groundnuts and it products derived from them are very susceptible to contamination [66]. An analysis of 1168 samples of Gambian peanuts over ten years indicated that 42% had aflatoxin contamination at levels greater than the Codex-recommended limit of 15 µg/kg [67]. According to a study done in Mali, aflatoxin builds up in peanuts at every step of production, although it is most noticeable during postharvest storage [46]. Aflatoxin levels in the 92 samples that were analyzed ranged from 0.014 to 48.67 µg/kg, with 6.5% of the samples exceeding the codex limits for aflatoxins in peanuts [56]. Another investigation conducted in Zambia between

2012 and 2014 looked for the prevalence of aflatoxin in locally and imported peanut butter and found that 73%, 80%, and 53%, of the samples collected in 2012, 2013 and 2014 respectively, were polluted at values ranging from 20 to 1000 µg/kg [68]. According to a study by Kortei et al. in Ghana, 49 (61.25%) of the 80 samples examined tested positive for AFB1, with results ranging from 0.38 to 230.21 µg/kg [69]. The ratio of total aflatoxins, which ranged from 0.38 to 270.51 µg/kg, was also observed [70].

2.4. Maize

One of the most widely grown crops in the world is maize [71]. In Asia, maize is one of the top three crops, and it is the main contributor to food security and economic growth in Latin America, the Caribbean, and sub-Saharan Africa (SSA) [72]. Between 1993 and 2013, the harvested maize increased at an average annual growth rate of 2.7% in Africa, 3.1% in Asia, and 4.6% in Latin America [73]. In terms of caloric intake, cereals like maize are the key staple crops in sub-Sahara Africa. The most widely grown grain in Sub-Sahara Africa, maize, is crucial to this region's food security and livelihoods [74]. In West Africa, maize is a significant crop for food. An estimated 18.5 million tonnes of maize is produced annually. Nigeria makes up roughly 54.5% of the overall production, followed by Ghana, Burkina Faso, Mali, and Benin with contributions of 9.25%, 8.95%, 7.88%, and 7.05%, respectively [75], [76]. Maize yields have doubled over the past ten years [73]. This is attributed to the use of improved maize varieties and the use of fertilizers aided by agricultural policies for the improvement of crop productivity [75]. Human consumption accounts for over 60% of maize production [77]. For many years, aflatoxin contamination has raised concerns regarding the quality of the maize sold in markets.

Maize (*Zea mays* L.) has been grown in Ghana for so many years and is the most widely grown and consumed cereal crop [78]. Most farmers in Ghana plant common maize varieties like

'Obaatanpa', 'Mamaba', 'Dadaba', and 'Aburohoma' and maize is a significant source of calories in Ghana [79]. According to a report, sorghum and pearl millet, two traditional food crops, are grown in northern Ghana [80]. Ghana produces a million metric tons of maize each year, most of which is used as a primary staple food in the homes of the producers [81]. The maize grain is used as food for birds in the poultry business and is consumed in a variety of ways across traditions and cultures [82]. Only 20% to 25% of the entire amount of maize sold is used for processing and other industrial applications [82].

Many food products, including feed, maize grit, alcoholic beverages, baby food, and morning cereal, all contain maize. Along Ghana's transitional regions, there are essentially two maize growing seasons (major and minor) [24], and typically one harvest season falls during a wet season, endangering grain quality in relation to fungal growth and insect pest infestation since the majority of farmers rely on the sun for drying their crops [83]. Storage of maize is a significant problem for maize production [24] and a major cause of post-harvest losses of maize worldwide. Post-harvest losses of maize are estimated to be 30% and include inadequate post-harvest management, late harvesting, insufficient drying, and improper storage systems as primary factors [84]. The location of markets and the time of year affect the wholesale price of maize, with prices typically being highest during the off-seasons [85].

2.5. Groundnut

Groundnut is an important monoecious legume [86] crop widely cultivated for its oil, food, and its use as animal feed [87]. It is primarily grown in tropical, subtropical, and warm temperate regions [88]. In developing countries, groundnut is an important source of cash revenue that considerably improves food security and reduces poverty [89]. Women cultivate and manage the crop to a large extent throughout many sub-Saharan African counties. As a result, groundnut farming directly

affects the general economic and financial well-being as well as the dietary status of women and children [90]. According to FAOSTAT, there are 26.4 million hectares of groundnut farms worldwide, producing a total of 38.2 million metric tons [86] of groundnuts. 97% of the world's groundnut farmland is in developing countries where 94% of the crop is produced [91]. Several abiotic, biotic, and socioeconomic factors affect how much groundnut can be produced. Aspergillus species, widespread saprophytic fungi that can be found in soil and seeds in all of the major groundnut-producing regions of the world, are the most well-known pollutants of groundnuts [92].

Ghanaian farmers rely heavily on groundnuts as a source of revenue and as a major supply of macro- and micronutrients [93]. According to FAOSTAT (2016), Ghana is one of the largest producers of groundnuts in the world [94] and 80% of Ghanaians eat groundnut at least once a week [95]. Groundnuts are a staple food in Ghana and the majority of other West African nations due to their versatility in preparation and consumption. A sufficient amount of attention has not, however, been paid to quantifying the use of groundnuts in Ghana [96]. An investigation is required into the frequency and variables affecting household consumption and trading practices for groundnuts at different stages of the marketing cycle. Many crops, including groundnuts, are prone to fungal infestation when cultivated under conditions of heat or drought stress or when improperly dried before storage [97].

2.6. Factors promoting fungal growth and aflatoxin production in food

Fungi development and mycotoxin generation are influenced by environmental factors [32]. The development of mycotoxin patterns can be deeply affected and drastically altered by factors like water activity, temperature, pH, and atmosphere.

2.6.1. Temperature

Whether the temperature is high or low, fungal growth and the subsequent mycotoxin formation are considered to be unavoidable [34]. Temperatures above 20°C promoted the growth of Aspergillus species whereas temperatures below 20°C favoured Penicillium and Cladosporium growth [98]. It has been noted that due to the storage temperatures, Aspergillus species were more likely to grow on food items like cereals and beans than on any other toxin-producing fungi [98]. When additional favourable conditions are present, it has been reported that fungal activity and toxin production are at their peak between 25 and 37 °C [99]. Ghana's average temperature falls between these ranges. The maximum level of aflatoxin production in maize was 2278-3082 µg/kg at 37 °C, and the highest Aspergillus growth rates were 6.9 mm/day at 35 °C were reported by Markus et al [100]. However, the impact of both temperature and moisture cannot be separated. Another study on chickpea grains showed that the maximum growth rate of *A. flavus* occurred at 30 °C. The study also showed that the concentrations of aflatoxin varied significantly with water activity and temperature [101].

2.6.2. Moisture content

As water content greatly controls microbial development and the formation of toxins, it is a critical component that affects both the grade and the ability to store grains and legumes [3]. Aflatoxin formation in food crops is thus significantly influenced by this factor. Aspergillus and other storage fungi need about 15% moisture or 65% relative humidity for growth and toxin generation [102]. The ideal level for growth and proliferation, however, is 77% or higher relative humidity [103]. When examining the impact of water activity (a_w) on both *A. flavus* and aflatoxin (AFB1) production in peanuts at 25 °C, it was discovered that maximum growth of *A. flavus* occurred at 0.95 a_w with the highest aflatoxin production at 0.90 a_w and 0.95 a_w after three weeks of storage

[100]. It has been demonstrated that water activity increases with storage duration, along with insufficient drying, making stored cereals and legumes more susceptible to fungal infestation, growth, and aflatoxin production [100]. The risk of *A. flavus* and its metabolites accumulating in Ghana may be significant due to the maturity and harvest of food crops at the end of the rainy season. Traditional drying methods use bare ground and field drying considerably increases the risk of fungal infection [104]. These methods take a lot of time and effort, need a lot of crop handling, and may not effectively dry the crop. It can be challenging to get the ideal moisture level for secure storage when this problem is exacerbated by continuous rain during harvesting and drying [105]. A study on the impact of environmental conditions on A. *flavus'* formation of aflatoxin during storage suggests that moisture and incubation time had a significant interaction effect on AFG1, AFB2, AFB1, and total aflatoxins [106].

2.6.3. Soil properties

A significant contributing element to the fungal contamination of agricultural produce is the soil [107]. As a result, there may be large variations in the frequency of aflatoxin in crops grown in various types of soil. In groundnut, fungi grow more quickly under light sandy soils, especially in dry conditions, while heavier soils produce less contamination because of their high water retention capacity, which lessens the stress of dryness [108]. Although Ghana has a variety of soil types, including sandy, loamy, and clayey, the precise type of soil in a given location may depend on the location in the country. The bulk of the country's soil is sandy or sandy loamy in the north, whereas clayey loamy to dark loamy soils are found in the south [104]. Nevertheless, the majority of the soils in Ghana are light sandy to sandy loamy and are used to cultivate grains and legumes such as maize, millet, groundnuts, bambara beans, and beans [3]. The significant aflatoxin contamination in some of these crops may be partly caused by the type of soil. Additionally,

according to Atongbiik et al 2017, light sandy soils, particularly under unfavourable dry circumstances, encourage the rapid expansion of *A. flavus* [3].

Studies have revealed that although excessive moisture weakens the seed coats and pods, severe drought stresses them, creating strains that act as entry places for fungi. In Ghana, particularly in the north, where rainfall occurs between May and September and the rest of the year is considered the dry season [109], droughts are a typical occurrence. As a result of the prolonged dry season, legumes like groundnuts and bambara beans which are often harvested during dry seasons experience stress on their seed coats and pods, opening up potential entry routes for fungi [3]. Conversely, certain leguminous crops that are occasionally harvested during a period of heavy rainfall may experience the weakening of the seed coat and pod due to an excess of moisture. The seeds may become extremely vulnerable to fungal infection as a result [3].

2.6.4. Heat

Studies have shown that aflatoxin is heat resistant and cannot be destroyed by normal cooking temperature, although their levels in food can be reduced [110]. According to a study by Kpodo et al 1996 in Ghana, after 3 hours of cooking, aflatoxins present in kenkey do not appear to be destroyed [111]. The findings revealed, aflatoxins B2 and G2 were more heat-resistant than B1 and G1 during cooking [111]. Aflatoxin B is stable to dry heat up to its melting temperature of 260°C in the solid state [112]. However, when heated with moist heat, the lactone ring is hydrolyzed, forming a terminal carboxylic acid that is then decarboxylated [113].

2.7. Aflatoxin exposure in children, and adults including pregnant women.

Humans are affected by aflatoxin exposure because they consume infected crops. Aflatoxin exposure in people affects all genders and age groups while being more common in children and

pregnant women [114]. The introduction of complementary meals leads to a rise in aflatoxin levels with age starting from the early childhood stage [3]. A study by Tunrner et al 2013 in Benin and Togo indicated that 99% (475 out of 479) of newborns and young children exposed to this toxin were between the ages of 9 months and 5 years old [115]. Similar results were seen in subsequent studies conducted in Benin, Kenya, and Tanzania on young children who were receiving supplemental feedings of porridges made from maize [10]. In a study conducted in nine African countries, it was shown that the prevalence of aflatoxin exposure was reported to have reached 100% with levels at 40.4 pg/mg and 5.4% at levels above 200 pg/mg [3]. Aflatoxin was also found in umbilical cords, which suggests that this microbial contaminant crosses the placenta, according to the study [3]. Aflatoxin levels in breast milk were also reported to positively correlate with maternal exposure.

Based on a study on aflatoxins in human breast milk and the placental membrane of persons in Ghana, Aflatoxin M2 was discovered in 34% (n = 90) out of the 264 milk samples, with levels ranging from 16 ng/l - 2075 ng/l [116]. Additionally, the milk samples contained 185 ng/l - 43,822 ng/l [116] of aflatoxin B1. Aflatoxins B1-lysine adduct (AF-ALB) levels in umbilical cord blood samples from 785 pregnant women in Kumasi, Ghana, ranged from 0.44 pg/mg to 268.73 pg/mg albumin in women [117]. In the Ashanti region of Ghana, 140 individuals had reported levels of aflatoxin B1 with a range from 0.12 to 2.995 pmol/mg albumin [49].

Aflatoxin levels have been shown to have a substantial positive connection with anaemia in an investigation by Shuiab et al [118] to determine the connection between anemia and aflatoxin in Ghanaian pregnant women. In the Ejura-Sekyedumase district of Ghana, urine samples taken from 28 children revealed the presence of aflatoxin M1 in concentrations ranging from 24.7 - 8368.1 pg/mg creatinine of aflatoxin M1[119].

Several studies have reported aflatoxin contamination in food intended for infants. Aflatoxin B1 levels ranging from 0.18 to 23.27 ngg^{-1} have been reported to be present in 72% of the packaged cereal-based food samples that were evaluated for aflatoxin contamination [120]. Additionally, the range of the total AF was 0.18 to 25.93 µg/kg. Findings showed that 52% of cereal-based meals surpassed the EU regulatory limits of 2 µg/kg for AFB1 and 4 µg/kg for total aflatoxins [121], while 96% of processed foods intended for infants had aflatoxin B1 levels over the 0.1 [121] µg/kg permitted limit by the European Union [120]. Aflatoxin exposure levels affect the insulin-like growth protein component and prevent children from absorbing minerals, which can result in low birth weight [126], growth impairment [30], immunological suppression, and mental disabilities [122]. Infants who were exposed to aflatoxins through their mothers had low ratings for their weight and height relative to their age, according to Lombard [123]. The results from this study, suggest that aflatoxins levels that babies and children were exposed to had levels greater than the daily maximum permissible limit. Depending on the type, length of exposure, and amount, aflatoxin can also result in various cancers and deaths [32].

2.8. Analytical instruments and techniques for determining the presence of aflatoxin in food.

As food production is increasing, one of the major problems is conducting meticulous and systematic monitoring of aflatoxins in various foods. Some of the aflatoxin measurement techniques and methodologies which have been developed include thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), enzyme-linked immunosorbent assay (ELISA) and test kits [124] such as AgraStrip® WATEX [125]. Analytical requirements have been developed at both the national and international levels. Any suggested method is verified in a group trial study before being adopted as an official procedure. Appropriate procedures explicitly describe the minimum method performance criteria [126], the framework for the conduct of joint

trial research, as well as the statistical evaluation of the outcomes. The contamination level determines the minimal conditions for method performance validation. The typical range for recovery is between 70% and 110%, with a relative within and between laboratory standard deviations of 20% and 30%, respectively. Any method that has been developed and examined following these guidelines may be accepted as an official method for use in court proceedings or purposes of international trade [127].

The main advancements are based on readily accessible, potent analytical techniques, such as the production of aflatoxin specific antibodies used in ELISA and immunoaffinity clean-up [128], as well as enhanced and well-established detection systems, such as those for increasing the fluorescence of aflatoxins [129], and other analytical methods. The latter can be accomplished with HPLC by UV light irradiation or post-column derivatization by various types of bromination [130]. Recently, a ring test conducted at an international level showed the exceptional performance of methods using immunoaffinity column clean-up with subsequent HPLC determination, even at very low levels of 0.1 ng/g [126]. The immunoaffinity techniques, in particular, improve performance by producing extremely clean extracts and by making aflatoxin determination simple because they may be used for automated sample clean up.

In Ghana, some of these techniques have been used in the detection of aflatoxin in various food products. An assessment of the quality of maize sold in four West African nations' markets including Ghana was monitored using enzyme-linked immunosorbent assay (ELISA) [76]. Most studies done on aflatoxin contamination in food products have been done using the high performance liquid chromatography (HPLC) [8], [31], [43], [111], [131]. With the use of AgraStrip® WATEX test kits, an investigation was conducted to assess the mycotoxin levels of maize cultivated on farms in the northern Ghanaian region in Tamale [132]. Thin layer

chromatography was used to examine the field efficacy of two atoxigenic biocontrol agents for lowering aflatoxin contamination in Ghanaian maize and groundnuts, and the results were measured using a scanning densitometer [15], [133].

2.9. Perspectives and way forward in reducing aflatoxin exposure in the population

Aflatoxin contamination of food can be decreased, if not eliminated, despite being difficult [134]. To combat the canker, some approaches have been proven successful and are therefore advised. Public awareness campaigns are crucial among them [135]. Increasing public understanding of aflatoxin, its health concerns, and management strategies may help reduce its occurrence while disseminating scientific discoveries to a larger audience for significant societal and individual advancement [32]. By informing food vendors and the public in a language they can understand and leverage important features and food items, the information's accessibility of propagation would be improved [136]. If agricultural, scientific, and medical professionals who are adequately knowledgeable about the toxin collaborate and actively participate, the awareness-raising effort would be more successful and the information would be well-received [137].

Furthermore, management methods such as timely planting, managing the effects of drought, controlling weeds and pests, early harvesting, maintaining high hygiene, and washing and sorting agricultural produce should be strongly encouraged [3]. These procedures would aid in removing or drastically reducing the circumstances and elements that encourage fungal growth and aflatoxin contamination [138]. Crop rotation can be used because it disrupts the cycles and accumulates populations of organisms that produce aflatoxin in crops [139]. Likewise, the development of resistant cultivars would aid in limiting the toxin's spread. This is particularly feasible if aflatoxin inhibitory compounds are found and applied in the development of novel grain and legume

varieties [140]. Before cultivation, plant breeders should be consulted for information on resistant variants [141].

To reduce aflatoxin contamination in agricultural output, the involvement of food regulatory agencies cannot be ignored [142]. These regulatory organizations must make sure that food products with aflatoxin contamination beyond the maximum permissible limits are taken off the market and destroyed without compromising the established standards. This can be accomplished by regularly checking the levels of aflatoxin in foods sold on the market and those that farmers keep, as well as by making timely data available [52]. Such information could be used as a tool for monitoring contamination levels over time as well as to guarantee that prompt and appropriate action is done to prevent the onset of problems connected to aflatoxins [143].

Reducing exposure to the toxin to a manageable level, maize and groundnut consumption frequency should be seriously reconsidered. Consuming millet, rice, guinea corn, beans, bambara beans, and soybeans, which are comparatively less vulnerable to the toxin than maize and groundnut [3], is advisable.

It is important to encourage the use of different food crops, such as root and tuber crops [3] for complementary feeding. An instance would be the suggested complementary meal combination based on orange-fleshed sweet potatoes [144]. Aflatoxin consumption by newborns and young children in developing countries may be reduced as a result of the removal of maize and groundnut from sweet potato-based recipes. There have been further discussions about sweet potato-based supplemental food as a superior substitute for infant meals [145]. But, given the short shelf life of orange-fleshed sweet potato cultivars' storage roots, more work needs to be done to guarantee all year's supply.

2.10. Risk Assessment

2.10.1. Estimated Daily Intake (EDI)

The dietary exposure of an adult and a child to aflatoxins was estimated based on the deterministic approach (JECFA, 2010). An EDI is obtained based on a comparison of data from the study with previous estimates in other studies [8], [69], [146]. Estimated exposure (µg/kg bw/day) is computed as a sum of aflatoxin exposure through the consumption of the crop under study. Multiplying the daily intake of crops and the concentration of aflatoxins in the consumed food and dividing the result by the body weight (1). Based on the assumption that processing of the food crops did not affect the levels of aflatoxins present.

$$\text{EDI} = \frac{\text{Daily intake of crop product} \times \text{Average concentration of aflatoxins}}{\text{Body weight}} \quad (1)$$

2.10.2 Population Risk Characterization

Margin of exposure (MOE) is used to characterize carcinogenic substances like aflatoxins in terms of their potential health risk to humans. The margin of exposure is given by the ratio of the Benchmark dose lower limit ($BMDL_{10}$) for aflatoxins – 0.34 $\mu g kg^{-1}$ bw day^{-1} to toxin exposure [131], [147].

$$\text{MOE} = \frac{\text{Benchmark dose lower limit}}{\text{EDI (Exposure)}}$$

2.10.3 Estimated Liver Cancer Risk

The estimated liver cancer risk is determined by [0.01 (%$HBsAg^-$) + 0.30 (%$HBsAg^+$)], where %$HBsAg^+$ represents the proportion of populations with hepatitis B and %$HBsAg^-$ represents the proportion of the population without hepatitis B. $HBsAg^+$ prevalence rate of 12.3% and 87.7% for

$HBsAg^{-}$ groups for the Ghanaian population was adopted in this study [148]. The average potency for cancer in the population is estimated as:

Average potency = (0.3 × 0.123) + (0.01 × 0.877) = 0.04567 cancers per year per 100,000 persons per μg Aflatoxins $kg^{-1}bwday^{-1}$

The population risk is determined using the following formula:

Population risk = Exposure (EDI) × Average potency

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. Geographical description of the sampling area

Ghana is a country in West Africa that is situated between Togo and Cote d'Ivoire. The Gulf of Guinea forms the southern boundary, while Burkina Faso forms the northern border [149]. It is located in the middle of Africa's Gold Coast and has a 535 km long coastline that features lagoons and mangrove forests [150]. The relative humidity is between 77% and 85% is present throughout the day, with temperatures averaging between 30°C during the day and 24°C at night [27]. There are two distinct rainy seasons in Ghana's southern region: from September through November and from April through June. Rain fall starts in Ghana's northern region in March and April, then there is occasional rain until August and September when the amount of precipitation reaches its peak.

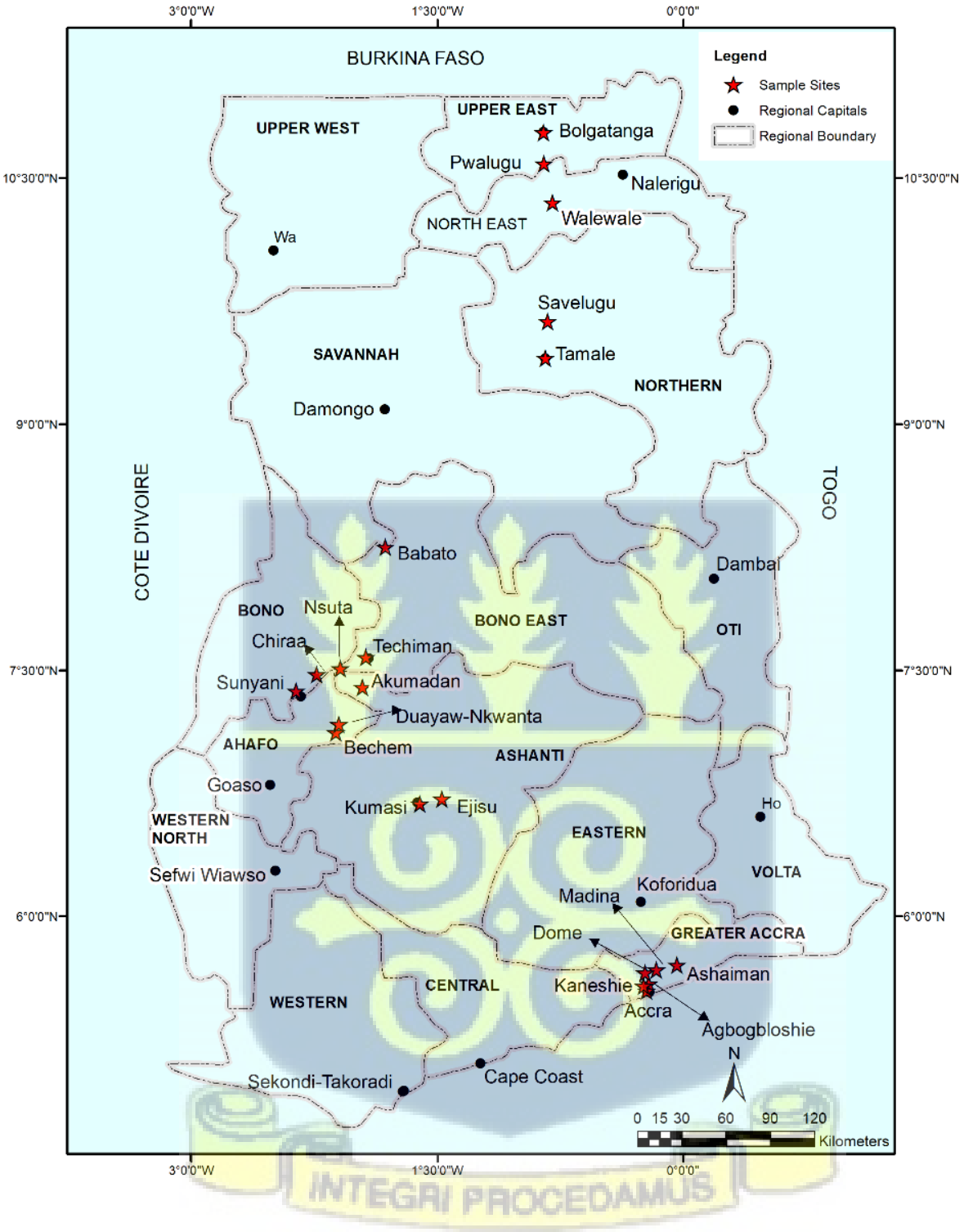


Figure 3.1 Map showing sampling sites for the study.

3.2. Sampling and Preparation

In total, 303 samples were collected from twenty-one (21) different markets in eight out of the sixteen regions of Ghana, which is made up of 165 maize samples and 138 groundnut samples. The eight regions were Greater Accra, Ashanti, Ahafo, Bono, Bono East, Northern, North East, and Upper East. The samples were collected from twenty-one different areas in the various regions as shown in the table below.

Table 3.1 Sampling regions and towns where groundnut and maize were collected.

Region	Town
Northern	Savelugu Tamale
North East	Walewale
Upper East	Bolgatanga Pwalugu
Bono East	Techiman Nsuta Babato
Bono	Sunyani Chiraa
Ahafo	Bechem Duayaw Nkyanta
Ashanti	Kumasi Akumadan Ejisu
Greater Accra	Agbogbloshie Madina Ashiaman Tema community 1 Dome Kaneshie

The samples were taken to the lab for sample preparation and analysis while being kept in clear Ziploc bags. The samples were milled using a blender for about 2 minutes in order to increase the surface area. The milled samples were then kept in a refrigerator at 4 °C before analysis.

3.3. Extraction of Aflatoxins from maize and groundnut.

The extraction and analysis were done adopting the procedure used by Blankson et al [121] with some modifications. Before analysis, milled maize and groundnut samples were kept at 4 °C in plastic containers. ISO method (ISO 16050, 2003) was used to extract the aflatoxins AFB1, AFB2, AFG1 and AFG2 from the maize and groundnut. A mass of 2 g of NaCl and 100 mL of an extraction solvent (methanol: water 80:20, v/v) were added to a 20 g of each groundnut and maize samples. Each of the samples were mixed thoroughly before weighing to unsure uniform mixture. The mixture was homogenized for 3 minutes to ensure a uniform mixture and filtered using a Whatman filter paper. Using glass microfiber filter paper, 20 mL of the filtrate was filtered after being thoroughly combined with 60 mL of 10% phosphate-buffered saline (pH =7.4).

3.4. Cleanup of aflatoxin extract.

A volume of 20 mL of the diluted extract was run through the AflaStar immunoaffinity column (IAC) at a flow rate of 1-3 mL/min to improve selectivity and sensitivity. The IAC contains a buffer at a pH of 7.4 and antibodies which traps the aflatoxins (antigens). The immunoaffinity column was washed with 10 mL of deionized water following the passage of the extract through the column. Air pressure was used to push any remaining water content in the IAC after washing. Methanol (1.5 mL) which breaks the antibodies and antigens interactions, and 0.5 mL of water were used to elute the aflatoxin in the IAC from the column and transferred to a 5 mL volumetric flask for analysis. A further 0.5 mL of methanol was then used to rinse to ensure that all the aflatoxins have been eluted. A 2 mL volume of the extract was then poured into a valve. To ensure a uniform mixture of the final extract, a Stuart SA7 vortex mixer was used to mix the extract thoroughly before being subjected to HPLC analysis.

3.5. HPLC analysis conditions and Aflatoxin content determination.

The levels of aflatoxins in the samples were determined by HPLC at the Mycotoxin Laboratory of the Ghana Standards Authority. A Shimadzu Nexera 40 Series HPLC was utilized for the aflatoxin analysis. The Shimadzu LC software package for HPLC real-time and post-operative analysis, a computer, an automated injection system, a pump with a flow rate of 1 mL/min, a column heater with a constant temperature of 40 °C, a chromatographic column made of non-polar stationary phase (silica and C18), 250 mm × 4.6mm, 5 µL post-column derivatization system, and a column oven were used. At a flow rate of 1 mL/min, a mobile phase made up of a mixture of water, acetonitrile and methanol in a ratio of 60:20:20 were used. The sample injector had a 50 µL sample loop. A fluorescence detector configured at 435 nm emission and 365 nm excitation was used to detect the eluent [151].

Before the aflatoxins levels were determined, a prepared standard, a blank and certified reference material (CRM) were run through the system to ensure better recovery and accuracy. The certified reference materials were obtained from Food Analysis Performance Assessment Scheme (FAPAS T04381QC), Sand Hutton, York. The samples in 2 mL sample vials were loaded onto the autosampler and then run for 13 minutes. The aflatoxins were eluted with the more polar being eluted first, thus AFG2, followed by AFG1, AFB2 and AFB1. The results appeared as peaks on a computer monitor screen and their respective levels in µg/kg. The percentage recoveries were determined based on the levels recorded multiplied by a dilution factor of 2. The standard values for the types of aflatoxins (AFG2, AFG1, AFB, and AFB1) from the CRM data sheet obtained from FAPAS were used in ratio and proportion calculation.

3.6. Chemicals and reagents

The chemicals and reagents used were concentrated phosphate buffer-PBS, deionized water of HPLC grade, methanol, acetonitrile, and sodium hydrogen phosphate dehydrate ($Na_2HPO_4.2H_2O$). All chemicals were purchased from the German company VWR International. The Association of Official Analytical Chemists (AOAC) method was used to prepare the stock and working standard solutions in acetonitrile. The produced solutions were stored in an amber glass vial at a temperature of about 20 °C until analysis.

3.7. Apparatus

AflaStar immunoaffinity columns (IAC), an HPLC system, a column heater, a chromatographic column 250 × 4.6 (Silica and C-18) with a pool size of 5 microns, a 5 µL post-column derivatization system, and a fluorescence detector were used.

3.8. Quality control

The reagents used for extraction and assessment of aflatoxin levels in the maize and groundnut samples were of analytical grade. Spiking was done by adding known concentrations of the various types of aflatoxins in order to evaluate the performance of the analytical method used. AFB1, AFB2, AFG1, and AFG2 standards were used during the entire aflatoxin analysis as a means of quality control checks. For every batch of samples run, the aflatoxin standards were first run, followed by the blank and a central reference material (CRM) to know the percentage recovery of the aflatoxins in the extract. The HPLC analysis of the reagent blanks revealed no aflatoxins. Before the quantification, all of the extracts were stored in a refrigerator.

Using regression analysis, correlation coefficients (r^2) were obtained from standard curves of all analytes. The standard curves for both groundnut and maize samples were linear with ranges for

aflatoxins G2 (0.125 to 2.5 µg/kg), aflatoxins G1 (0.5 – 10 µg/kg), aflatoxins B2 (0.125 – 2.5 µg/kg) and aflatoxins B1 (0.5 – 10 µg/kg).

Liquid of quantification (LOQ) and limit of detection (LOD) were estimated by adding known concentrations of aflatoxins to the sample matrix in other to evaluate the performance of the analytical method. LOQ was determined by 10 times the ratio of standard deviation to the slope of the curve and LOD by 3.3 times the ratio of standard deviation to the slope of the curve. The instrument used for this analysis was able to quantify and detect aflatoxin concentration as low as 0.1 µg/kg.

Percentage recovery for the study was evaluated based on the certified reference material (CRM) for each type of aflatoxin. The prepared samples were diluted by two fold, hence the aflatoxin levels measured were multiplied by dilution factor of two (2). For instance, the % recovery of aflatoxin B1 in maize samples is determined by the ratio of the measured value multiplied by two (2) to CRM value of aflatoxin B1 for the maize samples.

$$\%\text{Recovery} = \frac{2 \times \text{Measured value}}{\text{CRM value for aflatoxin}}$$

CHAPTER FOUR

4.0. RESULT AND DISCUSSION

4.1. Aflatoxins levels in maize

Out of 165 maize samples, aflatoxins were present in 80.6% of the analysed samples, aflatoxin B1 was present in 77.6% of the sample, aflatoxin B2 was present in 64.2% of the sample, aflatoxin G1 present in 27.9% and aflatoxin G2 present in 19.4% of the samples. Aflatoxins levels in maize range from 0.2 – 1057.70 µg/kg for AFB1, 0.1 – 79.7 µg/kg for AFB2, 0.1 – 446 µg/kg for AFG1, 0.1 – 13.4 µg/kg for AFG2 and 0.2 – 1064.7 µg/kg for total aflatoxin for the studied samples with a mean concentration for total aflatoxins varying from 3.87 to 320.64 µg/kg. Among the 165 maize samples analyzed, 50.9% of the samples exceeded the concentration of 10 µg/kg the limit set by the Ghana Standards Authority for total aflatoxins. A study done to assess the levels of aflatoxin in maize from six (6) regions in Ghana gave a range of 4.27– 441.02 µg/kg and 64.44% out of 180 maize samples exceeding the GSA standard limit of 10 µg/kg [152]. A previous study done to determine the occurrence of aflatoxins conducted on 90 samples of maize which were collected from markets throughout Ghana's regions showed that 80% tested positive for total aflatoxins and varied from 0.78±0.04 to 445.01±8.9 µg/kg which is lower than levels recorded in this study. A total of 41.25% of samples were above the limit of 10 µg/kg of GSA standard in their study compared to 50.9% of the samples in this study exceeding the GSA standard limit [8]. When the results of this study are compared to other earlier ones [152], it can be concluded that a higher percentage of the maize samples examined contained aflatoxins levels above the GSA standard of 10 µg/kg.

Table 4.1. Repeatability and accuracy of HPLC method used for aflatoxins determination in maize samples.

Aflatoxin	LOQ(µg/kg)	R^2	Recovery (%)
AFB1	**0.1**	**0.9999664**	**95**
AFB2	**0.1**	**0.9999732**	**94**
AFG1	**0.1**	**0.9999761**	**82**
AFG2	**0.1**	**0.9999698**	**84**

Table 4.2. Average levels of aflatoxin in maize samples in all the studied regions.

Aflatoxins	Average(µg/kg)
AFB1	119.78
AFB2	6.94
AFG1	9.81
AFG2	0.37
TOTAL AF	137.03

4.3. Aflatoxin levels in maize from homes, markets and storage centres in the studied regions.

Aflatoxin in maize from the various studied markets showed diverse concentrations. In the Northern region, maize samples were collected from two (2) major markets, Savelugu and Tamale markets. The level of total aflatoxins in these markets ranges from 0.3 - 550.1 µg/kg with an average level of 66.56 µg/kg. Out of the 30 samples collected from these markets, 53.3% exceeded the standard limit of 10 µg/kg for total aflatoxin and 56.7% above the limit of 5 µg/kg for aflatoxin B1 set by the Ghana Standard Authority (GSA). 60% of the samples exceeded the European Food Safety Authority (EFSA) standard limit of 2 µg/kg for aflatoxin B1 and 56.7% were above the EFSA limit of 4 µg/kg for total aflatoxin. A total of six (6) maize samples were collected from

Walewale market in the Northeast region. The results gave a range of 0.8-8.0 µg/kg and a mean level of 3.87 µg/kg. All the maize samples were below the GSA standard limits for total aflatoxins and 50% above the standard limit for aflatoxin B1.

Maize samples were collected from two markets in the Upper east region, namely Bolgatanga and Pwalugu markets. A range of 0.2 - 690.7 µg/kg and a mean value of 52.8 µg/kg were recorded from the studied samples. 22.2% and 37.0% out of the 27 samples collected were beyond the GSA limit for total aflatoxin and aflatoxin B1, respectively. 37.0% and 44.4% of the samples were also above the EFSA limit for total aflatoxin and aflatoxin B1, respectively.

In the Bono East Region, maize samples were collected from Techiman and Babato markets. The levels of total aflatoxin range from 0.2 - 98.0 µg/kg and an average concentration of 16.87 µg/kg. 27.8% and 33.3% out of the 18 samples collected exceeded the GSA standard limit of total aflatoxin and aflatoxin B1 respectively. For EFSA standards, 33.3% and 38.9% exceeded the limit for total aflatoxin and aflatoxin B1, respectively.

Sunyani and Chiraa are towns in the Bono region where maize samples were taken. The results obtained from the samples collected gave a range of 0.5 - 1129.7 µg/kg with an average level of 320.64 µg/kg. 64.4% and 78.9% of the samples were above the GSA standard limit for total aflatoxins and aflatoxin B1, respectively. 78.9% and 84.2% exceeded the EFSA limit for total aflatoxin and aflatoxin B1, respectively.

The study in the Ashanti region was focused on Kumasi, Akumadan and Ejisu markets. A total of 13 samples were collected. 0.2 - 262.3 µg/kg range was obtained with a mean concentration of 48.25 µg/kg. Out of these samples analyzed, 30.7% and 38.5% of them were above the GSA standard for total aflatoxins and aflatoxin B1, respectively. 38.5% and 46.2% were beyond the EFSA standard limit of total aflatoxin and aflatoxin B1, respectively.

In the Ahafo region, the maize samples were taken from Becham and Duayaw Nkyanta markets. The study in this region gave a range of 0.3 – 177.2 µg/kg and an average level of 69.6 µg/kg. 66.7% of the samples were above the standard limits of GSA and EFSA for total aflatoxin and aflatoxin B1.

In the Greater Accra region maize samples were taken from six different markets including Ahiaman, Agbogbloshie, Kaneshie, Madina, Dome and Tema Community 1 markets. A total of 48 samples were collected. The results gave a range of 0.6 – 1064.7 µg/kg and an average of 252.84 µg/kg. 79.2% and 81.3% were above the GSA standard limit of total aflatoxin and aflatoxin B1, respectively. 81.3% and 87.5% of the samples were beyond EFSA for total aflatoxin and aflatoxin B1, respectively.

The study revealed that Tema Community 1 market has the highest average level of aflatoxin in the maize (655.95 µg/kg) and the least level in samples from Ejisu market (1.00 µg/kg). Agbetiameh et al., (2020) studies showed that maize from various ecological zones in Ghana had aflatoxins levels ranging from 1-341 µg/kg [153], these levels are lower than the levels reported in this study. Another study done to assess levels of aflatoxins in selected locally processed cereal-based foods for human consumption from Ghana ranged from 1.77 ± 0.01 – 24.58 ± 0.05 µg/kg for aflatoxin according to Blankson et al., (2018) [121]. These levels were also lower than reported in this study. A study by Kpodo et al., (1996) reported aflatoxin levels in maize samples ranging from 20 – 355 µg/kg [111], these findings are similar to the level recorded in this study.

Table 4.3. Range of aflatoxin levels in maize samples in the studied regions.

Regions	No. of samples	AFB1 (µg/kg)	AFB2 (µg/kg)	AFG1 (µg/kg)	AFG2 (µg/kg)	Total AF (µg/kg)
Northern	30	0.3 – 501.5	0.2 – 47.2	0.5 – 446	0.1 – 12.3	0.3 – 550.1
North East	6	5.6 – 7.5	0.5 – 0.8	1.3 – 7.5	–	0.8 – 7.5
Upper East	27	0.2 – 670.2	0.1 – 20.5	0 – 23.9	–	0.2 – 690.7
Bono East	18	0.2 – 91.6	0.1 – 6.4	0.5 – 13.6	0.4 – 0.7	0.2 – 98
Bono	19	0.2–1110.1	0 – 45.3	0.1 – 47.8	0.4 – 3.7	0.5 – 1129.7
Ashanti	14	0.2 – 227.3	0.3 – 13.4	0.6 - 185.2	0.5 – 1.7	0.2 – 262.3
Ahafo	3	0.3 – 160.6	3.2 – 16.6	–	–	0.3 – 177.2
Greater Accra	48	0.4–1057.7	0.4 – 79.7	0.2 – 160.7	0.2 – 13.4	0.6 – 1064.7
All	**165**	**0.2–1057.7**	**0.1 – 79.7**	**0.2 – 446**	**0.2 – 13.4**	**0.2 – 1129.7**

Table 4.4. Average levels of aflatoxin in maize samples in studied regions.

Regions	AFB1 (µg/kg)	AFB2 (µg/kg)	AFG1 (µg/kg)	AFG2 (µg/kg)	Total AF (µg/kg)
Northern	41±20	4±2	21±10	0.7±0.4	67±30
North East	2±1	0.2±0.1	1±1	<0.1	4±2
Upper East	50±30	1.8±0.9	0.9±0.9	<0.1	53±30.
Bono East	13±6	0.8±0.4	1±1	1±1	17±7
Bono	303±90	12±3	6±4	0.2±0.2	321±90
Ashanti	24±20	1±1	23±10	0.2±0.1	48±20
Ahafo	63±50	7±5	<0.1	<0.1	70±50
Greater Accra	222±40	15±3	11±8	0.7±0.4	248±50

Table 4.5. Average levels of aflatoxin in maize samples in all the studied regions.

Regions	Towns	No. of samples	AFB1 (µg/kg)	AFB2 (µg/kg)	AFG1 (µg/kg)	AFG2 (µg/kg)	Total AF (µg/kg)
Northern	Savelugu	18	60±30	6±3	34±20	1.1±0.7	101±40
	Tamale	12	13±7	1.1±0.7	1.0±0.7	0.16±0.06	15±8
North East	Walewale	6	2±1	0.2±0.1	1±1	<0.1	4±2
Upper East	Bolgatanga	19	35±2	1.4±0.8	1±1	<0.1	38±20
	Pwalugu	8	86±80	3±3	<0.1	<0.1	88±80
Bono East	Techiman	11	9±8	0.6±0.6	0.05±0.04	<0.1	10±9
	Babato	7	21±8	1.2±0.5	4±2	0.2±0.1	28±10
Bono	Sunyani	9	80±40	6±3	0.06±0.03	0.02±0.01	86±40
	Chiraa	10	504±100	17±5	11±7	0.4±0.4	532±100
Ashanti	Kumasi	7	35±30	2±2	35±20	0.2±0.1	72±40
	Akumadan	5	15±10	0.4±0.3	12±10	0.3±0.3	28±20
	Ejisu	1	0.4±0.1	<0.1	0.6±0.1	<0.1	1.0±0.3
Ahafo	Becham	1	28±8	3.2±0.9	<0.1	<0.1	31±9
	Duayaw Nkyanta	2	80±80	8±8	<0.1	<0.1	89±80
Greater Accra	Agbogbloshie	10	245±100	15±4	17±16	1±1	279±100
	Madina	11	280±100	13±6	0.03±0.03	0.03±0.02	293±100
	Ashiaman	10	116±90	19±8	0.13±0.09	0.13±0.08	126±100
	Tema comm. 1	2	451±70	30±7	168±100	8±7	656±100
	Dome	4	372±200	24±10	0.23±0.10	<0.1	396±200
	Kaneshie	10	129±40	14±4	0.26±0.20	0.09±0.04	143±40

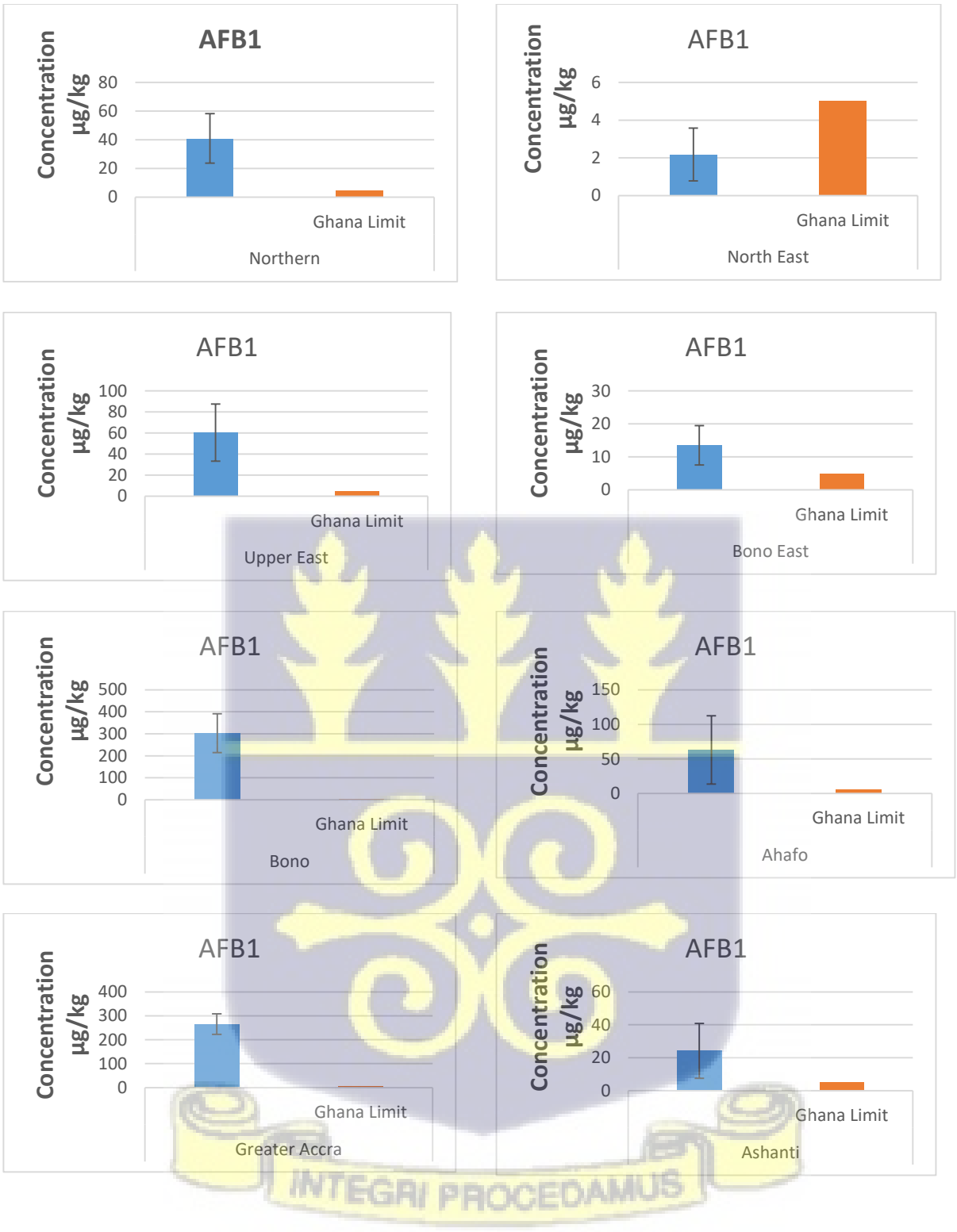


Figure 4.1. Graphs showing average levels of Aflatoxin B1 in maize samples at the various regions.

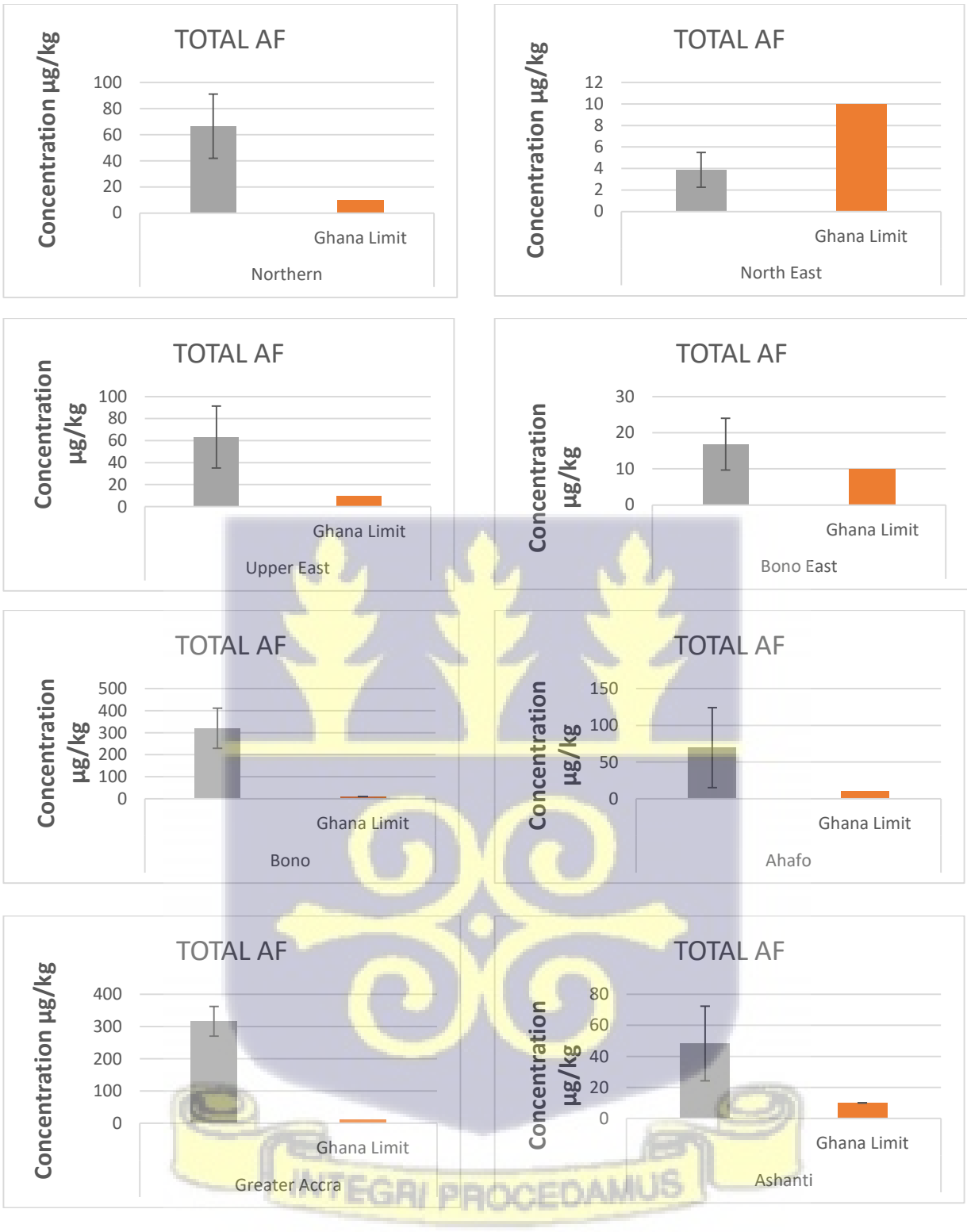
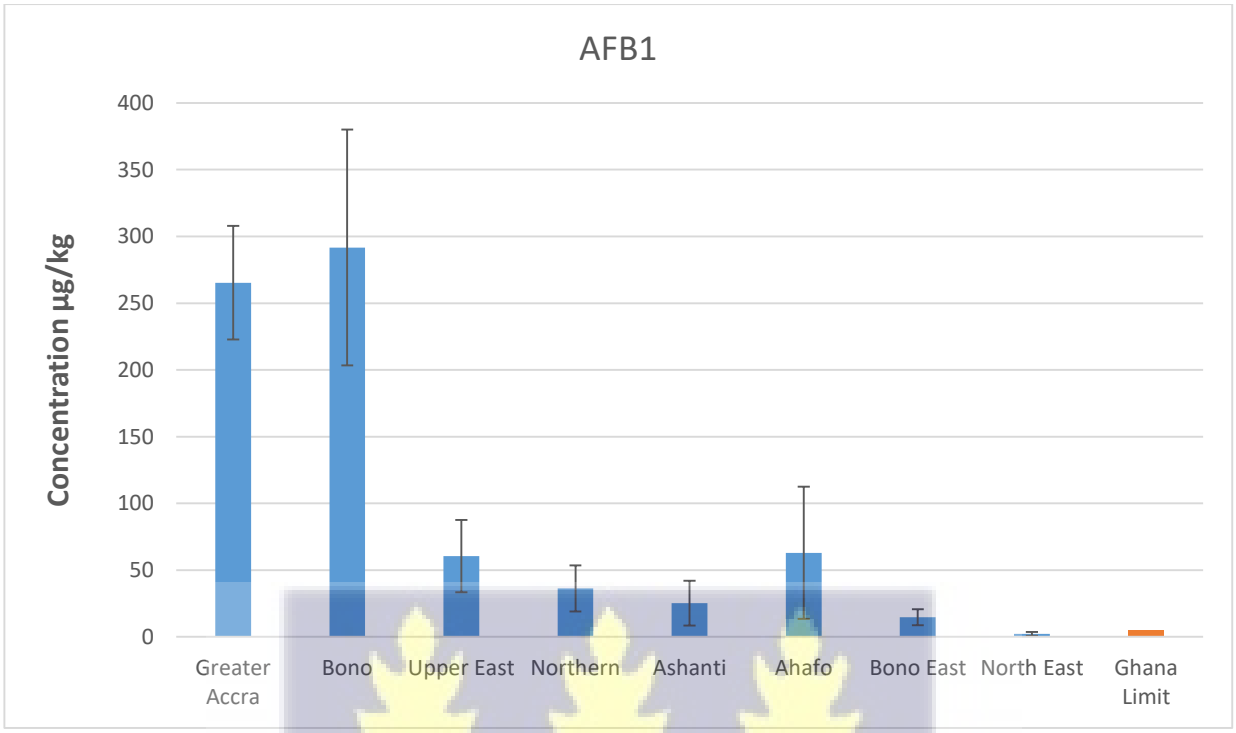


Figure 4.2. Graphs showing average levels of total aflatoxin in maize samples at the various regions.



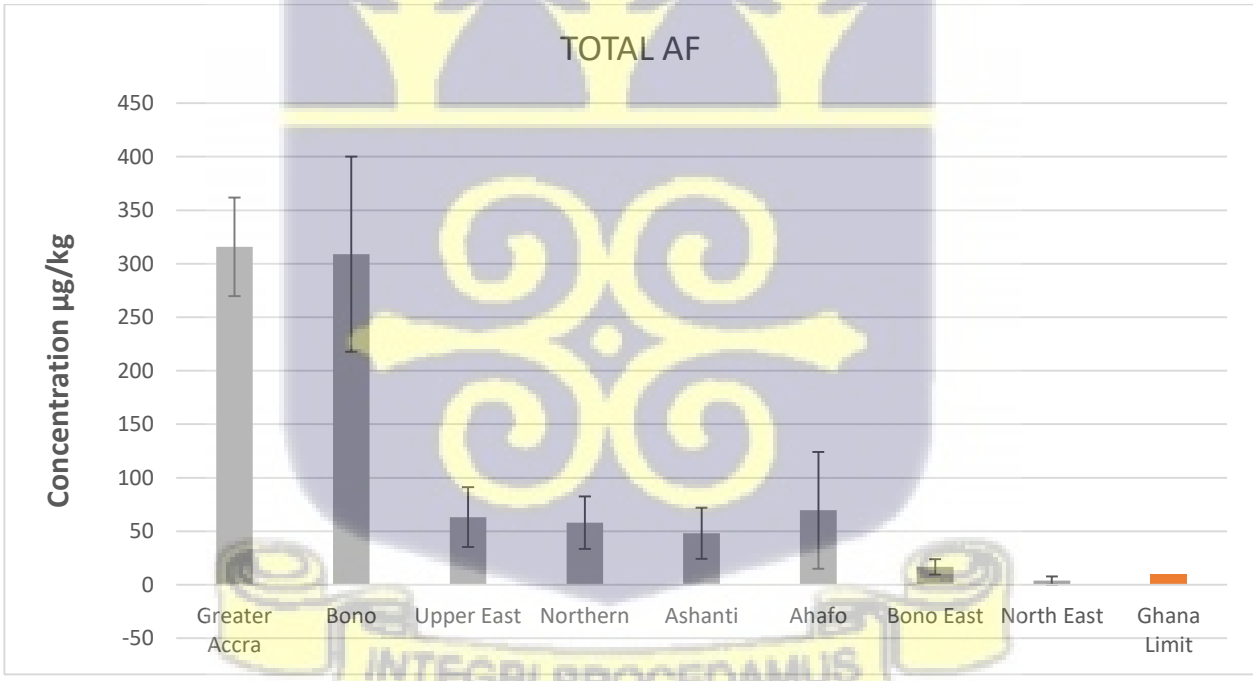


Figure 4.3. Graph showing the trend of the average levels of aflatoxins B1 and total aflatoxin in maize at the various regions.

4.4. Risk assessment

4.4.1. Estimated daily intake

In previous studies, the daily intake of maize for adults was estimated as 107 g/day and 54 g/day for children [8], [131].

Estimated exposure (µg/kg bw/day) is computed as a sum of aflatoxin exposure through the consumption of the crop under study. Estimated daily intake (EDI) is given by:

$$\text{EDI} = \frac{\text{Daily intake of crop product} \times \text{Average concentration of aflatoxins}}{\text{Body weight}}$$

In this study, the average adult body weight was estimated as 60.7 kg and 26 kg for children [147]. The estimated daily intake of aflatoxins for persons who consume maize in the studied regions ranges from 0.0068 – 0.5652 µg/kg.bw/day for adults and a range of 0.0080 – 0.6659 µg/kg.bw/day for children. The highest estimated daily intake was recorded in the Bono region and North East region recorded the lowest daily intake for both adults and children. An estimated daily intake from the studied markets ranges from 0.0018 - 1.1563 µg/kg.bw/day for adults and 0.0021 - 1.3624 µg/kg.bw/day for children. Tema Community 1 in the greater Accra region recorded the highest estimated daily intake for the various studied markets, while Walewale market in the North east region recorded the least daily intake.

Table 4.6. Estimated daily intake of aflatoxin in maize at the various regions for children and adults.

Region	EDI - Adult (µg/kg.bw/day)	EDI – Children (µg/kg.bw/day)
Northern	0.1173	0.1382
North East	0.0068	0.0080
Upper East	0.0931	0.1097

Bono East	0.0297	0.0350
Bono	0.5652	0.6659
Ashanti	0.0851	0.1002
Ahafo	0.1227	0.1446
Great Accra	0.4364	0.5142

Table 4.7. Estimated daily intake of aflatoxin in maize at the various markets for children and adults.

Markets	**EDI – Adult (μg/kg.bw/day)**	**EDI – Children (μg/kg.bw/day)**
Savelugu	0.1781	0.2099
Tamale	0.0261	0.0308
Walewale	0.0068	0.0080
Bolgatanga	0.0666	0.0785
Pwalugu	0.1559	0.1837
Tachiman	0.017	0.0201
Babato	0.0496	0.0585
Sunyani	0.1508	0.1777
Chiraa	0.9382	1.1054
Kumasi	0.1272	0.1498
Akumadan	0.0498	0.0586
Ejisu	0.0018	0.0021
Becham	0.0552	0.065
Duayaw Nkyanta	0.1564	0.1843
Ashiaman	0.2219	0.2614
Agbogbloshie	0.491	0.5784
Kaneshie	0.2522	0.2972
Madina	0.5161	0.6080
Dome	0.6979	0.8223
Tema community 1	1.1563	1.3624

4.4.2. Margin of Exposures (MOEs)

Margin of exposure (MOE) was determined based on a threshold estimated from earlier studies, using a benchmark dose lower limit ($BMDL_{10}$) for aflatoxins of 0.34 $\mu g kg^{-1}$ bw day^{-1} [154]. The margin of exposure is given by the ratio of the Benchmark dose lower limit ($BMDL_{10}$) for aflatoxins [131], [147].

$$\text{MOE} = \frac{\text{Benchmark dose lower limit}}{\text{EDI (Exposure)}}$$

The margin of exposure values for the regions ranges from 0.6 – 11.45 for adults and 0.51 – 42.50 for children. In addition, MOE values for the markets range from 0.29 -188.89 and 0.25 - 161.9 for adults and children, respectively. MOE of 10 000 or more indicates a situation of low public health issues [155]. The results from this study showed that aflatoxin exposure in adults and children consuming maize from markets across the country presents a potential public health risk since all the MOE values were below 10 000.

Table 4.8. The margin of exposure values for aflatoxin in maize samples in the studied regions.

Region	MOE - adult	MOE - Children
Northern	2.8	2.46
North East	50	42.50
Upper East	3.65	3.10
Bono East	11.45	9.71
Bono	0.6	0.51
Ashanti	4	3.39
Ahafo	2.77	2.35
Great Accra	0.78	0.66

Table 4.9. The margin of exposure values for aflatoxin in maize samples in all the studied markets.

Markets	MOE - adult	MOE - Children
Savelugu	1.91	1.62
Tamale	13.03	11.04
Walewale	50	42.5
Bolgatanga	5.11	4.33
Pwalugu	2.18	1.85
Tachiman	20	16.92
Nsuta Tachiman	6.85	5.81
Babato	2.25	1.91
Sunyani	0.36	0.31
Kumasi	2.67	2.27
Akumadan	6.83	5.8
Ejisu	188.89	161.9
Becham	6.16	5.23
Duayaw Nkyanta	2.17	1.84
Agbogbloshie	1.53	1.3
Madina	0.69	0.59
Ashiaman	1.35	1.14
Tema community 1	0.66	0.56
Dome	0.45	0.41
Kaneshie	0.29	0.25

4.4.3. Liver Cancer risk valuation through the consumption of maize.

The population risk of liver cancer from the consumption of maize was estimated based on an average potency value of 0.04567 µg aflatoxins $kg^{-1}bwday^{-1}$ [148]. The average potency for cancer in the population is estimated as:

Average potency = (0.3 × 0.123) + (0.01 × 0.877) = 0.04567 cancers per year per 100,000 persons per μg Aflatoxins $kg^{-1}bwday^{-1}$ as explained previously.

The population risk is determined using the following formula:

Population risk = Exposure (EDI) × Average potency

The study gave cancer risk for adults ranging from 0.0003 - 0.0258 for the studied regions and a range of 0.0004 - 0.0341 for children in the regions. The results showed that the chance of developing liver cancer is highest in the Bono region and least in the North-east region for both adults and children. For the studied markets, cancer risk for adults ranged from 0.0003 - 0.1974 and a range of 0.0001 - 0.0622 for children. Kaneshie market in the greater Accra region gave the highest value for cancer risk and Ejisu market in the Ashanti region showed the lowest value for cancer risk.

An analysis of randomly chosen samples of maize from several Ghanaian markets for the presence of aflatoxins and an estimation of the dangers to human health revealed population risk for infants, children, adolescents, and adults were 3.35, 1.80, 1.01, and 0.77, respectively [8]. These values reported were higher than the cancer risks reported in this study. Chun et al 2006 estimate that high cancer risk values for liver cancer incidence from consuming these foods for AFB1 were 5.78×10^{-6} for people who tested negative for hepatitis B and 1.48×10^{-4} for people who tested positive in Korea [156] which suggest low cancer risks.

Table 4.10. Cancer risk for aflatoxin in maize samples in the studied regions.

Regions	Cancer risk - Adult	Cancer risk - Children
Northern	0.0052	0.0063
North East	0.0003	0.0004
Upper East	0.0043	0.0050

Bono East	0.0014	0.0016
Bono	0.0258	0.0341
Ashanti	0.0039	0.0046
Ahafo	0.0056	0.0066
Great Accra	0.0199	0.0235

Table 4.11. Cancer risk for aflatoxin in maize samples in all the studied markets.

Markets	Cancer risk - Adult	Cancer risk - Children
Savelugu	0.0304	0.0096
Tamale	0.0045	0.0014
Walewale	0.0012	0.0004
Bolgatanga	0.0114	0.0036
Pwalugu	0.0266	0.0084
Tachiman	0.0029	0.0009
Nsuta Tachiman	0.0085	0.0027
Babato	0.0258	0.0081
Sunyani	0.1602	0.0505
Kumasi	0.0217	0.0068
Akumadan	0.0085	0.0027
Ejisu	0.0003	0.0001
Becham	0.0094	0.0030
Duayaw Nkyanta	0.0267	0.0084
Agbogbloshie	0.0379	0.0119
Madina	0.0838	0.0264
Ashiaman	0.0431	0.0136
Tema community 1	0.0881	0.0278
Dome	0.1192	0.0376
Kaneshie	0.1974	0.0622

4.5. Aflatoxin levels based on groundnut composition.

A total of 138 groundnut samples were collected from twenty-one (21) different locations at marketplaces, homes and storage centres from eight (8) regions in Ghana. Out of the 138 groundnut samples, the results showed that 69.6% tested positive for AFB1, 44.2% for AFB2, 22.5% for AFG1, 13.0% for AFG2, and total aflatoxin 73.9%. Aflatoxins levels in groundnut range from 0 – 1221.6 µg/kg for AFB1, 0 – 104.9 µg/kg for AFB2, 0 – 450 µg/kg for AFG1, 0 – 15.6 µg/kg for AFG2 and 0 – 1242.9 µg/kg for total aflatoxin across all the studied markets with a mean concentration varying from 14.88 - 200.87 µg/kg. 26.8% of groundnut samples were beyond the standard limit of 10 µg/kg set by the Ghana Standard Authority. A similar study conducted to examine the presence of aflatoxins in groundnuts from different Ghanaian local markets [69] showed a range of 0.38 ± 0.02 – 270.51 ± 23.14 µg/kg. Their range was lower than reported in this study. Aflatoxin contamination at high levels in staple foods has been reported in studies from different parts of Africa. In Nigeria, aflatoxin levels have been found in groundnut samples with an average level of 216.1 µg/kg [157]. The level of aflatoxin reported in Nigeria was higher than the average concentration (200.87 µg/kg) reported in this study. Also, a study done in Kenya reported aflatoxin levels up to 159.3 µg/kg in groundnut samples [158].

Table 4.12. Repeatability and accuracy of HPLC method used for aflatoxins determination in groundnut samples.

Aflatoxin	LOQ(µg/kg)	R^2	Recovery (%)
AFB1	**0.1**	**0.9999729**	**97**
AFB2	**0.1**	**0.9999751**	**98**
AFG1	**0.1**	**0.9999653**	**92**
AFG2	**0.1**	**0.9999689**	**98**

Table 4.13. Average levels of aflatoxin in groundnut samples in all the studied markets.

Aflatoxins	Average(µg/kg)
AFB1	71.82
AFB2	7.72
AFG1	5.88
AFG2	0.24
TOTAL AF	85.87

4.7. Aflatoxin levels in groundnuts from homes, storage centres and markets in the studied regions.

Aflatoxin contamination was observed in all the markets in the studied regions. In the Northern region groundnut samples were taken from two local markets namely; Savelugu and Tamale markets. A total of 18 samples were collected from this region. The results gave a range of 0.2 - 1057.2 µg/kg with an average concentration of 200.87 µg/kg. 33.3% and 38.9% of the samples were above the standard limit of 10 µg/kg for total aflatoxin and 5 µg/kg for aflatoxin B1, respectively set by the Ghana Standard Authority (GSA). 44.4% and 50.0% of the samples were beyond European Food Safety Authority (EFSA) standard limit for total aflatoxin (4 µg/kg) and aflatoxin B1 (2 µg/kg), respectively.

A total of 12 samples were taken from the Walewale market in the North-east region. The results gave a range of 0.6 - 1242.9 µg/kg and a mean level of 148.68 µg/kg. 41.7% and 50% of the samples had levels above GSA standard limit for total aflatoxin and aflatoxin B1, respectively. For the EFSA standard, 50% of the samples were above the set limits.

Bolgatanga and Pwalugu markets in the Upper east region recorded a range of 0.2 - 1224.5 µg/kg and an average level of 109.65 µg/kg. Out of the 18 samples, 16.7% were beyond the GSA standard limit for total aflatoxin and aflatoxin B1. 22.2% and 27.8% of the samples were above the standard limit of EFSA for total aflatoxin and aflatoxin B1, respectively.

Groundnut samples obtained from the Bono east region were sampled from Techiman, Nsuta and Babato markets. A range of 0.3 - 977.0 µg/kg and a mean concentration of 124.33 µg/kg was obtained from the studies done in this region. 30.8% out of the 15 samples collected exceeded the GSA standard limit for total aflatoxin and aflatoxin B1. 30.8% and 33.3% were above the standard limit of EFSA for total aflatoxin and aflatoxin B1, respectively.

In the Bono region, 10 groundnut samples were collected from the Sunyani market. An analysis of these samples gave a range of 0.2 - 154.9 µg/kg and an average concentration of 27.08 µg/kg. 40.0% and 50.0% of the samples had levels above GSA standard limit for total aflatoxin and aflatoxin B1, respectively. 50.0% of the samples had levels beyond the standard limit of EFSA for total aflatoxin and aflatoxin B1.

Kumasi, Akumadan, and Ejisu markets were markets in the Ashanti region where groundnut samples were taken. A total of 14 samples were collected and the results obtained gave a range of 0.2 - 90.7 µg/kg and a mean level of 20.97 µg/kg. 35.7% of the samples had levels beyond GSA

standard limit for total aflatoxin and aflatoxin B1. 42.9% of the samples were above the standard limit of EFSA for total aflatoxin and aflatoxin B1.

The Ahafo region gave a range of 1.4 - 86.4 µg/kg for total aflatoxin from the 6 samples collected at Bechem and Duayaw Nkyanta markets. An average concentration of 14.88 µg/kg was recorded from the study in this region. 16.7% of the samples were above the GSA and EFSA standards for total aflatoxin and aflatoxin B1.

In the Greater Accra region, groundnut samples were collected for Agbogbloshie, Madina, Ashiaman, Tema community 1, Dome, and Kaneshie markets. The results from this study gave a range of 0.2 - 627.2 µg/kg for total aflatoxins and an average level of 44.03 µg/kg. 20.5% and 22.7% out of the 44 samples were above GSA standard limit for total aflatoxin and aflatoxin B1, respectively. 25% and 29.6% of the samples exceeded the EFSA standard for total aflatoxin and aflatoxin B1, respectively.

The results from this study show that aflatoxin B1 recorded the highest level of contamination in the groundnut samples, followed by AFB2, AFG1, and AFG2, respectively. A study by Asare-Bediako et al., (2020) and Agbetiameh et al., (2002) revealed mean values of 928.7 µg/kg [17] and 145.6 µg/kg [157] in groundnut samples from the Upper West and Brong Ahafo regions of Ghana, respectively. The mean level of aflatoxins in groundnut samples studied in the Upper West reported by Asare- Bediako et al., (2020) was higher than levels recorded in the Northern region (200.87 µg/kg) and in the Upper East region (109.65 µg/kg) in this study. Similarly, the mean level of aflatoxin reported by Agbetiameh et al., 2008 in the Brong Ahafo region was higher than levels recorded in the Bono region (124.33 µg/kg) in this study. According to research by Kumi et al., (2015) in Ejura- Sekyedumase District of Ghana, 36 food samples that were locally produced for infants from a blend of groundnut, beans, and maize contained aflatoxins in the range of 7.9–500

µg/kg [119], of which 30 (83%) had exceeded the 20 µg/kg standard. The range reported by Kumi et al., (2015) is higher than that reported in the Ashanti region (0.2 – 90.7 µg/). Another study done to assess the prevalence of aflatoxin contamination in groundnut in Ghana [153] showed levels of aflatoxin up to 3,868 µg/kg and a mean level of 58 µg/kg.

Table 4.14. Ranges for aflatoxin in groundnut samples in the studied regions.

Regions	No. of samples	AFB1 (µg/kg)	AFB2 (µg/kg)	AFG1 (µg/kg)	AFG2 (µg/kg)	Total AF (µg/kg)
Northern	18	0.2 – 949.7	0.2 –175.2	0.2 – 5.6	0.1 – 0.6	0.2–1057.2
North East	12	0.6–1221.6	1.9 – 28.4	–	–	0.6 – 1242.9
Upper East	18	0.4 – 724.8	0.1 – 82.5	0.2 – 450	0.2 – 5.2	0.2 – 1224.5
Bono East	15	0.2 – 887.3	0.1 – 104.9	15.6 – 15.7	0.4 – 1.1	0.3 – 977
Bono	10	0.2 – 137	1.1 – 17.9	0 – 9.7	0 – 3	0.2 – 154.9
Ashanti	15	0.2 – 83.7	0.2 – 12.6	0.2 – 8.7	0.2 – 0.2	0.2 – 90.7
Ahafo	6	1.2 – 78.4	0.2 – 8.0	–	–	1.4 – 86.4
Greater Accra	44	0.2 – 483.3	0.2 – 64.6	0.1 – 166.1	0.1 – 15.6	0.2 – 627.2
All	138	0.2 – 1221.6	0.1 – 104.9	0.1 – 450	0.1 – 15.6	0.2 – 1242.9

Table 4.15. Average levels of aflatoxin in groundnut samples in studied regions.

Regions	AFB1(µg/kg)	AFB2(µg/kg)	AFG1(µg/kg)	AFG2(µg/kg)	TOTAL(µg/kg)
Northern	176±80	25±10	0.4±0.3	0.06±0.03	201±90
North East	142±100	7±3	<0.1	<0.1	149±100
Upper East	77±50	6±5	25±25	0.3±0.3	110±80
Bono East	109±70	13±8	2±1	0.10±0.08	124±70
Bono	23±10	3±3	1±1	0.3±0.3	27±10
Ashanti	18±8	3±1	0.6±0.6	0.01±0.01	21±9
Ahafo	14±10	1±1	<0.1	<0.1	15±10
Greater Accra	33±10	4±2	7±4	0.5±0.4	44±20

Table 4.16. Average levels of aflatoxin in groundnut samples in all the studied markets.

Regions	Towns	No. of samples	AFB1 (µg/kg)	AFB2 (µg/kg)	AFG1 (µg/kg)	AFG2 (µg/kg)	TotalAF (µg/kg)
Northern	Savelugu	**7**	204±100	23±20	0.8±0.7	0.13±0.08	228±100
	Tamale	**11**	157±100	26±20	0.10±0.06	0.02±0.01	183±100
North East	Walewale	**12**	142±100	7±3	<0.1	<0.1	149±100
Upper East	Bolgatanga	**12**	114±70	8±7	38±30	0.6±0.4	163±100
	Pwalugu	**6**	34±3	0.3±0.2	<0.1	<0.1	4±4
Bono East	Techiman	**9**	120±90	11±8	3±2	0.2±0.1	134±100
	Nsuta Techiman	**2**	1.6±0.4	0.25±0.05	<0.1	<0.1	1.9±0.5
	Babato	**4**	138±100	26±20	<0.1	<0.1	164±100
Bono	Sunyani	**10**	23±10	3±2	1±1	0.3±0.3	27±20
Ashanti	Kumasi	**9**	12±6	2±1	1±1	0.02±0.02	15±8
	Akumadan	**5**	32±20	4±3	0.04±0.03	<0.1	36±22
	Ejisu	**1**	0.4±0.1	<0.1	<0.1	<0.1	0.4±0.1
Ahafo	Becham	**5**	16±10	2±2	<0.1	<0.1	18±17
	Duayaw Nkyanta	**1**	<0.1	<0.1	<0.1	<0.1	<0.1
Greater Accra	Agbogbloshie	**10**	83±50	8±5	27±19	2±2	121±80
	Madina	**8**	6±6	1.0±0.8	0.4±0.2	0.01±0.01	8±6
	Ashiaman	**8**	0.5±0.3	0.03±0.02	0.06±0.04	<0.1	0.6±0.3
	Tema Comm. 1	**4**	14±7	3±2	4±3	0.8±0.6	22±10
	Dome	**5**	0.2±0.1	<0.1	<0.1	<0.1	0.2±0.1
	Kaneshie	**9**	56±53	8±7	0.5±0.5	0.02±0.02	64±60

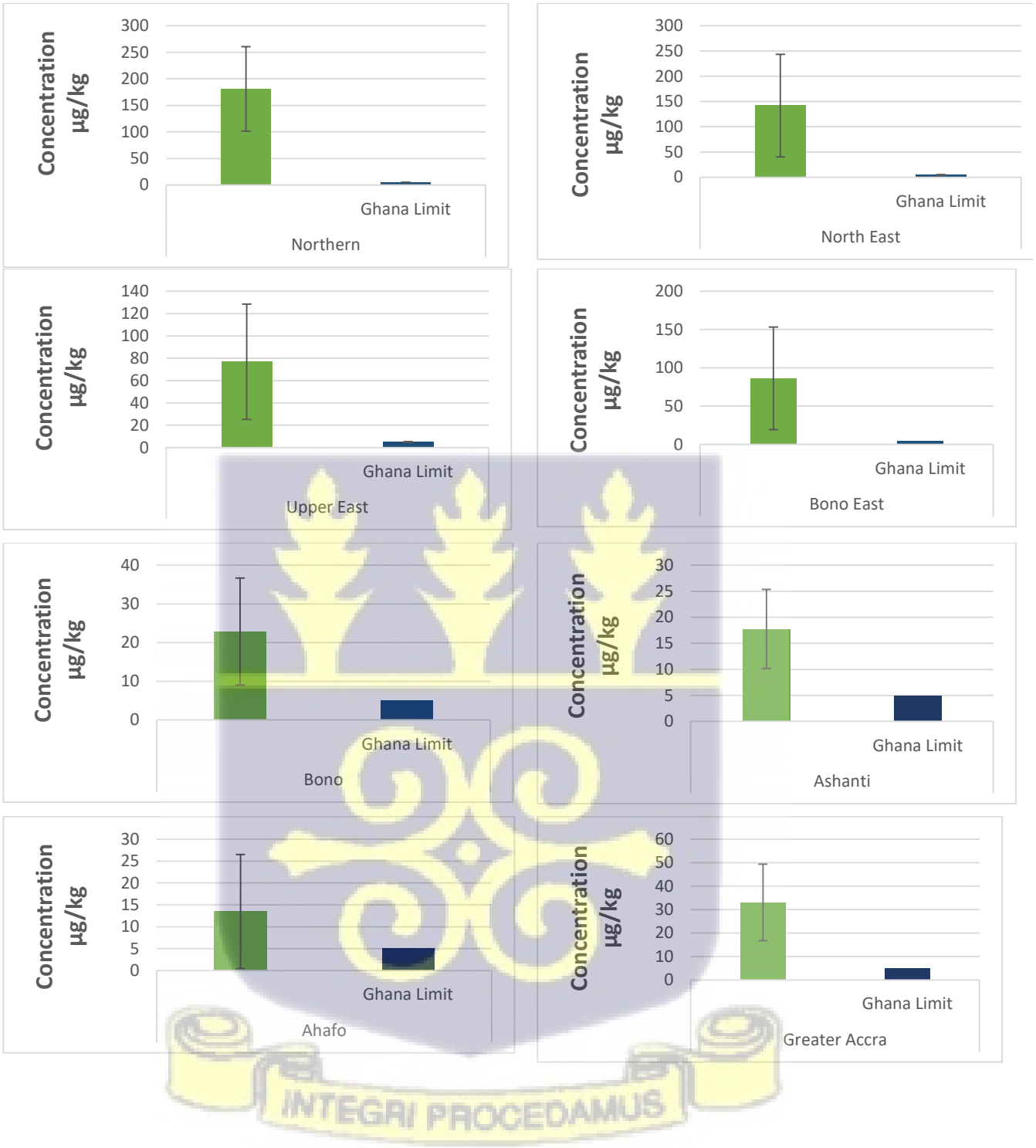


Figure 4.4. Graphs showing average levels of aflatoxin B1 in groundnuts at the various regions.

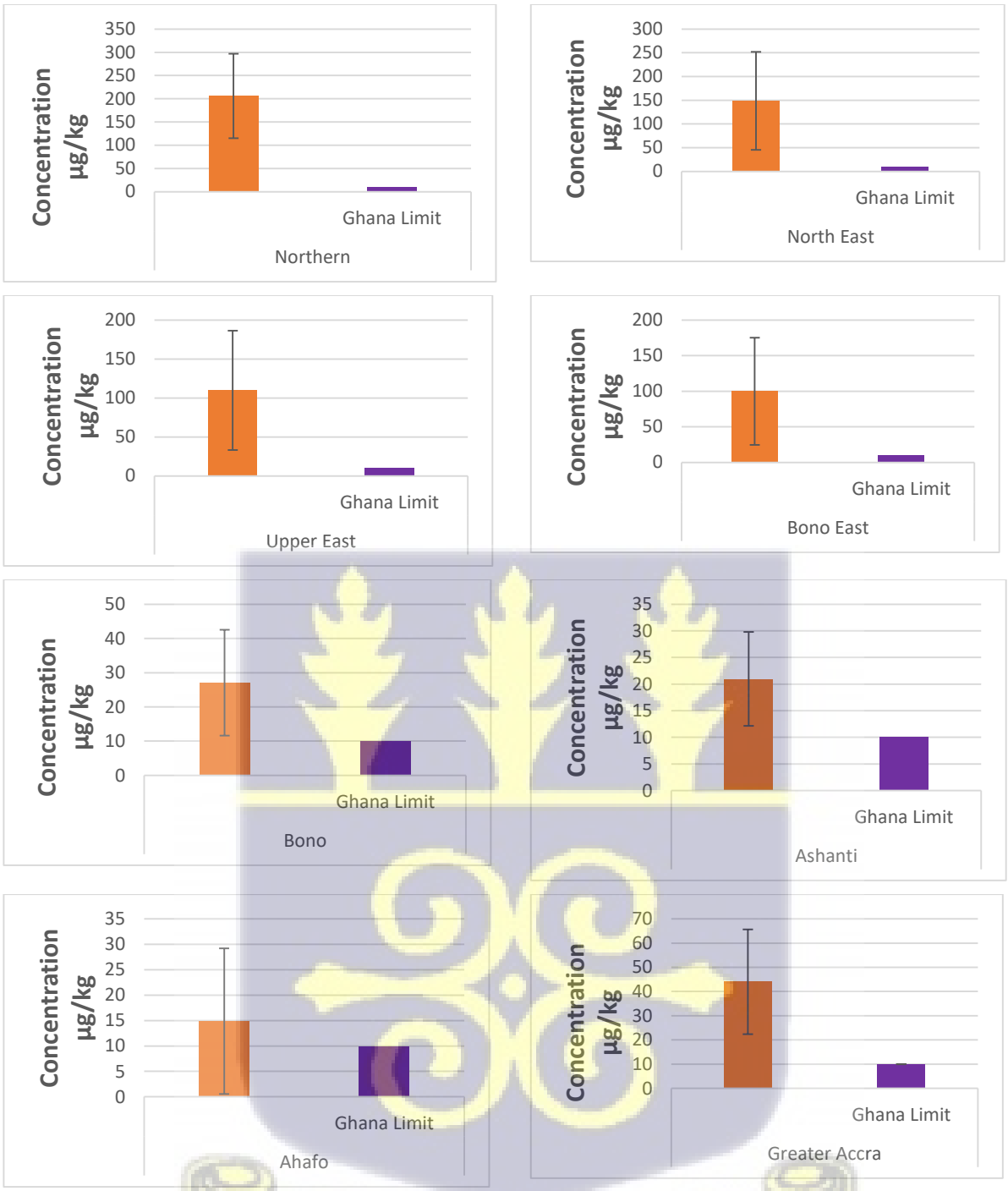
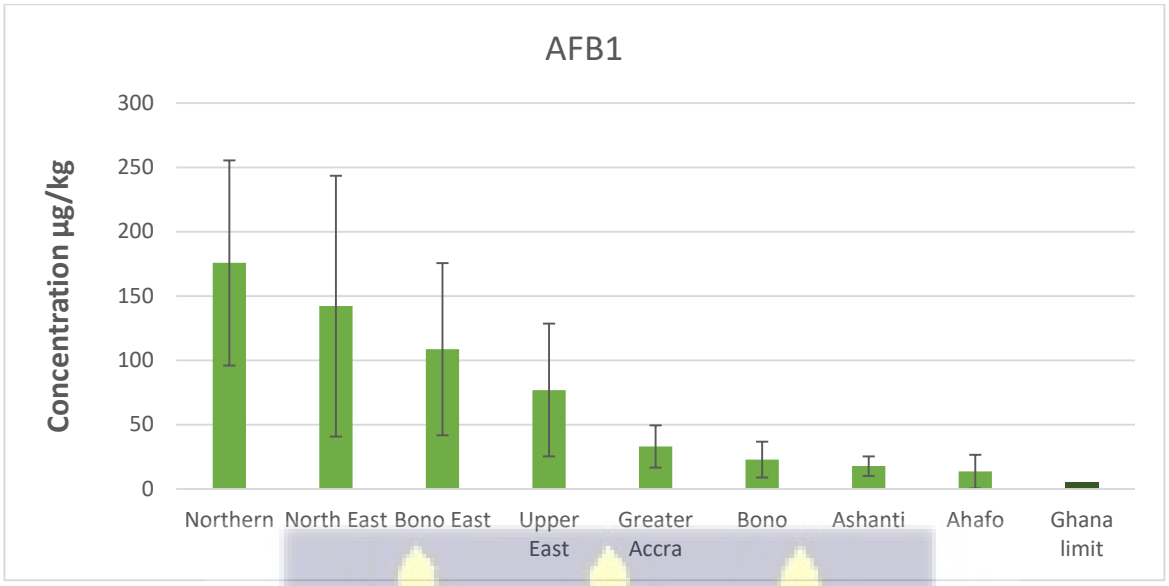


Figure 4.5. Graphs showing average levels of total aflatoxin in groundnut at the various regions.



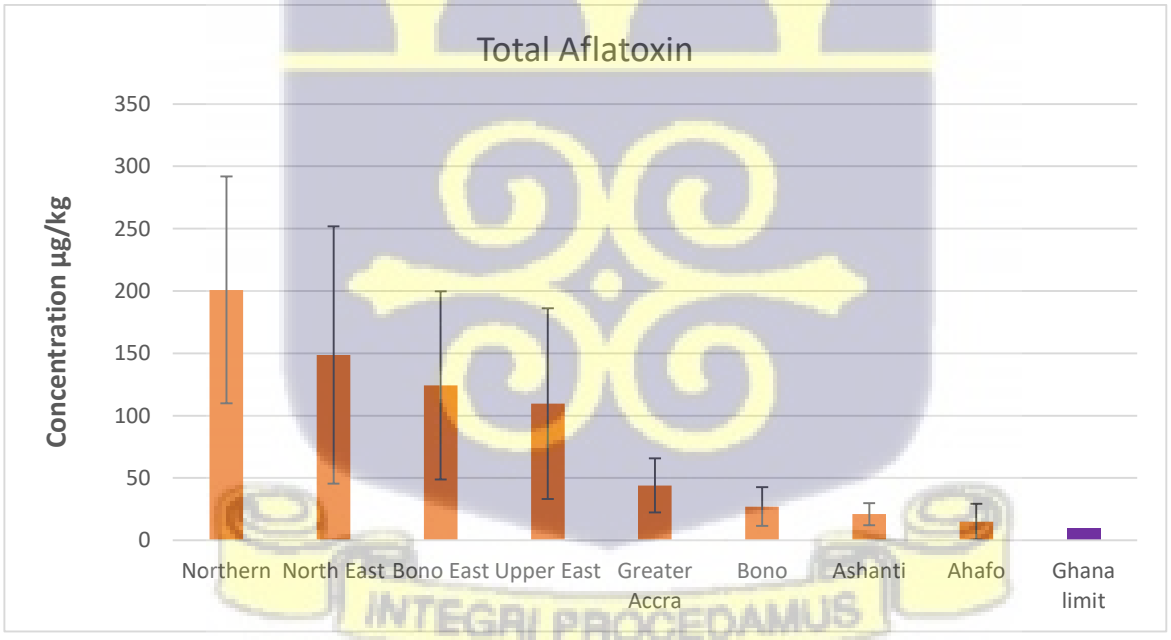


Figure 4.6. Graph showing trend of average levels of aflatoxin B1 and total aflatoxin in groundnut at the various regions.

4.8. Risk assessment for groundnut samples.

4.8.1. Estimated daily intake

The daily intake of groundnut was estimated based on information from the Ministry of Agriculture in Ghana and other studies to be 87 g/day for an adult and 46 g/day for children [69], [159]. An average adult body weight of 60.7 kg and 26 kg were used in this study [160]. Based on the mean concentrations of total aflatoxin in groundnuts obtained in this study, an estimated daily intake was determined for the various markets and regions. The EDI values of the studied regions range from 0.0213 - 0.2879 µg/kg.bw/day for adults and 0.0263 - 0.3554 µg/kg.bw/day for children. The highest estimated daily intake of groundnut was recorded in the Northern region and the lowest intake in the Ahafo region.

Moreover, EDI values for the markets range from 0.0003 - 0.3270 µg/kg.bw/day for adults and a range of 0.0004 to 0.4037 µg/kg.bw/day for children. The highest EDI value was recorded at Savelugu in the Northern region and least EDI value was recorded at Dome in the greater Accra region. An earlier study done in Ghana showed relatively higher estimated daily intakes (EDI) for total aflatoxins in the groundnut samples for infants, children, adolescents, and adults were 0.38, 0.2, 0.1, and 0.087 µg/kg.bw/day respectively [69]. According to Kooprasertying et al., (2016), Thailand's average daily intake of aflatoxins [161] for raw, roasted, and ground peanuts was 0.00049, 0.00040, and 0.00213 µg/kg bw/day [162], respectively which is lower than reported in this study. Adetunji et al., (2018) reported an EDI for aflatoxin based on the consumption of groundnut in Nigeria gave a range of 0.02513 – 0.02928 µg/kg bw/day [163].

Table 4.17. Estimated daily intake values for aflatoxin in groundnut samples in the studied regions.

Regions	EDI – Adult (µg/kg.bw/day)	EDI – Children (µg/kg.bw/day)
Northern	0.2879	0.3554
North East	0.2131	0.2630
Upper East	0.1572	0.1940
Bono East	0.1782	0.2200
Bono	0.0388	0.0479
Ashanti	0.0301	0.0371
Ahafo	0.0213	0.0263
Great Accra	0.0631	0.0779

Table 4.18. Estimated daily intake values for aflatoxin in groundnut samples in all the studied markets.

Market	EDI – Adult (µg/kg.bw/day)	EDI – Children (µg/kg.bw/day)
Savelugu	0.327	0.4037
Tamale	0.2630	0.3246
Walewale	0.2131	0.263
Bolgatanga	0.2330	0.2877
Pwalugu	0.0054	0.0067
Techiman	0.1919	0.2369
Nsuta Techiman	0.0027	0.0033
Babato	0.2351	0.2902
Sunyani	0.0389	0.0479
Kumasi	0.0213	0.0263
Akumadan	0.0517	0.0638
Ejisu	0.0006	0.0007
Bechem	0.0256	0.0316
Duayaw Nkyanta	0.0000	0.0000

Agbogbloshie	0.1727	0.2132
Madina	0.0110	0.0136
Ashiaman	0.0008	0.0010
Tema community 1	0.0310	0.0383
Dome	0.0003	0.0004
Kaneshie	0.0922	0.1138

4.8.2. Margin of Exposures (MOEs)

A margin of exposure (MOE) was calculated based on a threshold estimated from previous studies. Based on a benchmark dose lower limit ($BMDL_{10}$) for aflatoxins of 0.34 μgkg^{-1} bw day^{-1} [154]. MOE value calculated ranges from 1.6 - 16 for adults and 0.96 - 12.93 for children in the studied regions. In addition, MOE values for the markets ranged from 1.04 - 1133.33 for adults and 0.84 - 850 for children. The results obtained show potential public health risk because none of the margins of exposure values exceeded 10 000.

MOE values ranging from 5.81 - 6.76 showed a potential risk from consuming groundnut [163] in a study done in Nigeria. The range reported in this study is lower than the range recorded in this study. The MOE for Turkish adults ranged from 995 - 860 for AFB1 exposure, suggesting a potential health risk [164].

Table 4.19. Margin of exposure values for aflatoxin in groundnut samples in the studied regions.

Region	MOE - adult	MOE - Children
Northern	1.18	0.96
North East	1.6	1.3
Upper East	2.16	1.75
Bono East	1.91	1.55
Bono	8.76	7.1
Ashanti	11.3	9.16
Ahafo	16	12.93
Great Accra	5.39	4.36

Table 4.20. Margin of exposure values for aflatoxin in groundnut samples in all the studied markets.

Market	MOE - adult	MOE - Children
Savelugu	1.04	0.84
Tamale	1.29	1.05
Walewale	1.6	1.29
Bolgatanga	1.5	1.18
Pwalugu	62.96	50.75
Techiman	1.77	1.44
Nsuta Techiman	125.93	103.03
Babato	1.45	1.17
Sunyani	8.74	7.1
Kumasi	15.96	12.93
Akumadan	6.58	5.33
Ejisu	566.67	485.71
Becham	13.28	10.76
Duayaw Nkyanta	0	0
Agbogbloshie	1.97	1.59

Madina	30.91	25
Ashiaman	425	340
Tema community 1	10.98	8.88
Dome	1133.33	850
Kaneshie	3.69	2.99

4.8.3. Liver Cancer risk valuation through the consumption of Groundnuts

The liver cancer risk from the consumption of groundnut was estimated based on an average potency value of 0.04567 µg aflatoxins $kg^{-1}bwday^{-1}$ [148]. Liver cancer risk was calculated based on this estimate. Cancer risk calculated ranges from 0.0010 - 0.0131 for adults and 0.0012 - 0.0162 for children in the studied regions. The study revealed that the chances of getting liver cancer for adults and children who consume groundnut from the studied regions were highest in the Northern region and lowest in the Ahafo region. For the various studied markets, cancer risk range from 0.00001 - 0.01490 for adult and 0.00002 - 0.01840 for children. The highest likelihood of getting liver cancer was recorded at the Savelugu market in the Northern region and the Dome market in the greater Accra region recorded the least. Population risk of 1.51×10^{-3}, 7.9×10^{-4}, 4.5×10^{-4}, 3.45×10^{-4} for infants, children, adolescents, and adults respectively was reported in aflatoxin levels in a study of randomly chosen groundnuts from some local markets in Ghana [69].

Table 4.21. Cancer risk for aflatoxin in groundnut samples in the studied regions.

Regions	Cancer risk - Adult	Cancer risk - Children
Northern	0.0131	0.0162
North East	0.0097	0.0120
Upper East	0.0072	0.0089
Bono East	0.0081	0.0100
Bono	0.0018	0.0022
Ashanti	0.0014	0.0017
Ahafo	0.0010	0.0012
Great Accra	0.0029	0.0036

Table 4.22. Cancer risk for aflatoxin in groundnut samples in all the studied markets.

Markets	Cancer risk - Adult	Cancer risk - Children
Savelugu	0.01490	0.01840
Tamale	0.01200	0.01480
Walewale	0.00970	0.01200
Bolgatanga	0.01060	0.01310
Pwalugu	0.00020	0.00030
Techiman	0.00880	0.01080
Nsuta Techiman	0.00010	0.00020
Babato	0.01070	0.01330
Sunyani	0.00180	0.00220
Kumasi	0.00100	0.00120
Akumadan	0.00240	0.00290
Ejisu	0.00002	0.00003
Becham	0.00120	0.00140

Duayaw Nkyanta	0.00000	0.00000
Agbogbloshie	0.00790	0.00970
Madina	0.00050	0.00060
Ashiaman	0.00004	0.00005
Tema community 1	0.00140	0.00170
Dome	0.00001	0.00002
Kaneshie	0.00420	0.00520

4.8.4. Risk assessment based on the average concentration of aflatoxin in maize and groundnut.

Following the average concentration of aflatoxin in maize (137.03 µg/kg) and groundnut (85.87 µg/kg) for all the analysed samples, the estimated daily intake (EDI), margin of exposure and cancer risk were determined.

The daily intake of maize for an adult was estimated to be 107 g/day and 54 g/day for children, 87 g/day for adults and 46 g/day for children in groundnut as reported by the Ministry of Agriculture and Kortei et al., (2021) [159], [8]. The average body weight for an adult is 60.7 kg and 26 kg for children [160]. The EDI for adults based on the average concentration of aflatoxin in maize and groundnut were 0.241547 µg/kg.bw/day and 0.118976 µg/kg.bw/day, respectively. In children, the EDI was obtained as 0.284595 µg/kg.bw/day and 0.151928 µg/kg.bw/day for maize and groundnut respectively. Also, an EDI based on the consumption of both crops was determined to be 0.732263 µg/kg.bw/day for adults and 0.857305 µg/kg.bw/day for children. In earlier research in Ghana, a slightly higher EDI of 0.087 µg/kg.bw/day has been reported for groundnuts [69]. Likewise, a study by Omari et al (2020) reported EDI of aflatoxins ranging from 0.014 - 0.55 µg/kg bw/day based on the consumption of maize-groundnuts complementary foods in children [165].

A margin of exposure (MOE) was determined using a threshold estimated from previous studies and a benchmark dose lower limit ($BMDL_{10}$) for aflatoxins - 0.34 $\mu g kg^{-1}$ bw day^{-1} [154]. For groundnut samples, a MOE value of 2.858 for adult and 2.238 for children were estimated. Similarly, maize gave a MOE value of 1.408 for adults and 1.195 for children. MOE for the consumption of both crops were 0.464 and 0.397 for adults and children, respectively. Based on a study done by the European Food Safety Authority (EFSA), a margin of exposure, MOE of 10 000 or more indicates an occurrence of low public health issues [155]. The MOE values obtained in this study showed that aflatoxins exposure in adults and children consuming maize and groundnut from homes, markets and storage centres in the studied regions present a potential public health risk.

The chance of developing liver cancer from the consumption of both crops was determined using an average potency value of 0.04567 µg aflatoxins $kg^{-1}bwday^{-1}$ [148]. An adult population risk of 0.011 was estimated for maize, 0.005 for groundnuts, and 0.033 for the consumption of both crops. In children, a population risk of 0.013 was obtained for maize, 0.152 for groundnuts, and 0.039 for the consumption of both crops. The results obtained from these estimates suggest a higher chance of developing liver cancer from the consumption of maize, groundnuts, or both. Earlier studies have reported lower estimates of 0.000269 [69] and 0.0039 [165], indicating lower risks.

4.9. Discussion on aflatoxin levels in groundnut and maize crops.

The findings from this study show that there are relatively high levels of aflatoxin in maize and groundnut samples collected from markets in the greater Accra region compared to the other studied regions. However, aflatoxin levels in groundnut samples were recorded to be highest at the Northern region. There is no explicit trend between aflatoxin contamination in the groundnut and

maize samples from the various studied regions. All the maize crops sold at markets in the greater Accra region are produced from other regions such as Bono, Bono east, Ashanti and Northern regions [166]. Previous studies have shown that aflatoxin contamination occurs in three different phases: pre-harvest, during harvest and post-harvest. However, considerable contamination tends to occur after harvest as a result of poor storage conditions and transportation [153]. These factors appear to contribute to the high levels obtained in this study, particularly in the greater Accra region for the maize samples.

Some factors such as temperature, moisture content, insect pest infestation, nut or kernel integrity, and storage conditions influence the quality and levels of aflatoxins in grains and nuts. Aflatoxin levels in low-quality and damaged grains or nuts are considerably high as reported in a study done by Awauh and Kpodo et al., (1996). Undamaged kernels in their study gave aflatoxins levels up to 154.2 µg/kg compared to damaged kernels which recorded levels to be 22,168.0 µg/kg, almost 144 times higher than in the undamaged kernels [167].

Additionally, a report by Nyarko et al., (2021) suggests that groundnut and maize kernels stored in polypropylene sacks have lower grain quality and a significant increase in aflatoxin contamination compared to the use of hermetic sacks [79]. As such, this could be a contributing factor to the high levels of aflatoxin recorded in the studied groundnut and maize samples, since almost all these staples are stored in polypropylene sacks. An earlier study by Mutambuki et al., (2019) explained that hermetic bags work by limiting O_2 access to insects and microbes already present in the grains upon storage [168]. The primary goal of removing oxygen from the air is achieved by the exchange of gases among cereals, insects, and microbes inside a tightly sealed bag. Respiration inside the airtight bag results in a decrease in oxygen volumes with an increase in carbon dioxide volumes, which kills insects and microbes by suffocation.

Some major challenges leading to high levels of aflatoxin in most staples are poor transportation and storage conditions. *A. flavus* and *A. parasiticus* responsible for the production of aflatoxins flourish under conditions such as moisture, environmental stress, and relatively high temperatures. It turns out that most sellers store these groundnut and maize kernels under such poor conditions. Besides, during the transportation of farm produce the condition that influences the growth of aflatoxins are experienced as a result of poor road network leading to crops staying longer in transport vehicles than expected. Findings have shown that aflatoxin levels found in on-field kernels are significantly lower compared to stored kernels [169].

In the Bono region, a storage centre at Chiraa recorded relatively higher levels of aflatoxin in maize samples collected. The maize kernel from this storage centre was unsorted and seems unpleasant in appearance. Thus the high levels of aflatoxin might be ascribed to the maize kernel being contaminated on the field before and during harvest. Most people in rural areas consume damaged, insect pest infested and low-quality maize and other farm produce. On the contrary, sellers in the cities tend to display and sell the best quality crops for better attraction and pricing for consumers [76]. Hence the populace in these communities may be exposed to potentially high levels of aflatoxin than reported in this study. Also, the risk assessment analysis from the study showed that adults and children are at a potentially high health risk when consuming these crops, with a higher potential health risk in children. It is important to note that, these risk assessments were based on conservative estimates. Consequently, the potential health risk could be higher than estimated, especially in rural areas where the average intake of staple foods may be significantly higher than in the cities.

Aflatoxin B1, the most toxic aflatoxin [8] was reported in higher concentration in the studied groundnut and maize samples. This finding agrees with the report by Dadzie et al., (2019) which

suggest that aflatoxin B1 is the predominant toxin [170]. Aflatoxin B1's high incidence in both crops could be a result of the high levels of *A. flavus* S-strain occurrence in the studied crops [6].

CHAPTER FIVE

5.0. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

Given the reliance of Africans on aflatoxin-vulnerable staples such as maize and groundnuts, aflatoxins may continue to be an urgent and pressing challenge. Part of addressing this challenge is knowledge about aflatoxin occurrence and potential exposure in consumers. Unfortunately, knowledge of aflatoxins in major crops remains low, significantly mirroring general awareness of these toxins in major crops. This study has provided significant data on aflatoxin occurrence in aflatoxins in maize and groundnut samples across Ghana.

The study reports on a comprehensive analysis of aflatoxin contents (AFB1, AFB2, AFG1, AFG2) in 303 samples comprising 165 samples of maize and 138 samples of groundnut from homes, markets, and storage centres in eight regions across the country. The method employed the use of an immunoaffinity column to ensure reliable extraction and purification of aflatoxin congeners. Additionally, High-Performance Liquid Chromatography coupled to a fluorescence detector was used to assess the levels of aflatoxins in both maize and groundnut matrixes. The results from the study have shown that, aflatoxins contamination may vary widely across congeners, crops, markets, and regions. Nonetheless, significantly high levels of aflatoxins were observed in samples from across the country. Based on the findings, 50.9% of the maize samples and 26.8% of the groundnut samples exceeded the permissible limits established by the Ghana Standards Authority (GSA) 10 µg/kg for total aflatoxins. The study demonstrates extensive potential exposure to aflatoxins among Ghanaians, based on the estimated consumption of food made from these crops. Of huge relevance in this study is the fact that, the levels found in this study are among some of the highest frequency of occurrence and concentrations found in the country. Moreover, exposure

assessment and risk characterization indicate that both children and adult consumers of groundnut and maize from the various homes, markets, and storage centres across the country may be at potentially high risks of aflatoxin exposure and consequently higher risks of developing liver cancer. This study will help inform the policy on prioritization of aflatoxin as a significant food safety concern in Africa and provide essential data for the newly established Partnership for Aflatoxin Control in Africa (PACA) by the African Union Commission.

5.2. Recommendation

To ensure the safety of Ghanaians, precautionary measures must be put in place by the Food and Drugs Authority to make sure food products are examined for the presence of aflatoxin. Roots and tubers crops must be encouraged as substitute foods among Ghanaians since they are less prone to aflatoxin contamination. Moreover, aflatoxin awareness creation and education by the media, stakeholders and individuals must be intensified to help farmers, market women and consumers to appreciate the need to store maize and groundnut in good and favourable conditions. Farmers should use hermetic bags in storing kernels and nuts rather than polypropylene bags in an attempt to reduce aflatoxin contamination in crops.

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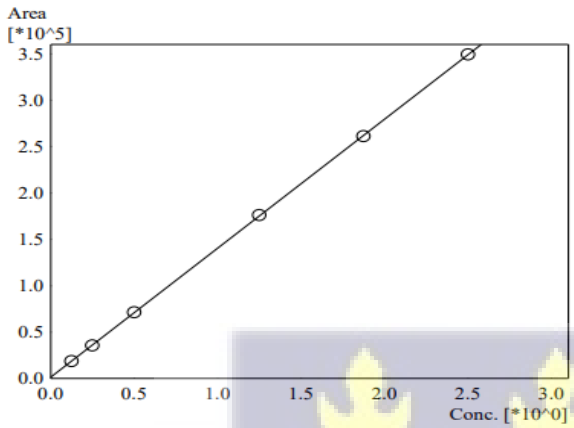
APPENDIX

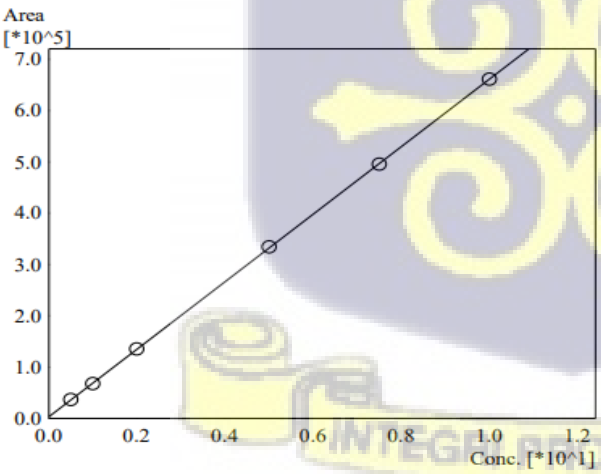
Calibration Curve

ID# : 1
Name : G2
Quantitative Method : External Standard
Function : f(x)=139034*x+1410.83
Rr1=0.9999849 Rr2=0.9999698 RSS=2.690606e+006
MeanRF: 1.424553e+005 RFSD: 3.888753e+003 RFRSD: 2.729807
FitType : Linear
ZeroThrough : Not Through
Weighted Regression : None
Detector Name : Detector B



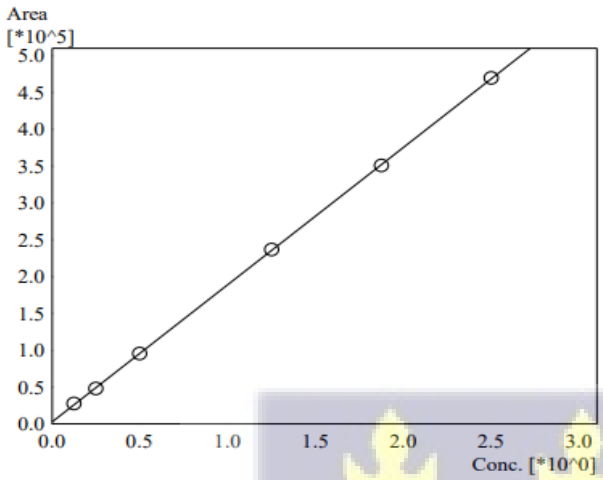
#	Conc.(Ratio)	MeanArea	Area
1	0.125	18737	18737
2	0.25	35553	35553
3	0.5	71345	71345
4	1.25	176246	176246
5	1.875	261098	261098
6	2.5	349206	349206

ID# : 2
Name : G1
Quantitative Method : External Standard
Function : f(x)=65750.3*x+3688.08
Rr1=0.9999881 Rr2=0.9999761 RSS=7.625430e+006
MeanRF: 6.807540e+004 RFSD: 2.774033e+003 RFRSD: 4.074942
FitType : Linear
ZeroThrough : Not Through
Weighted Regression : None
Detector Name : Detector B



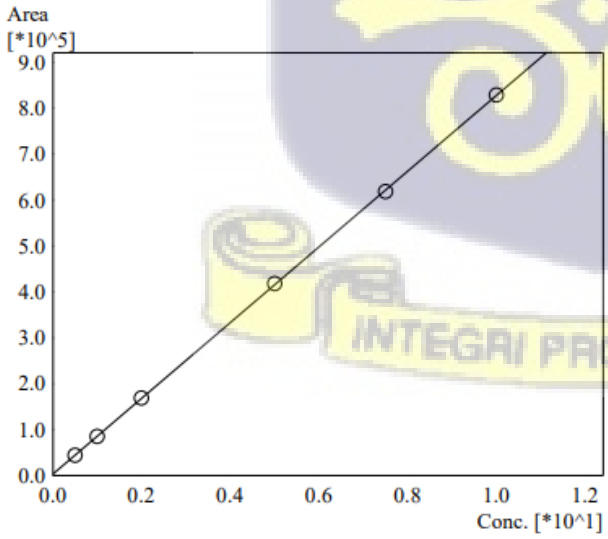
#	Conc.(Ratio)	MeanArea	Area
1	0.5	36739	36739
2	1	68081	68081
3	2	135611	135611
4	5	334473	334473
5	7.5	495618	495618
6	10	661115	661115

ID# : 3
Name : B2
Quantitative Method : External Standard
Function : f(x)=186476*x+2852.02
Rr1=0.9999732 Rr2=0.9999465 RSS=8.590941e+006
MeanRF: 1.950131e+005 RFSD: 1.303237e+004 RFRSD: 6.682815
FitType : Linear
ZeroThrough : Not Through
Weighted Regression : None
Detector Name : Detector B



#	Conc.(Ratio)	MeanArea	Area
1	0.125	27657	27657
2	0.25	48248	48248
3	0.5	95604	95604
4	1.25	236926	236926
5	1.875	350814	350814
6	2.5	469958	469958

ID# : 4
Name : B1
Quantitative Method : External Standard
Function : f(x)=82392.8*x+3549.38
Rr1=0.9999832 Rr2=0.9999664 RSS=1.683011e+007
MeanRF: 8.457682e+004 RFSD: 2.297401e+003 RFRSD: 2.716349
FitType : Linear
ZeroThrough : Not Through
Weighted Regression : None
Detector Name : Detector B



#	Conc.(Ratio)	MeanArea	Area
1	0.5	44364	44364
2	1	85397	85397
3	2	168908	168908
4	5	417839	417839
5	7.5	618423	618423
6	10	828577	828577

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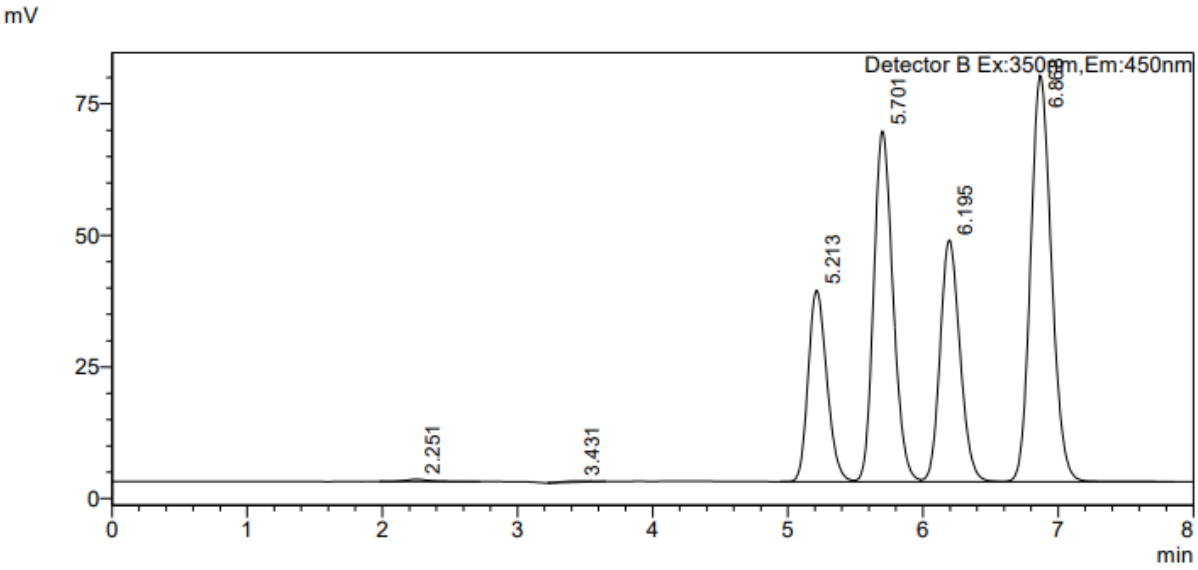

<Peak Table>

Detector A Channel 1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
Total							

Detector B Ex:350nm,Em:450nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	2.251	6426	435	0.000			
2	3.431	2685	189	0.000			
3	5.213	349206	36351	2.502	ug/L		G2
4	5.701	661115	66607	9.999	ug/L	V	G1
5	6.195	469958	45900	2.505	ug/L	V	B2
6	6.868	828577	77184	10.013	ug/L	SV	B1
Total		2317967	226666				

Levels of aflatoxin in maize samples in the studied regions.

Region	Towns		B1(µg/kg)	B2(µg/kg)	G1(µg/kg)	G2(µg/kg)	TOTAL(µg/kg)
Northern	Savelugu	Sample 1	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 2	19.1	1.2	<0.1	<0.1	20.3
		Sample 3	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 4	95.1	8.8	64.3	5.3	173.5
		Sample 5	117.4	4.8	56.8	0.8	179.8
		Sample 6	1.1	<0.1	0.5	<0.1	1.6
		Sample 7	22	18.2	446	12.3	498.5
		Sample 8	86.4	6.8	14	0.3	107.5

		Sample 9	1.1	<0.1	<0.1	<0.1	1.1
		Sample 10	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 11	0.3	<0.1	<0.1	<0.1	0.3
		Sample 12	11.2	0.7	<0.1	<0.1	11.9
		Sample 13	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 14	27.2	1.4	9.5	0.5	38.6
		Sample 15	50.8	3.1	5.2	<0.1	59.1
		Sample 16	501.5	47.2	1.4	<0.1	550.1
		Sample 17	102.6	9.2	<0.1	<0.1	111.8
		Sample 18	43.2	2.7	19	0.1	65
	Tamale	Sample 19	6.7	0.7	9	0.5	17.2
		Sample 20	1	0.2	0.9	0.3	2.4
		Sample 21	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 22	<0.1	<0.1	<0.1	0.3	0.3
		Sample 23	1.6	0.2	<0.1	0.2	2
		Sample 24	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 25	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 26	4.3	0.3	1	0.6	6.2
		Sample 27	12.1	0.4	<0.1	<0.1	12.5
		Sample 28	89.3	8.9	1.1	<0.1	99.3
		Sample 29	14	0.4	<0.1	<0.1	14.4
		Sample 30	21.9	1.6	<0.1	<0.1	23.5
North East	Walewale	Sample 31	7.5	0.5	<0.1	<0.1	8
		Sample 32	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 33	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 34	5.6	<0.1	1.3	<0.1	6.9
		Sample 35	<0.1	<0.1	7.5	<0.1	7.5
		Sample 36	<0.1	0.8	<0.1	<0.1	0.8
Upper East	Bolgatanga	Sample 37	3.8	<0.1	<0.1	<0.1	3.8
		Sample 38	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 39	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 40	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 41	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 42	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 43	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 44	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 45	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 46	92.6	0.2	23.9	<0.1	116.7
		Sample 47	98.3	5.6	<0.1	<0.1	103.9
		Sample 48	38.3	1.4	<0.1	<0.1	39.7
		Sample 49	0.2	<0.1	<0.1	<0.1	0.2

		Sample 50	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 51	327	14.4	<0.1	<0.1	341.4
		Sample 52	91.8	5.3	<0.1	<0.1	97.1
		Sample 53	6.1	0.1	<0.1	<0.1	6.2
		Sample 54	8.7	0.4	<0.1	<0.1	9.1
		Sample 55	<0.1	<0.1	<0.1	<0.1	<0.1
	Pwalugu	Sample 56	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 57	2	0.2	<0.1	<0.1	2.2
		Sample 58	0.3	<0.1	<0.1	<0.1	0.3
		Sample 59	670.2	20.5	<0.1	<0.1	690.7
		Sample 60	7.8	0.2	<0.1	<0.1	8
		Sample 61	5.9	0.3	<0.1	<0.1	6.2
		Sample 62	0.2	<0.1	<0.1	<0.1	0.2
		Sample 63	<0.1	<0.1	<0.1	<0.1	<0.1
Bono East	Techiman	Sample 64	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 65	0.2	<0.1	<0.1	<0.1	0.2
		Sample 66	0.8	0.1	<0.1	<0.1	0.9
		Sample 67	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 68	0.2	<0.1	0.5	<0.1	0.7
		Sample 69	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 70	5.6	0.2	<0.1	<0.1	5.8
		Sample 71	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 72	91.6	6.4	<0.1	<0.1	98
		Sample 73	0.7	<0.1	<0.1	<0.1	0.7
		Sample 74	<0.1	<0.1	<0.1	<0.1	<0.1
	Babato	Sample 75	11.5	0.6	<0.1	<0.1	12.1
		Sample 76	1	<0.1	<0.1	<0.1	1
		Sample 77	3	0.3	<0.1	<0.1	3.3
		Sample 78	53.6	2.3	13.6	0.7	70.2
		Sample 79	41	2.4	11.4	0.4	55.2
		Sample 80	33.4	2.8	<0.1	<0.1	55.2
		Sample 81	0.3	<0.1	<0.1	<0.1	0.3
Bono	Sunyani	Sample 82	49.2	3.4	<0.1	<0.1	52.6
		Sample 83	0.7	0.1	<0.1	<0.1	0.8
		Sample 84	0.2	<0.1	0.3	<0.1	0.5
		Sample 85	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 86	169.9	15.9	0.1	0.1	186
		Sample 87	2.3	0.1	<0.1	<0.1	2.4
		Sample 88	35.5	2.5	0.1	<0.1	38.1
		Sample 89	100.9	5.7	<0.1	<0.1	106.6
		Sample 90	358.1	24.8	<0.1	0.1	383
	Chiraa	Sample 91	1110.1	19.7	<0.1	<0.1	1129.7

		Sample 92	327.7	25.9	<0.1	<0.1	353.6
		Sample 93	1002	23.3	<0.1	<0.1	1025.3
		Sample 94	278.8	16	1.1	<0.1	295.9
		Sample 95	478.6	29	<0.1	0.4	508
		Sample 96	6.5	0.3	<0.1	<0.1	6.8
		Sample 97	53.4	5	47.8	3.7	109.9
		Sample 98	5.8	0.4	0.2	<0.1	6.4
		Sample 99	970.3	9.3	53	<0.1	1032.6
		Sample 100	804	45.3	4.6	<0.1	854
Ashanti	Kumasi	Sample 101	0.2	<0.1	<0.1	<0.1	0.2
		Sample 102	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 103	0.2	<0.1	<0.1	<0.1	0.2
		Sample 104	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 105	4.2	<0.1	35.9	<0.1	40.1
		Sample 106	227.3	13.4	21.1	0.5	262.3
		Sample 107	16	0.3	185.2	0.7	202.2
	Akumadan	Sample 108	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 109	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 110	6.3	0.3	<0.1	<0.1	6.6
		Sample 111	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 112	83.2	2	73.4	1.7	160.3
		Sample 113	1.3	<0.1	1.3	<0.1	2.6
	Ejisu	Sample 114	0.4	<0.1	0.6	<0.1	1
Ahafo	Becham	Sample 115	28.1	3.2	<0.1	<0.1	31.3
	Duayaw Nkyanta	Sample 116	0.3	<0.1	<0.1	<0.1	0.3
		Sample 117	160.6	16.6	<0.1	<0.1	177.2
Greater Accra	Ahiaman	Sample 118	28.1	1.4	0.9	0.2	30.6
		Sample 119	2.4	0.4	<0.1	<0.1	2.8
		Sample 120	<0.1	<0.1	<0.1	0.8	0.8
		Sample 121	0.4	<0.1	<0.1	0.3	0.7
		Sample 122	13.8	1.4	0.2	<0.1	15.4
		Sample 123	20.7	1.9	<0.1	<0.1	22.6
		Sample 124	921.9	79.7	0.2	<0.1	1001.8
		Sample 125	28	2.3	<0.1	<0.1	30.3
		Sample 126	138	12.2	<0.1	<0.1	150.2
		Sample 127	3.2	0.3	<0.1	<0.1	3.5
	Agbogbloshie	Sample 128	41	3.7	<0.1	<0.1	44.7
		Sample 129	1031.3	15.7	<0.1	<0.1	1047
		Sample 130	315.3	26.5	<0.1	<0.1	341.8
		Sample 131	101.3	9.8	<0.1	<0.1	111.1
		Sample 132	16.4	0.8	<0.1	<0.1	17.2

	Sample 133	150.7	16.2	<0.1	<0.1	166.9
	Sample 134	352.1	34.1	160.7	13.4	560.3
	Sample 135	317	32.8	5.8	0.6	356
	Sample 136	101.6	9.2	<0.1	<0.1	110.8
	Sample 137	26.6	2.8	<0.1	<0.1	29.4
Kaneshie	Sample 138	278.5	15	1.9	0.2	295.6
	Sample 139	63.8	7.8	0.5	0.2	72.3
	Sample 140	232.8	28.4	0.2	<0.1	261.4
	Sample 141	9.8	0.9	<0.1	<0.1	10.7
	Sample 142	0.5	<0.1	<0.1	0.2	0.7
	Sample 143	62.4	4.8	<0.1	<0.1	67.2
	Sample 144	190.2	21.4	<0.1	<0.1	211.6
	Sample 145	2.4	0.3	<0.1	0.3	3
	Sample 146	189.4	33.1	<0.1	<0.1	222.5
	Sample 147	255.7	30.5	<0.1	<0.1	286.2
Madina	Sample 148	57.8	3.8	<0.1	<0.1	61.6
	Sample 149	497.6	42.6	<0.1	<0.1	540.2
	Sample 150	888.6	59.7	<0.1	<0.1	948.3
	Sample 151	10.5	0.5	<0.1	<0.1	11
	Sample 152	0.6	<0.1	<0.1	<0.1	0.6
	Sample 153	196.6	13.2	<0.1	<0.1	209.8
	Sample 154	99.7	9.8	<0.1	0.2	109.7
	Sample 155	0.6	<0.1	<0.1	0.2	0.8
	Sample 156	<0.1	<0.1	<0.1	<0.1	<0.1
	Sample 157	70.3	4.9	<0.1	<0.1	75.2
	Sample 158	1057.7	7	<0.1	<0.1	1064.7
	Sample 159	478.3	12.4	0.4	<0.1	491.1
Dome	Sample 160	842.1	50.8	<0.1	<0.1	892.9
	Sample 161	120	9.2	<0.1	<0.1	129.2
	Sample 162	7.9	1	0.3	<0.1	9.2
	Sample 163	518.4	33.3	0.6	<0.1	552.3
Tema Community 1	Sample 164	521.7	37.2	0.6	<0.1	559.5
	Sample 165	379.3	22.8	335.1	15.2	752.4

Levels of aflatoxin in groundnut samples in all the studied markets.

Region	Towns		B1 (µg/kg)	B2 (µg/kg)	G1 (µg/kg)	G2 (µg/kg)	TOTAL (µg/kg)
Northern	Savelugu	Sample 1	0.2	<0.1	<0.1	<0.1	0.2
		Sample 2	0.3	<0.1	<0.1	<0.1	0.3
		Sample 3	2.7	0.2	<0.1	<0.1	2.9
		Sample 4	3.8	0.5	5.6	0.6	10.5
		Sample 5	382.8	42.8	<0.1	0.1	425.7
		Sample 6	123	9.1	<0.1	<0.1	132.1
		Sample 7	918.1	107.2	<0.1	0.2	1025.5
	Tamale	Sample 8	770.1	175.2	0.2	0.1	945.6
		Sample 9	4.5	1.1	<0.1	<0.1	5.6
		Sample 10	0.4	<0.1	<0.1	<0.1	0.4
		Sample 11	0.3	<0.1	<0.1	<0.1	0.3
		Sample 12	<0.1	<0.1	0.4	<0.1	0.4
		Sample 13	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 14	<0.1	<0.1	0.5	<0.1	0.5
		Sample 15	949.7	107.4	<0.1	0.1	1057.2
		Sample 16	6	1	<0.1	<0.1	7
		Sample 17	0.7	0.1	<0.1	<0.1	0.8
		Sample 18	0.6	<0.1	<0.1	<0.1	0.6
North East	Walewale	Sample 19	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 20	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 21	36.1	11.2	<0.1	<0.1	47.3
		Sample 22	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 23	302.8	28.4	<0.1	<0.1	331.2
		Sample 24	0.9	<0.1	<0.1	<0.1	0.9
		Sample 25	0.6	<0.1	<0.1	<0.1	0.6
		Sample 26	1221.6	21.3	<0.1	<0.1	1242.9
		Sample 27	111.8	11.6	<0.1	<0.1	123.4
		Sample 28	8	1.9	<0.1	<0.1	9.9
		Sample 29	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 30	23.3	4.7	<0.1	<0.1	28
Upper East	Bolgatanga	Sample 31	<0.1	<0.1	0.4	<0.1	0.4
		Sample 32	724.8	14.5	450	5.2	1224.5
		Sample 33	3.5	0.1	2.8	<0.1	6.4
		Sample 34	<0.1	<0.1	0.2	<0.1	0.2

		Sample 35	<0.1	<0.1	0.4	<0.1	0.4
		Sample 36	0.7	<0.1	0.4	<0.1	1.1
		Sample 37	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 38	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 39	0.4	<0.1	0.4	<0.1	0.8
		Sample 40	630.2	82.5	0.5	0.2	713.4
		Sample 41	3	0.3	<0.1	<0.1	3.3
		Sample 42	0.4	<0.1	0.2	<0.1	0.6
	Pwalugu	Sample 43	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 44	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 45	21	1.6	<0.1	<0.1	22.6
		Sample 46	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 47	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 48	<0.1	<0.1	<0.1	<0.1	<0.1
Bono East	Techiman	Sample 49	171.8	20.4	15.6	1.1	208.9
		Sample 50	1.2	0.1	<0.1	<0.1	1.3
		Sample 51	0.8	0.2	<0.1	<0.1	1
		Sample 52	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 53	0.2	0.1	<0.1	<0.1	0.3
		Sample 54	0.3	<0.1	<0.1	<0.1	0.3
		Sample 55	887.3	73.6	15.7	0.4	977
		Sample 56	14.1	1.5	<0.1	<0.1	15.6
		Sample 57	0.5	0.1	<0.1	<0.1	0.6
	Nsuta Techiman	Sample 58	1.2	0.2	<0.1	<0.1	1.4
		Sample 59	2	0.3	<0.1	<0.1	2.3
	Babato	Sample 60	549.1	104.9	<0.1	<0.1	654
		Sample 61	1.8	0.4	<0.1	<0.1	2.2
		Sample 62	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 63	<0.1	<0.1	<0.1	<0.1	<0.1
Bono	Sunyani	Sample 64	27.6	3.1	<0.1	<0.1	30.1
		Sample 65	137	17.9	<0.1	<0.1	154.9
		Sample 66	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 67	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 68	0.2	<0.1	<0.1	<0.1	0.2
		Sample 69	2.4	1.1	9.7	3	16.2
		Sample 70	6.3	1.1	<0.1	<0.1	7.4
		Sample 71	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 72	53.9	7.1	<0.1	<0.1	61
		Sample 73	1	<0.1	<0.1	<0.1	1
Ashanti	Kumasi	Sample 74	0.3	<0.1	<0.1	<0.1	0.3
		Sample 75	37.7	6.4	0.2	<0.1	44.3

		Sample 76	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 77	0.2	<0.1	<0.1	<0.1	0.2
		Sample 78	47.9	10.1	8.7	0.2	66.9
		Sample 79	1.6	0.2	<0.1	<0.1	1.8
		Sample 80	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 81	14.2	1.2	<0.1	<0.1	15.4
		Sample 82	3.6	1.2	<0.1	<0.1	4.9
	Akumadan	Sample 83	83.7	6.8	0.2	<0.1	90.7
		Sample 84	76.8	12.6	<0.1	<0.1	89.4
		Sample 85	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 86	0.2	<0.1	<0.1	<0.1	0.2
		Sample 87	<0.1	<0.1	<0.1	<0.1	<0.1
	Ejisu	Sample 88	0.4	<0.1	<0.1	<0.1	0.4
Ahafo	Becham	Sample 89	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 90	1.2	0.2	<0.1	<0.1	1.4
		Sample 91	78.4	8	<0.1	<0.1	86.4
		Sample 92	1.45	<0.1	<0.1	<0.1	1.45
		Sample 93	<0.1	<0.1	<0.1	<0.1	<0.1
	Duayaw Nkyanta	Sample 94	<0.1	<0.1	<0.1	<0.1	<0.1
Greater Accra	Agbogbloshie	Sample 95	31.2	5.6	2.4	0.2	39.4
		Sample 96	1.1	0.2	<0.1	<0.1	1.3
		Sample 97	15	2.5	<0.1	<0.1	17.5
		Sample 98	0.3	<0.1	<0.1	<0.1	0.3
		Sample 99	441.6	16.4	166.1	3.1	627.2
		Sample 100	346.4	50.7	106.3	15.6	519
		Sample 101	0.3	<0.1	<0.1	<0.1	0.3
		Sample 102	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 103	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 104	<0.1	<0.1	<0.1	<0.1	<0.1
	Madina	Sample 105	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 106	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 107	<0.1	<0.1	<0.1	<0.1	<0.1
		Sample 108	0.9	<0.1	1.8	<0.1	2.7
		Sample 109	1.4	0.2	0.7	0.1	2.4
		Sample 110	1.4	<0.1	<0.1	<0.1	1.4
		Sample 111	45.6	6.6	0.7	<0.1	52.9
		Sample 112	0.6	1.2	0.1	<0.1	1.9
	Ashiaman	Sample 113	2.5	0.2	0.1	<0.1	2.8
		Sample 114	0.5	<0.1	<0.1	<0.1	0.5
		Sample 115	0.2	<0.1	<0.1	<0.1	0.2
		Sample 116	0.2	<0.1	<0.1	<0.1	0.2

	Sample 117	<0.1	<0.1	<0.1	<0.1	<0.1
	Sample 118	0.3	<0.1	0.4	<0.1	0.7
	Sample 119	<0.1	<0.1	<0.1	<0.1	<0.1
	Sample 120	0.3	<0.1	<0.1	<0.1	0.3
Tema community 1	Sample 121	30.2	8.2	<0.1	<0.1	38.4
	Sample 122	5.8	0.9	2.4	0.6	9.7
	Sample 123	18.8	3.4	13.7	2.5	38.4
	Sample 124	<0.1	<0.1	<0.1	<0.1	<0.1
Dome	Sample 125	0.5	<0.1	<0.1	<0.1	0.5
	Sample 126	<0.1	<0.1	<0.1	<0.1	<0.1
	Sample 127	0.4	<0.1	<0.1	<0.1	0.4
	Sample 128	<0.1	<0.1	<0.1	<0.1	<0.1
	Sample 129	0.2	<0.1	<0.1	<0.1	0.2
Kaneshie	Sample 130	483.3	64.6	<0.1	0.2	548.1
	Sample 131	1.7	0.4	<0.1	<0.1	2.1
	Sample 132	0.4	<0.1	<0.1	<0.1	0.4
	Sample 133	0.2	<0.1	<0.1	<0.1	0.2
	Sample 134	<0.1	<0.1	<0.1	<0.1	<0.1
	Sample 135	0.2	<0.1	<0.1	<0.1	0.2
	Sample 136	<0.1	<0.1	<0.1	<0.1	<0.1
	Sample 137	2.9	0.3	<0.1	<0.1	3.2
	Sample 138	18.1	2.2	4.3	<0.1	24.6