OCCURRENCE AND HEALTH IMPLICATION OF MYCOTOXINS IN *Manihot esculenta* (CASSAVA) FLOUR COLLECTED FROM SELECTED AGRO-ECOLOGICAL ZONES OF NIGERIA

BY

,

AYENI, Ayomide Wemimo
MTech/SLS/2018/8823

DEPARTMENT OF BIOCHEMISTRY
FEDERAL UNIVERSITY OF TECHNOLOGY
MINNA

AUGUST, 2023

OCCURRENCE AND HEALTH IMPLICATION OF MYCOTOXINS IN *Manihot esculenta* (CASSAVA) FLOUR COLLECTED FROM SELECTED AGRO-ECOLOGICAL ZONES OF NIGERIA

BY

AYENI, Ayomide Wemimo
MTech/SLS/2018/8823

A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL FEDERAL UNIVERSITY OF TECHNOLOGY MINNA NIGER STATE NIGERIA, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTERS OF TECHNOLOGY IN BIOCHEMISTRY

AUGUST, 2023

DECLARATION

I hereby declare that this thesis titled: ‘‘**Occurrence And Health Implication of Mycotoxins in *Manihot esculenta* (Cassava) Flour Collected From Selected Agro-Ecological Zones of Nigeria’’** is a collection of my original research work and it has not been presented for any other qualification anywhere. Information from other sources (published or unpublished) has been duly acknowledged.

AYENI, Ayomide Wemimo
MTech/SLS/2018/8823
FEDERAL UNIVERSITY OF TEHNOLOGY
MINNA, NIGERIA

…….
SIGNATURE/DATE

CERTIFICATION

This thesis titled: **''Occurrence And Health Implication of Mycotoxins in *Manihot Esculenta* (Cassava) Flour Collected From Selected Agro-Ecological Zones of Nigeria"** by: AYENI, Ayomide Wemimo (MTech/SLS/2018/8823) meets the regulations governing the award of the degree of Master of Technology of the Federal University of Technology, Minna and it is approved for its contribution to scientific knowledge and literary presentation.

PROF. H. A. MAKUN
SUPERVISOR _____
SIGNATURE & DATE

PROF. A. ABUBAKAR
HEAD OF DEPARTMENT _____
SIGNATURE & DATE

PROF. F. O. ARIMORO
DEAN, SCHOOL OF LIFE SCIENCE _____
SIGNATURE & DATE

ENGR. PROF. O. K. ABUBAKRE
DEAN, POSTGRADUATE SCHOOL _____
SIGNATURE & DATE

DEDICATION

I dedicate this project work to God Almighty the giver of life and my all in all. This thesis is also dedicated to my Parents Mr and Mrs Tunde Ayeni, who did not relent in supporting me through the thick and thin of life especially in my academic pursuit and My Husband Dr Fagbemi Kayode, thank you for your support towards the completion of this project. Lastly, I dedicate this work to all in the world who will benefit from this work and also seek knowledge.

ACKNOWLEDGEMENTS

I acknowledge most importantly that only through the help of God was I able to carry out this work successfully.

I wish to express my profound gratitude to my supervisor, Prof. H.A. Makun (Center leader, Africa Center of Excellence for Mycotoxin and Food Safety ACEMFS), thank you very much sir for your mentorship, support and contribution towards the completion of this project, it has been a great privilege working with you sir.

I likewise want to acknowledge the immense contribution of Dr. H.L. Mohammad (Deputy Center leader, ACEMFS), thank you very much ma for all your help.

I sincerely also want to appreciate the full sponsorship for this work by Dr Maimuna Habib of Federal ministry of Agriculture and rural development and Africa Center of Excellence for Mycotoxins and Food Safety (ACEMFS) the contribution was invaluable.

In addition, I would like to appreciate National Agency for Food and Drug Administration and Control (NAFDAC) Central laboratories Oshodi, Lagos, for opening their doors to us to carry out this research using their. The following persons are worthy of mention as they made our work in the laboratory very productive; Dr. Charles Nwachukwu ,(Director laboratory services), Mrs Adebiyi Toyin (Mycotoxin Laboratory), Mr Mahmud Abdulkadri (Mycotoxin Laboratory), Dr. Okediran Oluwafemi (Pesticide Residue Laboratory) and Mrs Oluyide Oluwakemi (Microbiology Laboratory) thank you all I am very grateful.

My immense gratitude goes to my family for their prayers, support and encouragements throughout the programme.

ABSTRACT

Manihot esculenta flour (lafun) is a dry smooth granular product of cassava which is a staple food consumed daily in Nigeria. Cassava flour is exposed to fungi infestation due to poor processing and storage conditions, thus, this study seeks to determine the health risk associated with ingestion of lafun by the average consumer in Nigeria. Sixty-four (64) composite samples of cassava flour were formed from 130 samples, which were randomly purchased from open markets across the five Agro-Ecological zones in Nigeria (AEZs) and were aseptically transported to the NAFDAC laboratory for analysis. Fungal species isolation was carried out using dilution plate method and identification was based on the isolate's morphological characteristics using identification keys and atlas in literature. Aflatoxins (AFG_2, AFG_1, AFB_2, AFB_1, and AFT) were identified and quantified using high performance liquid chromatography (HPLC).Occurrence data obtained were used to estimate exposure level and risk associated with the studied mycotoxins. A total of 108 fungal isolates belonging to four genera: *Aspergillus spp., Fusarium spp., Penicillium spp., & Mucor Spp.* were isolated from the lafun samples, with *Aspergillus* spp. (55.56%) having the highest frequency compared to the population of other fungal genera in all the AEZs. AFG_2, AFG_1, AFB_2, AFB_1, occurred in all the tested samples at mean concentration of 12.62 µg/kg, 22.05 µg/kg, 2.12 µg/kg and 8.01 µg/kg respectively. The result indicated that 57.58 % of the total cassava flour samples that tested positive to AFB_1 exceeded the 2 µg/kg maximum regulatory limit set by European Union. Based on the estimated daily intake (EDI) calculated for average consumers, exposure to AFT were above acceptable limits. These findings raise public health concerns as the estimated risk of hepatocellular carcinoma per year is on the increase.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDIX

LIST OF ABBREVIATIONS

FB:	Detected Fumonisins
HPLC:	High Performance Liquid Chromatography
WHO:	World Health Organization
AF-alb:	Aflatoxin-Albumin Adduct
ND:	Non-Detectable
AFAR:	Aflatoxin Aldehyde Reductase
DNA:	Deoxyribonucleic Acid
RNA:	Ribonucleic Acid
IARC:	International Agency for Research on Cancer
ABPA:	Allergic Bronchopulmonary Aspergillosis
CPA:	Chronic Pulmonary Aspergillosis
HCC:	Hepatocellular Carcinoma
GC:	Gas Chromatography
RIA:	Radioimmunoassay (RIA)
ELISA:	Enzyme-Linked Immunosorbent Assay
TLC:	Thin-Layer Chromatography
FLD:	Fluorescent Detector
UV:	Ultraviolet
DAD:	Diode Array Detector

FAO:	Food and Agriculture Organization
PDA:	Potato Dextrose Agar
LOD:	Limit of Detection
LOQ:	Limit of Quantification
EDI:	Estimated Daily Intake
TDI:	Tolerable Daily Intake
AEZs:	Agro-Ecological Zones
CFU:	Colony Forming Units

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Cassava (*Manihot esculenta)* is a prominent root crop in the tropics, and its starchy roots provide calories to about 500 million people globally. In Nigeria, it is the most significant root crop in terms of food security, job development, and revenue generating for crop-producing households. It provides around 70% of the daily calories for over 50 million Nigerians (FAO, 2018).

Cultivation of cassava (*Manihot esculenta*) is becoming a global industrial and economic trend due to its flexible nature to grow under a wide range of soil and climatic conditions (Mtunguja *et al*., 2019) and allows for its use in several industries (Li *et al*., 2017). Cassava also has a competitive price when compared to many other food crop commodities, and its economic worth may be increased by developing and processing value-added products (Alene *et al*., 2018).With around 45 million metric tonnes, Nigeria is the world's largest cassava grower, and its cassava transformation is the most advanced in Africa (FAOSTAT, 2019). The natural high moisture content of fresh cassava roots increases microbiological degradation and unfavorable biochemical changes in the products, resulting in significant post-harvest deterioration. Processing is used to increase the shelf life, make transportation easier, and, most critically, detoxify the roots by eliminating the naturally occurring cyanogens (Nyirenda *et al*., 2021). As a result, cassava root is processed in Nigeria into garri, tapioca, lafun, fufu, and starch, among other products, with varying physical qualities due to differences in processing methods.

Cassava flour known as lafun in Yoruba, Akpu Nkpo in Igbo and Alubon Rogo in Hausa is a dry smooth granular product of cassava which is a staple food consumed daily in Nigeria. The production of lafun may or may not involve peeling of the cassava roots

before washing, fermenting in water (either in a flowing stream or stationary water) for softening, bagging/dewatering, drying, and milling (Ogunnike *et al*., 2015).

Tropical climate of some geographical areas of cassava production may contribute to fungal development of many species and subsequent toxinogenesis on such raw material (Bankole and Adebanjo, 2018). Moreover, the processing conditions and storage premises are not always well adapted to protect cassava products from secondary contamination and/or fungal development.

Mycotoxins may develop in almost any food or feedstuff during the growing season, at harvest time, or during processing or storage, depending on the environment and method of handling. Ingestion of high concentrations of mycotoxins can cause sickness or death in humans and animals. There are three major genera of fungi that produce mycotoxins: *Aspergillus*, *Fusarium*, and *Penicillium spp*

Evidence of contamination of cassava products with pathogenic fungi has been documented in several studies (Nweke, 2015) suggesting possible occurrence of their secondary metabolites (mycotoxins) in the products. Ediage *et al*. (2014) and Makun *et al*. (2013) also reported the occurrence of toxigenic fungi and mycotoxins especially those belonging to *Aspergillus* species in some common cassava products. A study by Nyaka *et al*. (2015) revealed the occurrence of other toxigenic fungi such as the *Fusarium spp.* as a causative agent of cassava root rot disease in Cameron, and this corroborates with the study of Manjula *et al*. (2009), who detected fumonisins (FB) in cassava products in Tanzania. This study therefore seeks to not only detect the presence of fungi and mycotoxin in cassava flour but also determine the health risk associated with the consumption of the staple in Nigeria.

1.2 Statement of the Research Problem

Microbial mycotoxins are frequently studied in food and feedstuffs across the world, and maximum limits are imposed to protect consumer safety (Juan *et al*., 2017). The toxic effects due to human and animal mycotoxins exposure have been in the limelight since the last decade (Zain, 2011). Mycotoxins have been reported to cause health effects ranging from acute to chronic disorders such as carcinogenic, estrogenic, and immunosuppressive effects. Few studies have been conducted on the contamination of cassava products with regulated mycotoxins when compared with the number of studies of toxin contamination of cereals, peanuts, dairy products, wheat, and dried chilies (Sulyok *et al*., 2015). On the other hand, a larger range of data has been published on the occurrence in cereals and cereal products of emerging mycotoxins, such as enniatins, beauvericin, moniliformin, fusaproliferin, fusaric acid, culmorin, butenolide, sterigmatocystin, emodin, mycophenolic acid, alternariol, alternariol monomethyl ether, and tenuazonic acid (Gruber-Dorninger *et al*., 2016). The lack of reliable data may have contributed to Africa's major cassava-producing countries, including Nigeria, the world's largest producer and consumer of cassava products, being unable to establish regulatory limits for mycotoxins in cassava products calculated based on per capita consumption of the cassava products, as well as the prevalence and concentrations of the various mycotoxins in the products.

1.3 Justification for the Study

Researchers have attempted to examine cassava product contamination with moulds and mycotoxins, given the importance of cassava crop in poor nations and the potential for fungal toxin generation. Thus, varieties of potentially mycotoxigenic fungi have been identified from cassava products, and cassava contamination with mycotoxins has been described. However the potential hygienic danger of such contamination has not been

adequately investigated (Westby, 2012). Knowledge of the levels of contaminants in food products is needed to assist food regulatory agencies in estimating possible exposure of consumers to such contaminants, health implications of consumption of the contaminated food and in setting maximum allowable levels for food control purposes. It should be noted that aflatoxins are genotoxic carcinogens. Therefore, the maximum limits for total aflatoxin content in a food or feed product (the sum of aflatoxins B1 and G1) is controlled or regulated, depending on the form in which the product is consumed or further processed before consumption. As a result, the goal of this study is to assess the presence of fungi and major mycotoxins in cassava flour consumed in selected agroecological zones of Nigeria, using a versatile and precise mycotoxin quantitation methodology based on the well-established principle of High Performance Liquid Chromatography (HPLC) (Sulyok *et al*., 2015).

1.4. Aim and Objectives of the Study

1.4.1. Aim of the research

The aim of the study is to determine the level, types, and risk of consumption of cassava flour contaminated with fungi and mycotoxins in some selected agro-ecological zones of Nigeria.

1.4.2 The objectives of the research are to;

1. isolate and identify fungi species in cassava flour using their morphological characteristics.
2. analyse the level of mycotoxins in Cassava flour samples using High Performance Liquid Chromatography
3. estimate human exposure and determine the risk of consumption of Cassava flour from the study area.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Mycotoxins in Food

According to the FAO (2018), approximately 25% of the world's agricultural products are contaminated with mycotoxins, and this contamination may be caused by saprophytic fungi before harvest of these crops while they are still in the field, during harvest, and even after harvest during storage of these products by endophytic fungi (Streit *et al*., 2017). This suggests that mycotoxins are found everywhere, including stored food and ill-kept food; when food is not adequately maintained, fungal growth can occur, leading to the creation of mycotoxins. Furthermore, mycotoxins can occur naturally, making it simpler for them to survive in human surroundings and become dangerous. Humans can be exposed to mycotoxin through the consumption of contaminated plant-derived foods, from the carry-over of mycotoxins and their metabolites to animal products such as meat and eggs, or from exposure to air and dust contaminated with toxins (Niculita-Hirzel *et al*., 2016).

Exposure to these toxins is more likely to predominate in parts of the world where there are poor methods for the pest infestation control of crops, transportation facilities, and storage facilities; however, it can also be rampant in societies with a high level of poverty, where people consume only the available and not the preferable. Constant exposure to mycotoxins is also frequent in areas where there are no rules or laws protecting the population's food consumption. In developed countries, particularly in areas where improper food handling is frequent, there is a danger of mycotoxin exposure, putting the population vulnerable to mycotoxin-induced diseases. However, because mycotoxins are naturally occurring pollutants, their presence in food may be inevitable (Francis *et al.*, 2020).

2.1.1 Types of mycotoxins

2.1.1.1 *Ochratoxins*

Ochratoxins, produced by *Penicillium, Fusarium,* and *Aspergillus* species, are found naturally in various plant products such as cereals, coffee, beans, pulses, and dried fruits (Xianjiang *et al*., 2021). It has been found in rice and maize samples obtained from west Africa, specifically Nigeria. Ochratoxin causes nephropathy in humans; it is also suspected to be the cause of Tunisian nephropathy and human Balkan endemic nephropathy (BEN) (Lulamba, 2016). This mycotoxin is known for its teratogenic action; it can harm the fetus in the pregnancy due to its capacity to cross the placenta and induce central nervous system abnormalities and brain damage. (Laura *et al*., 2012). Darwish *et al*. (2014) reported that ochratoxin toxicity was widespread in Africa.

2.1.1.2 *Fumonisins*

Fusarium species that generate these include *Fusarium verticillioides* and *Fusarium proliferatum. F. verticillioides*, a significant economic species, grows as a corn endophyte in both vegetative and reproductive tissues, frequently without causing disease symptoms in the plant. It can be found in almost all corn samples. It has been discovered in Nigerian and South African maize and corn sample. (Rheeder *et al*., 2016). However, dependent on the weather conditions, insect damage, and the fungal and plant genotype, they can cause seedling blight, stalk rot, and ear rot. Fumonisin has been linked to cancer of the esophagus in humans, although it affects animals in different ways: it has been known to be the cause of various illnesses, such as leukoencephalomalacia in equines and rabbits (Giannitti *et al*., 2019).

2.1.1.3. *Zearalenone*

Zearalenone is a secondary metabolite from Fusarium graminearum. It was found to be present in addition to other mycotoxins in samples of rice, maize, and peanuts obtained

from Cote d'Ivoire and Africa. Zearalenone is biosynthesized through a polyketide pathway by *F. graminearum, Fusarium culmorum,* and *Fusarium equiseti* (Sangare-Tigori *et al*., 2016). These species are well-known cereal crop contaminants all over the world. Small amounts of zearalenone have been shown to alter animal health, creating major health risks and producing disorders such as hyperestrogenic syndrome in pigs. When zearalenone is present in high concentrations, it can interrupt fertilization, cause abortion, and create other issues (Misihairabgwi *et al*., 2019).

2.1.1.4 *Trichothecenes*

These are produced by several fungal genera, which include Fusarium, Trichoderma, Myrothecium, Trichothecium, Verticimonosporium, Stachybotrys, and Cephalosporium. Trichothecenes have been reported in Middle East Africa, where they were found to be present in feed and feed ingredients. They are a large group of mycotoxins that consist of more than 180 structurally related sesquiterpenoid mycotoxins produced from essential raw materials used in animal food and feeds such as maize, wheat, and oats. They are known to be strong inhibitors of protein synthesis that can be absorbed into the body through the skin to inhibit protein synthesis in the body, harming the health of its host. Trichothecenes, such as other mycotoxins, are resistant to heat (Rodrigues *et al*., 2011).

2.1.1.5 *Patulin*

Patulin is commonly found in rotting apples, but it has also been identified in vegetables and other types of fruit, and it has been detected in South African apples and apple products. It is created especially by Penicillium and Aspergillus and is persistent even at high temperatures; hence, thermal denaturation cannot eradicate it. Fermentation, on the other hand, may impair its stability. (Ismaiel and Papenbrock, 2015). In 1998, Llewellyn *et al*. (1998) reported that it can be destroyed by antioxidant and antimicrobial agents.

2.2 Aflatoxins

These are extremely toxic secondary metabolites of certain *Aspergillus* molds, which have been classified by the WHO as genotoxic and carcinogenic (FAO, 2018). Even at extremely low concentrations, aflatoxins are carcinogenic, hepatotoxic, teratogenic, and mutagenic when swallowed, breathed, or absorbed via the skin. (Wen *et al*., 2014). They were shown to be the cause of Turkey X disease, i.e. hepatic necrosis, in 1960 (José *et al*., 2018; Mateo *et al.,* 2017). Aflatoxins were also the cause of aflatoxicosis that occurred in 1981, 2001, 2004, and 2005 in Kenya (Africa). According to Makun *et al*. (2011), AFB1 is the most potent mycotoxin and is known to be hepatotoxic and hepatocarcinogenic.

Among more than few hundred known mycotoxins, aflatoxins represent the main threat worldwide. Aflatoxins are group of mycotoxins produced mainly by *A. flavus*, *A. parasiticus* and *A. nomius*. The word 'aflatoxin' came from '*Aspergillus flavus* toxin', because *A. flavus* and *A. parasiticus* are the predominant producers of aflatoxins (Yu *et al*., 2019). Aflatoxins are a class of naturally occurring carcinogens that have been found in a variety of human and animal foods. Aflatoxins are deadly byproducts of the soil-borne fungus Aspergillus, which is responsible for plant material degradation. (Bowman *et al.,* 2012).

Aflatoxins are a group of approximately 20 related fungal metabolites which belongs to a group of difuranocoumarins that are classified into two broad groups according to their chemical structure and they include the difurocoumarocyclopentenone series (AFB1, AFB2, AFB2A, AFM1, AFM2, AFM2A and aflatoxicol) and the difurocoumarolactone series (AFG1, AFG2, AFG2A, AFGM1, AFGM2, AFGM2A and AFB3) (Misihairabgwi *et al*., 2019), The four major naturally known aflatoxins produced by the Aspergillus species of mold include AFB1, AFB2, AFG1 and AFG2 where the "B" and "G" refer to the blue and green fluorescent colors produced under UV light on thin layer

chromatography plates, while the subscript numbers 1 and 2 indicate major and minor compounds, respectively. Whereas the B designation of aflatoxins B1 and B2 result from the exhibition of blue fluorescence under UV-light, while the G designation refers to the yellow-green fluorescence of the relevant structures under UV-light (Sarma *et al.*, 2017). The metabolic products of aflatoxins, M1 and M2 were first isolated from milk of lactating animals fed on Moldy grains contaminated with aflatoxin hence, the M designation. These toxins have closely similar structures (Figure 2.1) and form a unique group of highly oxygenated, naturally occurring heterocyclic compounds. Aflatoxins B2 and G2 were established as the dihydroxy derivatives of B1 and G1, respectively. Whereas, aflatoxin M1 is 4-hydroxy aflatoxin B1 and aflatoxin M2 is 4-dihydroxy aflatoxin B2. Of the four major aflatoxins (B1, B2, G1 and G2), G2 occurs in high quantities though less toxic while AFB1 is the most toxic of all the aflatoxin (Bennett and Klich, 2018).

The World Health Organization (WHO) classifies AFB1 as a class 1 carcinogen. The aflatoxins display potency of toxicity, carcinogenicity, mutagenicity in the order of AFB1> AFG1> AFB2> AFG2 (Sarma *et al.*, 2017). Aflatoxin B1 is the most harmful aflatoxin to humans and animals as it is carcinogenic due to its association with hepatocellular carcinoma which leads to liver cancer (Qureshi *et al*. 2015). Aflatoxins suppress the immune systems of humans and animals by interfering with the fickleness of those cells which are responsible to boost immunity. Large doses of aflatoxins lead to direct death and damage, while small longstanding doses lead to immunologic or nutritional effects, but both types of doses lead to liver cancer due to the accumulation of aflatoxin (Marroquín-Cardona *et al*. 2014). Children are more prone to the toxicity of Aflatoxin as it increases the risk of early infections by reduced immunization. The carcinogenic nature of aflatoxin is due to its ability to damage DNA either by lipid

peroxidation or by oxidation (Zhang *et al*., 2015). The extent of toxicity depends on the organ affected especially the liver. The lethal toxicity of aflatoxin B1 varies in different animals from extremely susceptible (Sheep, Rat, Dog) to resistant species (Monkey, Chicken, Mouse). However, there is no harm in humans, despite epidemiological data from studies in Africa, South Africa, South East Asia, and India implicating aflatoxins in the occurrence of liver cancer, particularly hepatobiliary carcinoma, and child mortality owing to malnutrition, kwashiorkor, and marasmus. (Thomas, 2018). Aflatoxins have been associated with various diseases like aflatoxicosis and other health problems in humans, livestock and domestic animals globally.

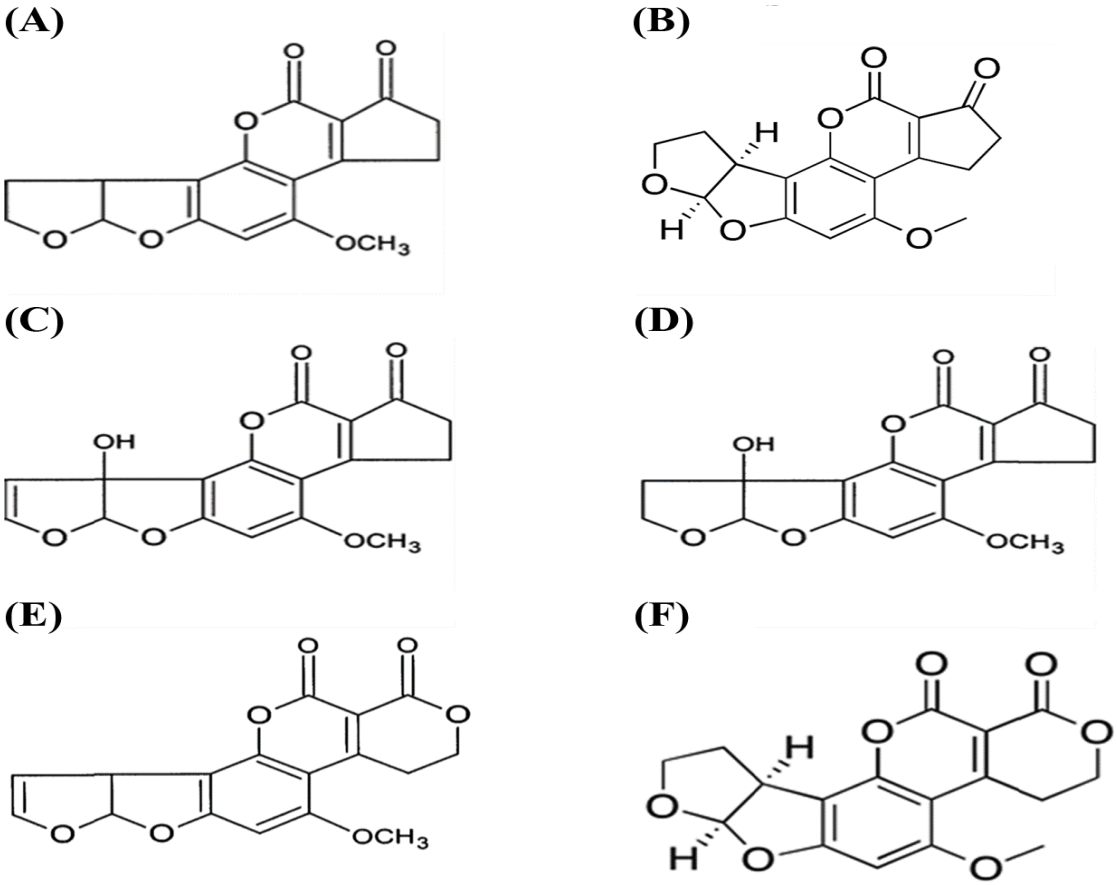


Fig. 2.1. Chemical Structure of Aflatoxin. (A) Aflatoxin B1, (B) Aflatoxin B2, (C) Aflatoxin M1, (D) Aflatoxin M2, (E) Aflatoxin G1, (F) Aflatoxin G2. (Sarma *et al.*, 2017)

2.2.1 Global aflatoxin exposure

Globally, aflatoxins contaminate about 25% of the worlds food supply (EAC Policy, 2018). An estimated 4.5 billion of the world's population is exposed to aflatoxins which accounted for one-sixth of the global deaths annually as Africa recorded more than half-billion cases (CDC, 2017). Increased exposure to aflatoxins is generally associated with acute or chronic toxicities depending on the amount and length of exposure, and often death in humans, other mammals, birds, and fishes. Being highly lipophilic, the primary target organ of aflatoxins is the liver which may spread to other organs like kidneys, lungs, heart, and brain (Gurav and Medhe, 2018). The cases are more likely to occur in African countries due to hot and humid climatic conditions coupled with poor storage practices (eg. Use of stores with leaky roofs and /or with poor ventilation) which promote the proliferation of aflatoxigenic fungi (Gong *et al*., 2016). This lead to the serious contamination of foods and feeds accordingly. Globally, Africa has the greatest rate of liver cancer (40% of the estimated 25,200- 155,000 cases per year) due to aflatoxins contamination. (Liu and Wu, 2019).

The aflatoxin-albumin adduct (AF-alb) biomarker has been used in a number of epidemiological studies to measure exposure and its associated health effects in many different populations groups. High risk populations are specifically those from rural subsistence farming communities in developing countries. In high-risk regions, AF-alb concentrations have previously been reported over a 2–3 log range from below the limit of detection (LOD) of 3 pg/mg to >1000 pg/mg (Turner *et al*., 2021). East and West Africa have the ideal climate of hot and humid conditions for *Aspergillus* growth and subsequent aflatoxin production, and intake of susceptible crops such as maize or groundnuts as staple foods is high. Reflecting this, it has previously been reported that over 95% of blood

samples collected from different parts of West Africa, across different age groups, had detectable concentrations of AF-alb (Felicia *et al.*, 2014).

More recently, the AF-alb biomarker has revealed high aflatoxin exposure in East African countries including Kenya where AF-alb was detected in 78% of 597 serum samples (non-detectable (ND) − 211 pg/mg); in Uganda (Asiki *et al*., 2014), where AF-alb was detected in 192/196 (98%) samples (range ND to 237.7 pg/mg) collected from adults and children; and in Tanzania (Shirima *et al*., 2018), where AF-alb was detected in 67% to 99% of samples collected from young children. The incidence of aflatoxin exposure is lower in North and South Africa, where the environment is dryer, than in East and West Africa. (Stephenie *et al*., 2021). Turner *et al*. (2021) found AF-alb in 31/46 (67%) samples from Egypt (range ND–32.8 pg/mg). Piekkola *et al*. (2017) found AF-alb concentrations in 34/98 (35.6%) serum samples from pregnant Egyptian women in their third trimester, and Stephenie *et al*. (2021) found no trace of AFM1 in urine samples collected from South African women.

Parts of Asia also have high prevalence of aflatoxin exposure (Felicia *et al.*, 2014); e.g., in Malaysia, where 97% of 170 samples had detectable AFB1-lysine adduct levels (detected by HPLC-fluorescence), ranging between 0.20 to 23.26 pg/mg (Leong *et al*., 2022). Furthermore, a study examining aflatoxin exposure in pregnant women in South Asia using isotope dilution mass spectrometry to measure AFB1-lysine (Groopman *et al*., 2014), found detectable levels of the biomarker in 94% of blood samples collected from Nepalese pregnant women, with levels ranging between 0.45 to 2939.30 pg/mg. In the same study, AFB1-lysine was detected in 63/63 (100%) samples collected from pregnant women in their first and third trimester from Bangladesh, as well as in 63/63 (100%) cord blood samples and in 63/63 (100%) infants who were born to the mothers exposed to aflatoxin during pregnancy. Rice is a nutritional mainstay in Nepal and Bangladesh;

nonetheless, aflatoxin contamination in rice is uncommon. (Roy *et al*., 2013). Other food commodities regularly consumed in Bangladesh such as betelnut, lentils and red chilli powder, however, have been shown to have high levels of aflatoxin. Maize is also a dietary staple in Nepal, and research has shown high levels of aflatoxin contamination present in maize commodities from Nepal (Karki and Sinha, 2019).

Aflatoxin exposure is not a major issue for developed regions, as there are strictly enforced regulatory limits in place and the diet is more diverse. AF-alb is rarely detected in blood samples from populations in these regions (Schleicher *et al*., 2018). For instance, in a subset of individuals (n = 2051) that participated in the 1999–2000 National Health and Nutrition Examination Survey (NHANES), which is a representative cross-sectional survey of the US population, only 1% had detectable levels (≥0.02 μg/L) of AFB1-lysine in their blood (Schleicher *et al*., 2018).

2.2.2 Occurrence of aflatoxins in food and crops

Aflatoxin levels in foods and food products fluctuate depending on geographic region, agricultural and agronomic activities. Food products are susceptible to fungal assault during pre-harvest, transit, storage, and processing (Sarma *et al.*, 2017). Aflatoxin contamination of food items is a prevalent concern in tropical and subtropical parts of the world, particularly in developing nations with inadequate practices and where the climatic circumstances of warm temperatures and humidity encourage the growth of fungus (Sarma *et al.*, 2017). The aflatoxins were initially isolated and identified as the causative agent in Turkey X disease that caused necrosis of the liver in 1960 and over 100,000 turkeys died in England and USA and the death was attributed to the consumption of a mould-contaminated peanut meal. Aflatoxin concentrations are commonly found in nutritious seeds such as maize, nuts, and cereal crops in Africa, and rice in China and Southeast Asia (Kitya *et al*., 2010).

Aflatoxin poisoning in crops is a global concern that jeopardizes food and feed safety while also affecting the agricultural economy and crop-dependent small-scale companies. Fungi can infect crops throughout the harvesting, storage, and shipping processes, resulting in the creation of numerous mycotoxins. Aflatoxin B1 and B2 are produced by *A. flavus*, while Aflatoxin G1 and G2 are synthesized by *A. parasiticus* and largely contaminate a wide range of food commodities including cereals (maize, sorghum, pearl millet, rice, and wheat), oilseeds (peanut, soybean, sunflower, and cotton), spices (chilies, black pepper, turmeric, coriander, and ginger), nuts (almond, Brazil nut, pistachio, walnut, and coconut), yam and various milk products (Rajarajan *et al*., 2013). The injuries caused by insects and nematode also paved the way for the entry of these fungi (Jeyaramraja *et al*. 2018). Fungus penetration into crops not only affects crop plant self-defense against fungal attack, but also contaminates crop seeds, resulting in aflatoxin formation. Fungal infections have an impact on crop growth and production, as well as market value.

2.2.3 Metabolism and toxicity of aflatoxins

2.2.3.1 *Aflatoxins and their metabolism*

The biotransformation of aflatoxin B1, the most prevalent type, has been studied in order to better understand the metabolism of aflatoxins (Vondracek *et al*., 2021). Aflatoxin B1 metabolism takes place in the microsome of the liver and is mediated by mixed function monooxygenases belonging to the cytochrome P450 super family of enzymes (Guengerich *et al*., 2018) (Figure 2.2). In humans, cytochrome P450 enzymes, CYP1A2 and CYP3A4, catabolize aflatoxin B1 through two separate electron transfer oxidation reactions. While CYP1A2 breaks down aflatoxin B1 to exoepoxide, endoepoxide, and aflatoxin M1, CYP3A4 breaks down aflatoxin B1 to aflatoxin B1-exo- 8,9-epoxide and aflatoxin Q1. AflatoxinsM1 and Q1 are not broken any further but are excreted in the

urine. Aflatoxin B1-exo-8,9-epoxide may be converted either to aflatoxinmercapturic acid via the glutathione *S*-transferase- (GST-) conjugate mediated route or into aflatoxin-glucuronide via the aflatoxin-dihydrodiol route described as follows. The activated form of aflatoxin B1 (*exo*epoxides and *endo*epoxides) is detoxified through GST- mediated conjugation by using reduced glutathione (GSH) to form AFB1 *exo*epoxide- GSH and *endo*epoxide- GSH conjugates, respectively (Johnson *et al.*, 2017). The reactive *exo*epoxides and *endo*epoxides also undergo rapid nonenzymatic hydrolysis to aflatoxin B1-8,9-dihydrodiol that slowly transforms into a dialdehyde phenolate ion. Dialdehyde phenolate ion is subsequently hydrolyzed by aflatoxin aldehyde reductase (AFAR) to a dialcohol, in the NADPH-dependent reduction reaction. Thereafter, dialcohol is excreted in urine as aflatoxin-glucuronide. Aflatoxin B1 dialdehydes also form Schiff bases with primary amine groups of amino acid residues such as lysine of such a protein as albumin to form aflatoxin B1-albumin conjugate (Wild and Turner, 2017). Because this conjugate stays in the systemic circulation as permanent and irreversible aflatoxin B1-albumin adducts, it is thought to be one of the causes contributing to the poor elimination of aflatoxins and their metabolites in urine (Wild and Turner, 2017).

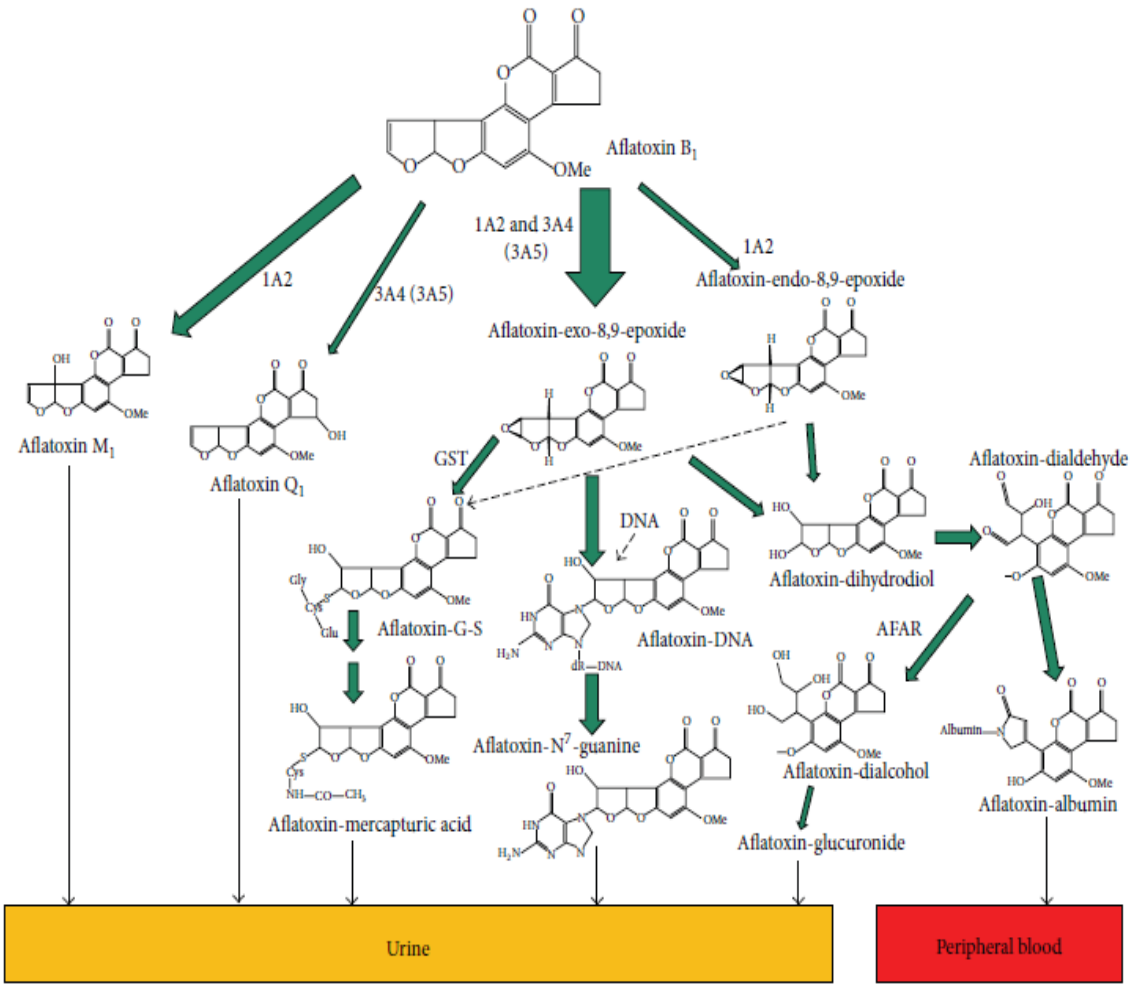


Fig 2.2: Principal metabolism of aflatoxin B1 leading to reactive metabolites and biomarkers. 1A2: CYP1A2; 3A4: CYP3A4; 3A5: CYP3A5; GST: glutathione S-transferase; AFAR: aflatoxin aldehyde reductase; aflatoxin-S-G, aflatoxin-glutathione conjugate (Wild and Turner, 2017).

2.2.3.2 *Toxicity of aflatoxins*

From the foregoing (Figure 2.1), it can be observed that the primary derivatives of aflatoxin B1 biotransformation comprise (a) aflatoxin M1 and aflatoxin-exo-8,9-epoxide (products of CYP1A2 activity) and (b) aflatoxin Q1 and aflatoxin-exo-8,9-epoxide (products of CYP3A4 activity). Aflatoxins M1 and Q1, although toxic, are less reactive with other molecules and are easily eliminated from the body in the urine (Wild and Turner, 2017). However, aflatoxin B1- 8,9-exo-epoxide is a known mutagen, which is extremely electrophilic and covalently reacts with nucleophilic sites of either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) or proteins, thereby introducing

mutations that may affect the normal function of cells. The formation of aflatoxin B1-DNA adducts is extremely associated with the carcinogenicity of aflatoxin B1. Typically, aflatoxin B1 reacts with DNA (methylation) resulting in G→T transversion mutation (Levy *et al*., 2012). Such a mutation has been associated with hepatocellular carcinoma, a type of cancer whereby aflatoxin B1 promotes AGG→AGT (Arg→Ser) transversion point mutation of p53 gene at codon 249 that alters *p53* gene, which is responsible for DNA repair. Apart from G→ T transversions, G→C transversions and G→A transitions have also been reported (Levy *et al*., 2012).

Nucleic acids and proteins interact covalently with aflatoxins and this results in alteration in base sequences in nucleic acids (both DNA and RNA) and in protein structures, leading to impairment of their activity. The highly reactive aflatoxin B1-8,9-exo-epoxide and its hydration product, dihydrodiol, bind covalently to DNA, RNA, and proteins to inhibit protein synthesis. Typically, RNA polymerase and ribosomal translocase have been demonstrated to be inhibited by aflatoxin B1-8,9-exo-epoxide. While the epoxide combines with the N7 position of guanine in both DNA and RNA, the dihydrodiol reacts with the bases' amino groups to generate a Schiff base (Bbosa *et al.*, 2013). Aflatoxin B1 has also been reported to negatively impact carbohydrate metabolism, which results in both the reduction in hepatic glycogen and also the increased blood glucose levels (Figure 2.3).

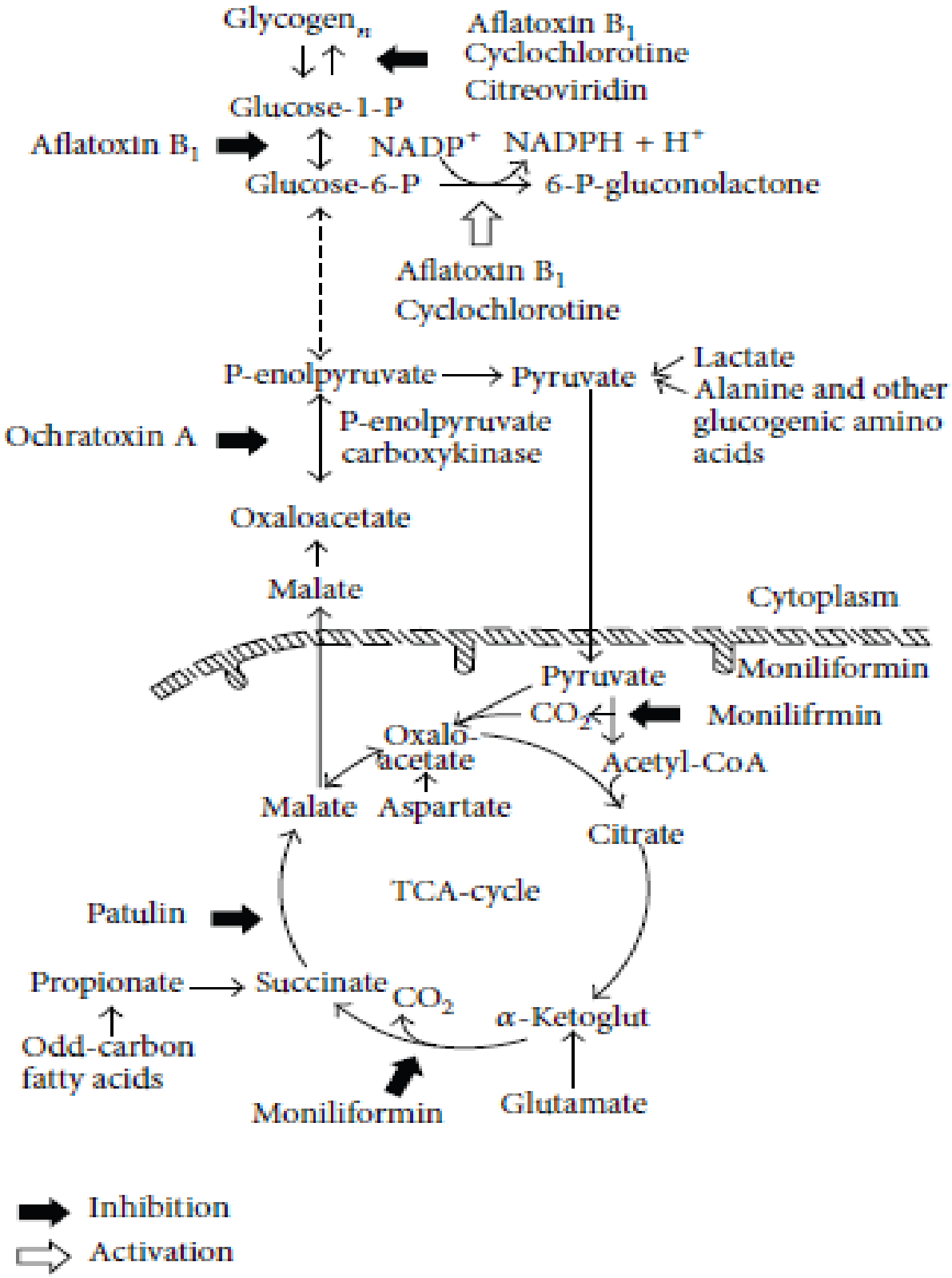


Fig 2.3: Inhibition of Oxidative Phosphorylation by Aflatoxins (Jimoh and Kolapo, 2018).

Notably, the detrimental effects of aflatoxin B1 on glucose metabolism appear to be due to its inhibitory effects on glycogen synthetase and transglycosylase enzymes, which results in a stop in glycogen synthesis (Bbosa *et al.*, 2013). Besides, aflatoxin B1 also inhibits phosphoglucomutase, an enzyme that reversibly catalyzes the conversion of glucose-6-phosphate into glucose- 1-phosphate, leading to a decrease in its activity, thereby promoting both the accumulation of glucose 6-phosphate and a decrease in glycogen synthesis. As a result, excess glucose cannot be stored as glycogen and instead accumulates in the circulation or is converted to glucose-6-phosphate for the creation of additional metabolic intermediates via the pentose phosphate pathway. Aflatoxin B1, G1, and M1 also impair the electron transport system in the mitochondria, especially cytochrome oxidase function (**Figure 2.4**) (Wild and Turner, 2017).

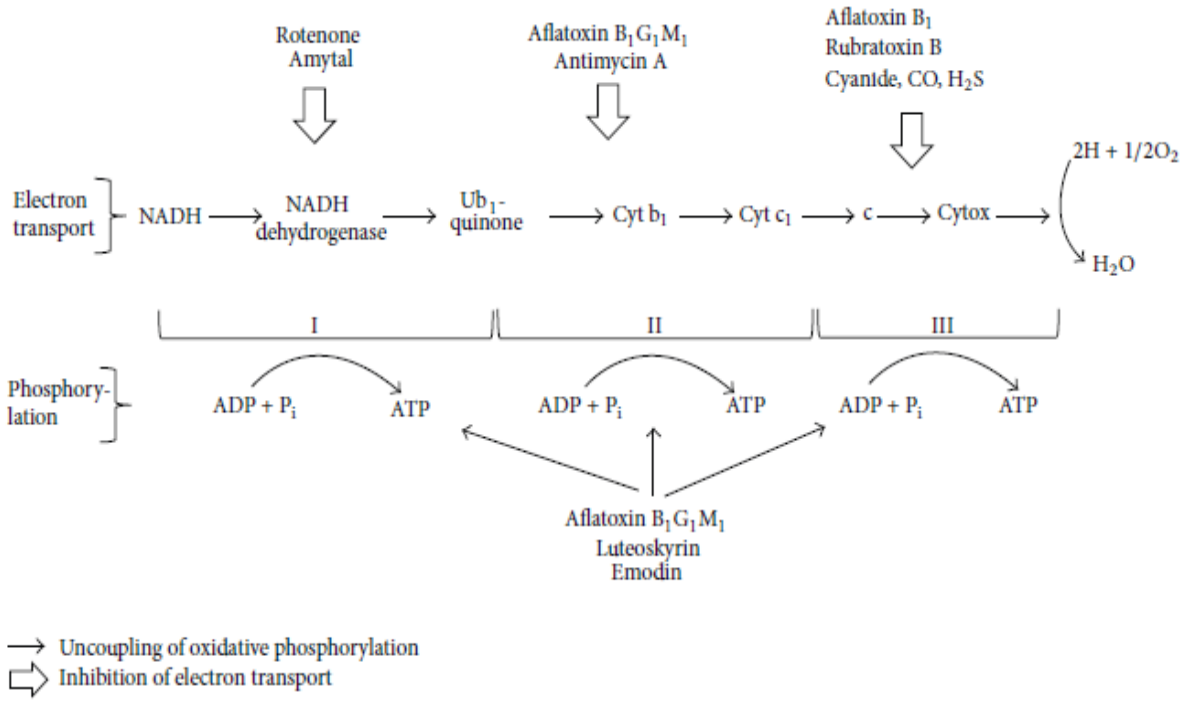


Fig 2.4: Sites of inhibition of the electron transport chain by aflatoxins (Wild and Turner, 2017).

Apart from inhibiting the electron transport chain, aflatoxins are also carcinogenic. As a result, the World Health Organization's International Agency for Research on Cancer (IARC) in 1993 classified aflatoxin B1 as Group 1 carcinogen (Zhang *et al.*, 2015). Since

then, aflatoxins concentration has become one of the most critical indicators of food and feed toxicity.

2.2.4 Impacts of aflatoxin contamination on agriculture and food security, trade, and health

2.2.4.1 *Agriculture and food security*

Aflatoxin contamination of major staples can have an impact on agricultural sector production in general, as well as each of the four pillars of food security (availability, access, usage, and stability). Contamination in staples including maize, sorghum, groundnuts, and cassava can affect food supply directly. Producers of the afflicted crop may also earn less as a result of product rejection, decreased market value, or inability to access higher-value foreign commerce and the formal market. Lower farmer income in turn limits ability to purchase food for the family, which translates into reduced access to food (Jimoh and Kolapo, 2018). Contamination reduces use options for the affected produce through complete rejection or need to put it to other safe uses, given the relationship between aflatoxin and negative human health impacts, including documented links to liver cancer, synergistic effects with Hepatitis B, and probable associations with stunting and immunosuppression, tainted food poses an obvious food security danger (Dragan *et al.*, 2010).

2.2.4.2 *Trade*

Many nations have put in place laws to limit aflatoxin exposure, which is usually represented in parts per billion (ppb). Some nations have differing limitations based on the intended purpose, with the most stringent governing human consumption and exports and the most stringent governing industrial goods. These laws may result in lost trade income due to increasing costs of meeting requirements, such as testing costs, cargo

rejection, and even ultimate loss of admissibility into foreign markets (Khlangwiset, 2021). The direct economic impact of aflatoxin contamination in crops is mostly due to a decrease in marketable volume, a loss in value in national markets, product inadmissibility or rejection by the international market, and losses incurred from animal sickness, morbidity, and death (Jimoh and Kolapo, 2018). Specifically, in the international market, products that do not meet the aflatoxin standards are either rejected at the border, rejected in channels of distribution, assigned a reduced price, or diverted to nonhuman or even non-fee uses. Similar economic losses may occur in domestic markets if consumer awareness about the problem rises, if leaders in marketing channels begin to pay more attention, and/or if regulations are either tightened or more strictly enforced (Jimoh and Kolapo, 2018).

Premiums for aflatoxin-free goods may be achieved for a short time under any of these conditions. In the long term, the premium will diminish when compliance becomes a prerequisite for being approved as a supplier. While it may appear that stricter phytosanitary standards imply more costs than benefits, in fact, once suppliers internalize and bear the economic costs of noncompliance, greater economic benefits for society will emerge in a variety of forms, including larger and more stable markets and a lower disease burden (Dragan *et al.*, 2010).

2.2.4.3 *Human and animal health*

Aflatoxins are considered not only hazardous for humans but also animals. They can cause different acute and chronic illnesses, which includes;

I. Aspergillosis

Aspergillosis is a lung infection caused by *Aspergillus* species in immunocompromised individuals. It is caused by twenty different species of Aspergillus, but *A. fumigatus* and

A. flavus are the main agents of aspergillosis in both humans and animals. Worldwide, most cases of aspergillosis infection in humans are caused due to excessive inhalation of *Aspergillus* spores, while the second main cause of infection is the transmission of spores through infected wounds, as well as through the smoking of contaminated tobacco or marijuana plants. Different animals such as rabbits, chickens, turkeys, and geese are also infected by aspergillosis. In addition, *A. flavus* also causes stone brood disease in honeybees. Clinically, aspergillosis has different forms, which include extrinsic asthma, allergic bronchopulmonary aspergillosis, extrinsic allergic alveolitis, saprophytic pulmonary, and extra-pulmonary colonizing, as well invasive pulmonary and extrapulmonary aspergillosis (Amare and Keller, 2014). Allergic bronchopulmonary aspergillosis (ABPA) accumulates in 1–15% of the world's population already infected with cystic fibrosis and also in 2.5% of asthma patients, which in total comprises 4.8 million people globally. Out of the 4.8 million of the world's population affected with ABPA, 400,000 people are also affected with chronic pulmonary aspergillosis (CPA). On the other hand, 1.2 million people with tuberculosis are also coinfected with chronic pulmonary aspergillosis (CPA) (Denning *et al*., 2013). Aspergillosis ranks in the list of the top four diseases that cause death in immunocompromised patients worldwide. Although *A. flavus* does not cause aspergillosis often, the rare cases of infection can be very severe. In North America, around 65% of aspergillosis in children is caused by *A. flavus*. Moreover, it is also the main causative agent of mycotic keratitis (Amare and Keller, 2014).

II. Cancer

Aflatoxins are reported as a Group 1 carcinogen and their long-term exposure may cause kidney, liver, lung, or colon cancer in both animals and humans. In Africa and Asia, the primary liver cancer known as hepatocellular carcinoma is related to aflatoxin B1, while

about 4.6–28.2% of hepatocellular carcinoma around the world is reported to be caused by aflatoxin consumption (Chandra, 2021). Moreover, aflatoxin B1, which is characterized as a Group 1 carcinogen, is found to be hazardous if a concentration of 20–120g/kg is consumed per day for 1 to 3 weeks (WHO, 2018). However, the level of aflatoxin toxicity is strongly dependent on the host's immunity. Hepatocellular carcinoma (HCC) is the most common result of aflatoxin exposure, accounting for 75-85% of all instances of liver cancer globally. In addition, 1480 additional instances of liver cancer caused by aflatoxins were discovered in Tanzania in 2016 (Kimanya *et al*., 2021).

III. Hepatocellular Carcinoma (HCC)

In 2012, HCC was recognized as the sixth most common cancer worldwide, with 83% of cases occurring in less developed regions (Odintsoya *et al*., 2015). Asian and African countries have the greatest incidence rates. Aflatoxin, along with the hepatitis B and C viruses, has been identified as a key risk factor due to its mutagenic and carcinogenic qualities (HCV). In fact, it has been shown that aflatoxin and hepatitis B, which is also highly prevalent in Africa and South Asia, can synergistically interact, resulting in an increased risk of HCC (Bosetti *et al*., 2014) (**Figure 2.5**).

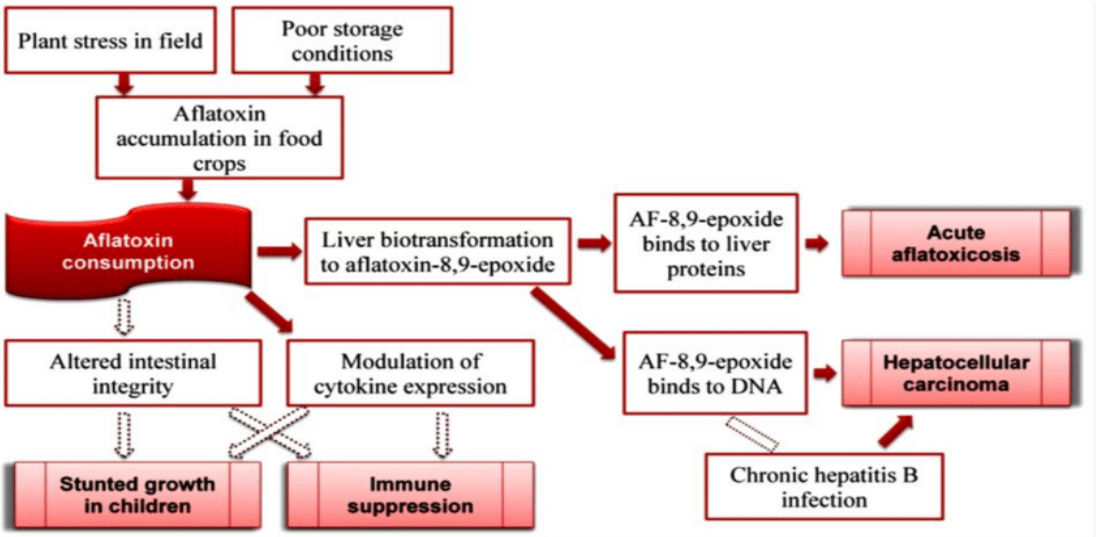


Fig 2.5: Aflatoxin disease pathways in Hepatocellular Carcinoma (HCC) (Wu, 2019).

The P53 gene hotspot mutation at codon 249 was associated with aflatoxin exposure. Han *et al*. (2020) observed a seasonal variation in levels of the R249S mutation in circulating cell free DNA in the serum of subjects in Gambia that reflected seasonal variations in aflatoxin exposure and markers of HBV infection, suggesting an interaction between these risk factors for HCC. A meta-analysis performed by Liu *et al.* (2017) concluded that in areas of high aflatoxin exposure and chronic HBV infection, aflatoxin exposure and HBV have a nearly perfectly multiplicative relationship in increasing HCC risk. In another research, about 25% of the 600,000 new cases of HCC reported annually worldwide were estimated by Liu and Wu (2019) to be attributed to aflatoxin exposure. Igetei *et al*. (2018), in a case-control study, evaluated 79 Nigerian HCC patients using cell free DNA in plasma for p53 gene codon 249 G – T mutation. In his study, none of the control individuals had this mutation while six (7.6%) of the HCC cases turned out positive. In Nigeria, an estimated 7,761 out of 10,130 annual liver cancer cases are attributed to Aflatoxin contaminated maize and groundnuts (Adetunji *et al*., 2019). This has led to the loss of about 5,000 lives and $155 million (Olayinka, 2018). A similar work was done in India by Murugavel *et al*. (2017), directly demonstrated aflatoxin in the liver cells by employing immunohistochemical techniques. The group evaluated 31 liver biopsy cases of HCC for the presence of AFB1 using in-house immunoperoxidase test directly on the paraffin embedded tissue sections and found 18 (58.1%) to be positive. Thirteen of the biopsies were positive for HBV surface antigen and 6 (46.1%) were also positive for AFB (Murugavel *et al., 2017*).

Aflatoxin has also been implicated in the aetiology of other liver diseases including cirrhosis and hepatomegaly (Gong *et al*., 2019). A study in Kenya by Gong *et al.* (2019) reported that the frequency of hepatomegaly, a severe form of liver enlargement, rose in

children exposed to more aflatoxin. This is consistent with the liver being the primary target organ for aflatoxin poisoning.

IV. Aflatoxicosis

Aflatoxicosis is the poisoning associated with the extensive consumption of Aspergillus species, mainly *A. flavus* in the form of spores or contaminated food that can cause chronic or acute aflatoxicosis in humans and animals. Acute aflatoxicosis includes high fever, vomiting, ascites, liver failure, edema of feet, and jaundice with a high mortality rate compared to chronic aflatoxicosis (Dhanasekaran *et al.*, 2021). Accurate values of the aflatoxin concentration that causes aflatoxicosis have not been confirmed; however, with the help of a few studies, it is estimated that generally 1000 g/kg of aflatoxin concentration in food can cause aflatoxin toxicity in humans. In the case of animals, a tolerable amount is 50–300 _g/kg (Sirma *et al*., 2018). Major outbreaks of aflatoxicosis were reported in India and Kenya in 1974 and 1981, respectively. It is worth mentioning that 500 cases and 200 deaths have occurred due to aflatoxicosis worldwide since 2004 (Kumar *et al*., 2017).

Several instances of aflatoxicosis have been reported in Nigeria, although only a handful have been recorded (Francis *et al.*, 2020). In the late 1900s, one of the most devastating outbreaks of acute aflatoxicosis occurred, with 40 children apparently dying at the Obafemi Awolowo Teaching Hospital complex in Ile-Ife, Nigeria. Aflatoxins were found in 81% (30 of the 40 children) of the children's brain tissues during autopsies (Oyelami *et al.*, 2017). Among the 30 aflatoxin positive children, 15 have kwashiorkor which is a sign of chronic aflatoxicosis while the remaining showed other symptoms (Oyelami *et al.*, 2017). Aflatoxins were also detected in the lung and kidney specimens of another set of 32 children during autopsy among which 18 suffered from kwashiorkor (Oyelami *et al.*, 2017). It was concluded that kidney and cardiac failures were the major cause of death

among such children (Oyelami *et al.*, 2017). Many other deaths due to acute aflatoxicosis have been occurring in various parts of Nigeria (Francis *et al.*, 2020). Aflatoxins were found in various tissues and biological fluids such as semen, blood, and urine of liver cancer patients in Nigeria (Francis *et al.*, 2020). According to a report from the National Hospital in Abuja, several post-mortem investigations on liver cancer have been connected to the impacts of aflatoxins (Francis *et al.*, 2020). Furthermore, multiple cases of aflatoxicosis and other mycotoxicoses have been reported in Nigerian specialty hospitals, according to sources (Francis *et al.*, 2020).

The effect of chronic exposure to aflatoxins is aflatoxin-induced hepatocellular carcinoma (HCC) which is mostly associated with hepatitis B or C virus (Lizarraga-Paulin *et al.*, 2022). Generally, aflatoxin exposure is the second largest environmental risk factor for cancer development worldwide (McCullough and Lloyd, 2019). Another debilitating exposure effect to aflatoxins is their ability to devitalize the immune system through modulation of cytokines, cell-mediated immune suppression, T-cells, B- cells and natural killer cells activities suppression (Bammler *et al.*, 2015). Many studies in Africa showed strong association between exposure to aflatoxins and a high risk of HCC, immunodeficiency, and development of infectious diseases, and as vaccine failure (Mupunga *et al*., 2017). In HIV-endemic areas, there is a substantial link between unsafe sex and the development of aflatoxicosis. This revealed that aflatoxins may have a role in AIDS-related immunosuppression and mortality, which might explain the increased occurrence of aflatoxicosis. In Nigeria, the HIV prevalence is 1.4%, accounting for around 1.9 million persons living with HIV and approximately 160, 000 AIDS-related deaths in 2016. (UNAIDS, 2017; 2019). These findings point to a probable link between aflatoxin exposure and AIDS.

Many studies have found a substantial link between aflatoxin exposure and childhood stunting. (Watson, 2018). Generally, aflatoxin exposure is more common in low-income families who cannot afford good quality food (Ahlberg *et al.,* 2018). According to the 2019 global child malnutrition estimate, about 149 million children under the age of five are stunted and the majority of which are found in Africa and Asia (UNICEF/WHO/World Bank Group, 2019). A study on exposure to aflatoxins among children with severe acute malnutrition was recently conducted in Nigeria (McMillan *et al.,* 2018). The median concentration of AFB1- lysin adducts detected in the serum samples was 2.6 pg/mg albumin with values ranging from 0.2pg/mg to 59.2 pg/mg albumin (McMillan *et al.,* 2018). Such AFB1-lysine was significantly higher in stunted children (4.6 pg/mg albumin) and children with severe acute malnutrition (4.3pg/mg) when compared to non-stunted (1.2 pg/mg albumin) and the controls (0.8 pg/mg). This AFB1-lysine was also found to be significantly higher in kwashiorkor children (median = 6.3 pg/mg albumin) compared to children with severe marasmus (median = 0.9 pg/mg). Several studies have established a strong link between aflatoxin exposure and kwashiorkor which is a severe form of protein-energy malnutrition (PEM), as well as increased morbidity in children. As observed by (McMillan *et al.,* 2018), the highest level of the serum aflatoxin was found in children with kwashiorkor. Similarly, considerably greater amounts of aflatoxins were found in the autopsies of several Nigerian and South African kwashiorkor children's livers (Lamplugh *et al.*, 2018).

A cross-sectional study of aflatoxins in the food plates, serum and urine samples of 36 kwashiorkor children (KC), 29 marasmic kwashiorkor children (MKC), 13 marasmic children (MC) and 33 healthy age-matched control subjects from the 3 agroecological zones of Nigeria was conducted (Makau *et al*., 2016). Aflatoxins were detected in 93.1% of MKC, 88.9% of KC, 76.9% of MC, and 63.6% of the healthy controls, with the

corresponding levels of AFB1 in the food plates as 82.4%, 69.4%, 53.8%, and 42.4% respectively. The median concentration of aflatoxins in the 3 groups of malnourished children is significantly higher than those in the control subjects for both serum/urine (p=0.013) and food plates (p=0.007), indicating that children in Nigeria are continuously being exposed (Lamplugh *et al.*, 2018).

2.2.5 Aflatoxin contamination and favorable conditions

The production of mycotoxins is influenced by the dietary supply, enzymes, and numerous environmental conditions. However, the circumstances that favor aflatoxigenic fungus may not always support the formation of aflatoxins (Mannaa and Kim, 2017). These factors are summarized as:

2.2.5.1 *Physical factors*

Physical factors like pH, light, moisture, temperature, water, relative humidity, and atmospheric gases are responsible for aflatoxin contamination. Aflatoxin-producing molds/Fungi can grow in a wide range of pH (1.7–9.3), but the optimum range of pH is (3–7) (Yoshinari *et al*., 2019). The lower pH (3 > pH> 1) minimizes the fungal growth and a slightly higher pH (6 > pH> 3) promotes both fungal and aflatoxins production (Eshelli *et al*., 2015). Initial pH (pH = 5) promote AFB (Aflatoxin B) production while higher pH (pH = 7) promote AFG (Aflatoxin G) production, however, the composition of media in which fungi grow also influence pH (Sneh *et al*., 2021).

The presence of light influences fungi growth and aflatoxin formation. Aflatoxin synthesis is increased by darkness, whereas it is inhibited by sunshine (Rushing and Selim, 2019). High moisture content always favors the aflatoxin contamination because moist conditions are favorable for fungal growth. Relative humidity (85%) is optimal for aflatoxins production, while 95% relative humidity increases aflatoxins production to a

considerable level (Ding *et al*., 2015). However, there has been no observed influence of water level on aflatoxin contamination. Aspergillus flavus may grow in a wide variety of temperatures ranging from 12 °C to 48 °C, however the optimal temperature range for its growth is 28 °C to 37 °C (Hawkins *et al*., 2020). Aflatoxins production can occur at a wide range of temperature; however, the optimal temperature for aflatoxin production is 25-35 °C (Siciliano *et al*., 2017). Normally at high temperatures, AFB production is high than AFG, but at low temperatures both AFB and AFG production is equal. Availability of O_2 and CO_2 also influence the aflatoxins productions. Aflatoxins production and fungal growth are inhibited at a higher level of CO_2 and a lower level of O_2 (Mahbobinejhad *et al*. 2019).

2.2.5.2 *Nutritional factors*

The substrate and numerous nutritional variables such as carbon, amino acids, nitrogen, lipids, and a few trace components also have a large impact on aflatoxin synthesis. Carbohydrate-rich substrates promote greater output than oil-rich substrates because carbohydrate easily offers carbon, which is required for effective fungal development (Ma *et al*., 2014). Among carbohydrates glucose, ribose, sucrose, xylose, and glycerol acts as excellent substrates, while peptone, lactose, and sorbose are unable to promote aflatoxins production (Liu *et al*., 2016). Nitrogen in the form of nitrite and nitrate promotes aflatoxin formation by A. flavus in a variety of ways (Wang *et al*., 2019). Lipids also play an important role in aflatoxin production. Aflatoxins biosynthesis in toxigenic fungi leads by lipophilic epoxy fatty acids and fungal growth as well as aflatoxin production induced by ergo-sterol oxidation (Reverberi, 2019). Consequently, lipids also act as a substrate to obtain an acyl-CoA starter as well as a signaling molecule. The aflatoxin production and accumulation increase in full-fat substrates as compared to the low-fat substrate. In comparison to media without the addition of maize oil, the inclusion of corn oil in defatted

wheat infected with A. flavus enhances aflatoxin formation (Liu *et al*., 2016). Vitamins, amino acids, and metal ions also promote aflatoxin production in combination. Amino acids like glycine, glutamate, and alanine along with some bivalent metals like zinc and magnesium promote aflatoxin production, while it is inhibited by tryptophan.

2.2.5.3 *Biological factor*

Fungal species, weeds, and insect damage are examples of biological influences. Weeds grow primarily as a competitor and induce plant stress, which is linked to aflatoxin formation. The amount of aflatoxin produced is mostly determined by the kind of fungus; insects wound in the plant generate stress and serve as a breeding ground for aflatoxigenic fungi (Kinyungu, 2019). Aflatoxin production also depends on the types of strains. *A. flavus* produces fewer aflatoxins as compare to *A. parasiticus* (Manjunath and Mohana, 2018). Aside from the factors mentioned above, A. flavus is the main species responsible for aflatoxin production and crop contamination because it is the most abundant mold found in soil and has the saprobe character, which allows it to grow on a variety of organic nutrient substrates such as compost piles, plant debris, cotton, dead insects, stored grains, field crops, animal corpses, and animal fodder. Pre-harvest contaminations of field crops are common because of the natural existence of *A. flavus* in soil, while post-harvest contamination also occurred by *A. flavus* during storage because it spoils the food grains. Due to the lack of host specificity *A. flavus* contaminate both monocot and dicot seeds (Leger *et al*., 2000).

2.2.6 Methods for detection and quantification of aflatoxins

Aflatoxins' quantitative detection technologies have advanced dramatically since their discovery. One of the oldest techniques for analyzing contaminated materials is chromatography, which includes thin layer chromatography (Fallah *et al*., 2016). Other methods are also used such as Gas Chromatography (GC), and high-performance liquid

chromatography (HPLC) with fluorescent detector, or with fluorimetric detector. Aflatoxins are also detected by Spectroscopic Methods; Fluorescence Spectrophotometry, Frontier Infrared Spectroscopy coupled to a mass spectrometer (Sulyok *et al*., 2017). Other methods such as immunochemical methods; Radioimmunoassay (RIA), Enzyme-Linked Immunosorbent Assay (ELISA), Lateral Flow Devices (Immunodipsticks) and Immunosensors are also used in Aflatoxins detection.

2.2.6.1 *Chromatographic methods*

Chromatographic techniques are based on the physical interaction between a mobile phase and a stationary phase. The components to be separated are distributed between the two phases (stationary phase and mobile phase) (Braithwaite *et al*., 2018). The mobile phase is usually a fluid that penetrates through or along the stationary bed (liquid or solid). Liquid, gas, and supercritical fluids are currently used as mobile phase and chromatographic techniques derive their names from the nature of the mobile phase: liquid chromatography, gas chromatography, and supercritical fluid chromatography, respectively. In practice, the sample to be analyzed is dissolved in the mobile phase and applied as a spot on the stationary phase. The analyte or sample is carried along by the mobile phase and partitions between the solid and liquid stationary phase are called the sorbent. The various constituents in the analytes travel at different speeds resulting in differential partitioning of the constituents between the mobile and the stationary phases. The most commonly used chromatography techniques for analysis of aflatoxins are thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), and gas chromatography (GC). Although many chromatographic procedures are extremely sensitive, they need highly professional technicians, sample processing is time-consuming, and apparatus/equipment are expensive (Sapsford *et al*., 2016).

High-Performance Liquid Chromatography (HPLC)

High-performance liquid chromatography (HPLC) is the most popular chromatographic technique for separation and determination of organic compounds. About 80% of organic compounds in the world are determined using HPLC (Zhang *et al*., 2015). The HPLC technique makes use of a stationary phase confined to either a glass or a plastic tube and a mobile phase comprising aqueous/organic solvents, which flow through the solid adsorbent. When the sample to be analyzed is layered on top of the column, it flows through and distributes between both the mobile and the stationary phases. This is achieved because the components in the samples to be separated have different affinities for the two phases and thus move through the column at different rates. The liquid (mobile) phase emerging from the column yields separate fractions containing individual components in the sample. In practice, the HPLC technique employs a stationary phase such as C-18 chromatography column, a pump thatmoves the mobile phase(s) through the column, a detector that displays the retention times of each molecule, and mobile phases (**Figure 2.6**).

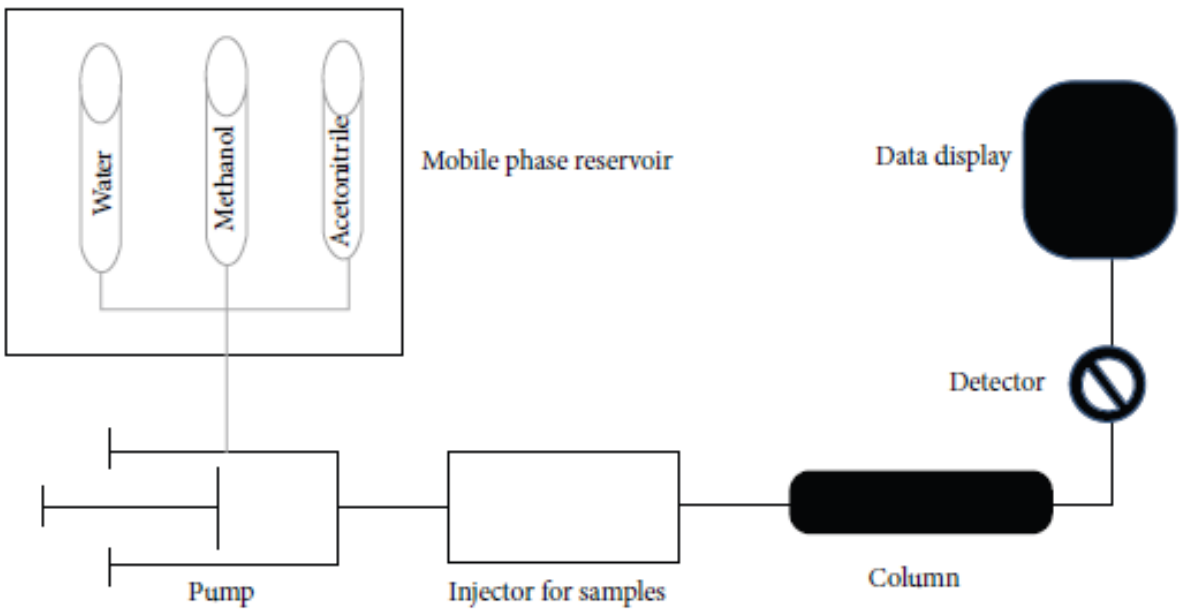


Fig 2.6: Schematic diagram of components of high-performance liquid chromatography

The sample to be analyzed is usually injected into the stationary phase and the analytes are carried along through the stationary phase by the mobile phase using high pressure delivered by a pump. The analytes are distributed differently within the stationary phase through chemical as well as physical interactions with the stationary and mobile phases (Rahmani *et al*., 2019). The time at which a specific analyte elutes is recorded by a detector as its retention time. The retention time depends on the nature of the analyte and composition of both stationary and mobile phases. Programmable detectors such as either the fluorescent detector (FLD) or the ultraviolet (UV) detector or the diode array detector (DAD) may be used in the detection and identification of aflatoxins. Normal-phase and reversed-phase high pressure liquid chromatography techniques are employed for the detection of aflatoxins in foods (Rahmani *et al*., 2019). The reversed phase HPLC method is the most widely used for separation and determination of aflatoxins. Occasionally, chemical derivatization of aflatoxins B1 and G1 may be required to enhance sensitivity of HPLC during analysis since the natural fluorescence of aflatoxins B1 and G1 may not be high enough to reach the required detection limit (Kok *et al*., 2016). The derivatization reactions of aflatoxin B1 with both the acid and halogens are presented in Figure 6. While in the first reaction step, the second furan ring of aflatoxin B1 is hydrolyzed by trifluoroacetic acid (TFA) into a highly fluorescent aflatoxin B2a, in the second and the third derivatization reaction steps, bromine and iodine are used as reagent, respectively. They react with aflatoxin B1 to form highly fluorescent aflatoxin B1 derivatives of these halogens, respectively.

Papadopoulou-Bouraoui *et al*. (2002) compared two post column derivatization methods for the determination of aflatoxins B1, B2, G1, and G2 by fluorescence detection after liquid chromatographic separation (Figure 2.7). The results showed that both bromination and irradiation by UV light were suitable for the determination of aflatoxins in various

foods and animal feed matrices and both generated comparable results for fluorescence amplification and repeatability. The fluorescence of aflatoxins B1 and G1 was significantly enhanced after derivatization reaction either by bromination or by irradiation by UV light. High-performance liquid chromatography provides fast and accurate aflatoxins detection results within a short time. A sensitivity of detection as low as 0.1 ng/Kg using FLD has been reported (Herzallah, 2019). However, the disadvantage of using HPLC for aflatoxins analysis is the requirement of rigorous sample purification using immunoaffinity columns. In addition, HPLC requires tedious pre- and post-column derivatization processes to improve the detection limits of aflatoxins B1 and G1 Therefore, to overcome the challenges associated with derivatization processes in aflatoxins analysis, a modification of the HPLC method, whereby the HPLC is coupled to mass spectroscopy, has been made and is currently employed in the determination of aflatoxin (Abdallah *et al.*, 2019). Since the mass spectrometer requires neither use of UV fluorescence nor the absorbance of an analyte, the need for chemical derivatization of compounds is eliminated. The HPLC-MS/MS uses small amounts of sample to generate structural information and exhibits low detection limits (Rahmani *et al*., 2019). However, HPLC-MS/MS is bulky and very expensive equipment which can only be operated by trained and skilled personnel. Besides, this also limits its use to only laboratory environment and not field conditions.

2.3 The Cassava Plants

Cassava *(Manihot esculenta* [Crantz]) belongs to the family *Euphorbiaceac*. which includes rubber *(Hevea brasilensis)* and castor bean *(Ricunus communis)*. Before the discovery of the New World, the genus *Manihot* grew only in the Americas between about 30'N and 30'S latitude (James, 2018). There are two main centers of diversity-a major one in Brazil and a secondary one in Central America. Cassava is farmed in the majority of

regions where Manihot species thrive, although it is not found in the wild. The wild progenitor of cassava is unknown, and the places where cassava was domesticated have not been located with certainty (James, 2018).

2.3.1 Species or taxonomic group of cassava

2.3.1.1 *Classification and nomenclature*

The scientific name of cassava is *Manihot esculenta* Crantz (Cheek *et al.*, 2020), synonym *Manihot utilissima* Pohl (Parmar *et al.*, 2019). Cassava is a member of the spurge family, and its taxonomic hierarchy is:

Order; Malpighiales

Family; Euphorbiaceae

Genus; *Manihot*

Species; *Manihot esculenta* Crantz

Subspecies; *M. esculenta* Crantz ssp. *esculenta*

M. esculenta Crantz ssp. *flabellifolia* (Pohl)

M. esculenta Crantz ssp. *peruviana* (Müeller) (Allem, 2017)

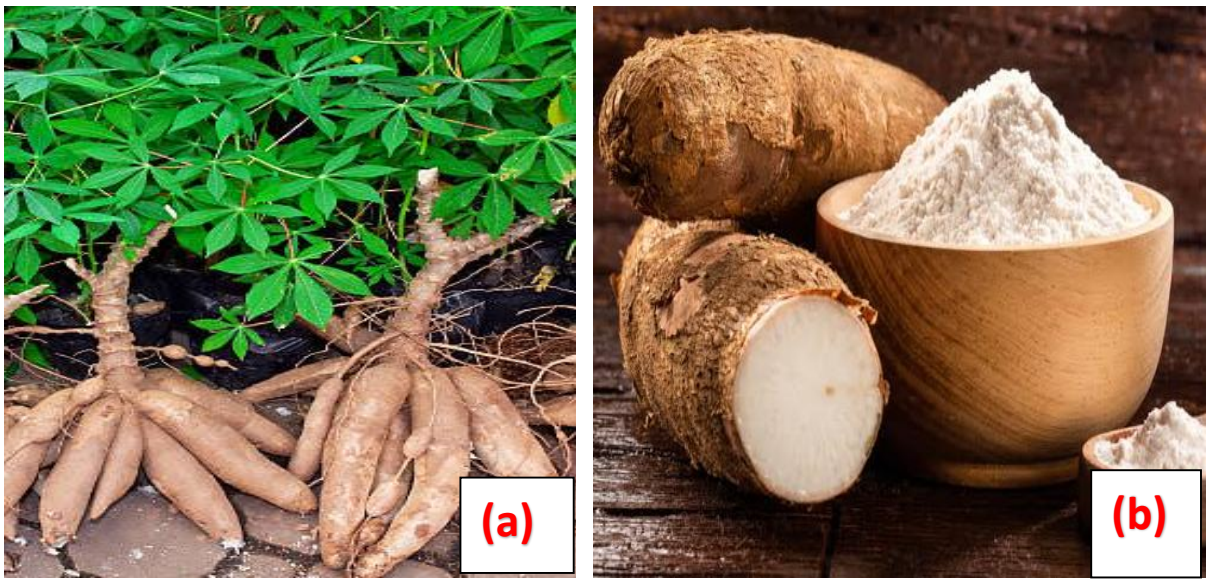


Fig. 2.7: (a). Cassava plant with the roots, (b). Cassava tubers and cassava flour (www.shutterstock.com)

Three subspecies of cassava have been recognized: *Manihot esculenta* ssp. *Esculenta* is the cultivated strain, and *M. esculenta* ssp. *flabellifolia* and *M. esculenta* ssp. *Peruviana* are wild forms (Allem, 2017). In this chapter, "cassava" will be used to refer to the cultivated strain, *M. esculenta* ssp. *esculenta*. Common synonyms in other languages are *manioc* (French); *mandioca*, *macaxeira* and *aipim* (Portuguese); *yuca* (Spanish); and *manioca* (Italian).

2.3.2 Botanical description and propagation of cassava

The cassava plant is a perennial woody shrub. Commercially. It is grown by planting a cutting taken from the woody part of the stem. After the cutting is planted, one or more of the axillary buds sprout and roots grow principally, from the base of the cutting. The shoots show strong apical dominance, which suppresses the development of side shoots (James, 2018). When the main shoot becomes reproductive and begins to flower, the apical dominance is broken and several (two to four) of the axillary buds immediately below the apex begin to develop, giving the typical branching habit found in the plant. The time at which branching occurs is extremely variable: some clones never branch.

The cassava plant primarily produces branches and a fibrous root structure during the early months. At the start of the growth cycle. Plants, on the other hand, store modest quantities of starch in their roots. Three months after planting. Some of the fibrous roots begin to quickly swell, accumulating significant amounts of starch. The thicker storage roots contain a fleshy heart that accounts for around 85 percent of their overall weight, as well as a harder, more fibrous peel (James, 2018).

The propagation of cassava is unusual for several reasons. First, the economically useful part. the root, is not used for propagation. To propagate other major food staples, such as grain crops or potatoes, part of the harvest must be kept for replanting. Second, the

multiplication rate of cassava is low from 3 to 30 times per crop cycle, depending on conditions. Third, although cassava is normally propagated through stem cuttings, which produce genetically identical progeny or clones, it can also be reproduced from true seeds. The progeny obtained from true seeds have great genetic variability, permitting superior varieties to be selected. Which can then be reproduced by stem cuttings and hence have their new characters fixed in a stable form (James, 2018).

2.3.3 Cassava plant production

Cassava is a starchy root crop produced nearly exclusively in the tropics. Although it is one of the most important food crops in tropical nations, it is seldom recognized outside of the tropics, and it is frequently regarded as a low-grade subsistence crop inside the tropics (James, 2018).

2.3.3.1 *World production of cassava*

Globally, cassava has experienced consistent growth of well above 3% annually (FAO, 2018). According to FAO (2018), as of 2018, world cassava production stood at about 278 million tons. Since the cassava root is about 65 percent water, that production level yields about 42 million tons of dry matter, which is equivalent, in energy terms, to 40 to 50 million tons of grain. Because much of the cassava grown is produced by small farmers in marginal agricultural areas and because most of it does not enter commercial marketing channels, it is difficult to obtain data on production. Food and Agriculture Organization (FAO) statistics are the best available guide to global production: however, errors in the estimates for individual countries can be quite large. FAO figures for Brazilian production for example, are much greater than estimates obtained from data in Brazil's agricultural census.

2.3.3.2 *Africa and Asian production*

Africa total production was about 170 million tonnes (about 56% of world production) (FAOSTAT, 2019). Africa though it has slightly more than half the world's cassava land. produces only 37 percent of the world crop. FAO estimates show that yields have been nearly constant at 6.4 t/ha in recent years but between 1969-1971 and 1980. Plantings increased from 5.8 million hectares to 7.3 million hectares, and annual production climbed from 39.4 million tons to 46.5 million tons. Zaire, Nigeria and Tanzania are the major African cassava producers. Average yields in these countries range from 9 t/ha in Tanzania to less than 6.5 t/ha in Zaire.

Asia accounts for only 27 percent of the world cassava area. but it produces 37 percent of the crop. Yields vary from less than 8 t/ha in Vietnam to over 16 t/ha in India (James, 2018). Increasing Asian output during the last 20 years has been due mainly to expansion in Thailand. Production in Thailand grew from less than I million tons in 1957 to 4 million tons in 1972 to an estimated 17.9 million tons in 1981. 1he second largest producer in Asia is Indonesia, which harvests over 13 million tons annually. Its land area in cassava had declined slightly in recent years. But gains in yields, which now average about 9.6 t/ha. have more than compensated for that decline. Evidence from FAOSTAT (2019) revealed that, as at 2018, Indonesia made the most tremendous improvement in terms of output per hectare between the periods of 1970 and 2017. This is closely followed by Vietnam, Ghana and Benin.

2.3.3.3 *Cassava production in Nigeria*

Cassava products are increasingly becoming popular in Nigerian food and agricultural markets. Nigeria produced about 60 million tonnes of Cassava annually (FAOSTAT, 2019) Thus, it provides a strong incentive for more economic agents to be involved in the cassava market. Despite being the largest producer of cassava in the world, more than

90% of cassava produced in Nigeria are consumed locally (Denton *et al*., 2019). According to FAO (2018), cassava is a choice crop for rural development, poverty alleviation, economic growth and ultimately, food security. It is in view of the above that critical stakeholders have continued to contribute immensely to shaping the development of cassava sub-sector in Nigeria. Eke-Okoro and Njoku (2019) captured the phases in efforts to improve cassava production in Nigeria as the emergent stage that spread from 1940 to 1953; a primitive stage that stretched from 1970 to 1990 and the anticipatory stage that spanned from 1995 to date (Edamisan, 2020).

2.3.4 Consumption patterns

Cassava is mostly used as a food. Because of its size and perishability, it is often consumed in or around the area where it is cultivated. The FAO food balance sheet has the only global statistics on cassava consumption, which is merely an approximate approximation of the true situation. They indicate that in the mid-1970s, 65 percent of the total production was used as a basic source of energy in human diets. About equal amounts were consumed as cooked fresh cassava or as processed cassava (mainly flours and meals). The main non-food uses of cassava are animal feed and starch. About one-fifth of world production is fed to animals mainly in producing countries, and the amount is rising. Almost all exports are pelleted animal feed destined for Europe. About 6 percent of world production goes into Starch for industrial processes and food processing (James, 2018).

Consumption patterns vary greatly among geographies. Almost all cassava used in Africa is for human consumption. Although cassava is considered a subsistence crop in Africa, significant amounts are traded. Throughout Africa, cassava and cassava products can be found in the rural and urban markets. In Nigeria, *garri* a fermented cassava flour, is transported up to 700 kilometers to urban markets, and each year the equivalent of

120,000 tons of fresh cassava enters the Lagos market as *garri.* A study in Ghana in the 1950s showed that 22.000 tons of fresh cassava and 27.000 tons of cassava products were entering Accra annually, providing 41 percent of the calories brought into the city. In Zaire, an estimated 55 percent of total production is sold off the farm. While it is hard to quantify how much cassava is traded and how much is consumed by producers, it is evident that a large amount of African output enters the market economy (James, 2018).

2.3.5 Importance of cassava in diets

The FAO *Food balance sheets* give estimates of cassava consumption for whole countries. but consumption patterns may vary substantially from area to area within a country. In Brazil for example, cassava has far more dietary significance in the Northeast than it does in the South. Similarly, in India the national average figures suggest that cassava is unimportant. but in fact, in Southern India cassava provides more than 700 cal/ day for 20 to 30 million people. Thus, national averages tend to overestimate the number of people who consume cassava as a major staple while underestimating the amounts consumed by actual cassava eaters. That notwithstanding, the food balance sheet data indicate that in 1975-1977. 500 million people in 24 countries consumed more than 100 cal/day in the form of cassava. with an average intake of' over 300 calories (James, 2018).

In the countries of' tropical Africa. cassava provides an average of' 230 calories per person per day. In Zaire and Congo. However, the average intake is over 1000 cal/day, or close to 1 kilogram of fresh cassava per day**.** This quantity accounts for around half of the entire energy use in the two countries. Cassava provides more than 500 calories per day to an estimated 70 million individuals. (James, 2018).

The main value of cassava is the starchy roots, but the leaves are also eaten in Africa. Cassava leaves contain about 7 percent protein on a fresh weight basis (20 to 30 percent

protein on a dry weight basis). Cassava leaves compare favorably with soybeans in protein quality and are considerably higher in lysine. However, methionine and possibly tryptophan are deficient. The leaves are used as a base in making sauces and soups, particularly in the Congo basin. Tanzania and parts of West Africa. Consumption levels in Zaire have been estimated to be 500 g/day, 40 to 170 g/day in Congo, and 30 to 100 g/day in Cameroon. Consumption of cassava leaves at these levels can contribute significantly to total protein intake (James, 2018).

2.3.6. Cassava: a crop for sustainable agriculture and food security in developing countries

Cassava is currently the most important food source for carbohydrate, after rice, sugarcane and maize, for over 500 million people in the developing countries of the tropics and sub-tropics. Its main value is in its storage roots with dry matter containing more than 80% starch. Due to the very low protein content in storage roots (values range among cultivars from 5 to 19 g per kg dry matter, based on an average conservative Kjeldahl nitrogen to-protein conversion factor of 2.49–3.67 (Oresegun *et al.*, 2016), human requirements for protein and other essential nutrients are commonly fulfilled by other food sources.

Young leaves are gathered and processed for human use as a vegetable or as a constituent in a type of sauce consumed alongside primary staple meals in several locations where the crop is produced, notably in Africa (Latif & Muller, 2015). Also, cassava leaves have value as a protein supplement (leaf crude protein content on a dry basis ranges among cultivars from 21% to 39% (Ravindran, 2019) in animal nutrition either in feed formulations for monogastric animals or as a fresh forage to supplement low-quality roughages in ruminant feeds (Ravindran, 2019). Worldwide, the crop occupies the sixth place as a source of energy; it is not generally consumed outside the tropics and subtropics

where it is produced. About 70% of world cassava root production (which is estimated to be over 45 million metric tons of dry root annually) is used for human consumption either directly after cooking or in processed forms; the remaining 30% is used for animal feed and other industrial products such as starch, glucose, and alcohol.

To reduce health risks, cultivars low in cyanogen are preferred in countries where cassava is frequently used directly for human food, notably in Africa and Latin America. Much of the hydrocyanic acid in cassava roots and leaves is removed from cultivars rich in cyanogen (bitter) during food processing and preparation using a combination of sophisticated traditional methods and contemporary technologies (Essers, 2015).

Due to root perishability and rapid deterioration after harvest, fresh roots have to be used immediately after harvesting, either eaten on the farm, marketed for consumption, processed for starch extraction, dried for flour production, roasted for food products and/or used for animal feed. However, pruning three weeks before harvest reduces root degradation due to increases in the total sugar/starch ratio in the roots (Van Oirschot *et al.*, 2020).

2.3.7 Nutritional value of cassava

Cassava roots have 30 to 40 percent dry matter, which is a higher proportion than most other roots and tubers. The dry matter content depends on such factors as the variety, the age of the root at harvest, the soil and climatic conditions and the health of the plant. Starch and sugar are the predominant components (approximately 90 percent) of the dry matter: starch is by far the most important. The metabolizable energy of dry cassava. 3500 to 4000 kcal/g. is similar to that of maize flour (James, 2018).

The crude protein content is less, because up to half the nitrogen in the root is non-protein nitrogen. The quality of the protein is reasonably good in cassava young leaves; leaf crude

protein content on a dry basis ranges among cultivars from 21% to 39%, though sulfur amino acids are deficient. The roots contain significant quantities of vitamin C, thiamine, riboflavin, and niacin. A person obtaining more than 250 cal/day from cassava would satisfy the daily vitamin C requirement. But boiling reduces the vitamin C content by 50 to 70 percent and processing to such products as *garri and foufou* reduces it by 75 percent or more. Nevertheless in areas in which cassava consumption is high even processed cassava can supply sufficient vitamin C to satisfy minimum daily requirements (James, 2018).

In nutritional terms, cassava is low in essential amino acids such as methionine, lysine, tryptophan, phenylalanine and tyrosine (Parmar *et al.*, 2019). A cassava-based diet therefore requires an adequate protein source of good quality to prevent nutritional deficiency symptoms (Aditya *et al.*, 2017). It is well established that cassava is not edible raw due to the presence of toxic compounds. Cassava contains two cyanogenic glucosides, linamarin and lotaustralin, which are found in all sections of the plant but are most abundant in the root peel. Normal cyanoglucoside levels vary from 31 to 630 ppm estimated as mg HCN/kg of fresh cassava root, however the concentration fluctuates greatly depending on variety, climate, and environmental circumstances. Sweet cassava cultivars have lower cyanide levels than bitter ones, although there is no confirmed link between flavor and toxicity (Parmar *et al.*, 2019).

2.3.8 Toxicity of cassava roots

Raw cassava contains the glycosides linamarin and lotaustralin which are converted to hydrocyanic or prussic, acid, a poison, when they come in contact with linamarase, an enzyme that is released when the cells of cassava roots are ruptured. Although occasionally deaths from consuming raw cassava roots have been reported the traditional processing and cooking methods reduce the cyanide levels. If normal preparation

procedures are used, acute cyanide toxicity does not occur. Chronic cyanide toxicity occurs in some localities in Africa where cassava consumption is high up to the equivalent of 1 kilogram or of fresh more roots per day over a long period, and where the consumption of iodine and protein, particularly animal protein, is extremely low. In Nigeria and Zaire. Ataxic neuropathy (nervous degeneration) and goiter (which leads to cretinism in severe cases) have been associated with high levels of cassava consumption (James, 2018).

When cyanide enters the bloodstream, it is converted to thiocyanate, a sulfur-containing compound, by the enzyme rhodanase. The thiocvanate is later excreted in the urine. The compound plays its toxic role by using up body sulfur in detoxification. Thus, increasing the body's demand for sulfur containing amino acids, or by interfering with the iodine uptake of the thyroid, resulting in goiter. In both cases, high cassava consumption aggravates problems associated with low levels of sulfur amino acids and iodine in the diet (James, 2018). Chronic cyanide toxicity has not been reported in areas of high cassava consumption in Latin America or Asia, which reinforces the hypothesis that goiter and ataxic neuropathy are caused by a complex interaction of several factors.

2.3.9 Processing of cassava

Cassava root is processed in Nigeria into garri, tapioca, lafun, fufu and starch, among other products, with varying physical qualities due to differences in processing methods (Awoyale *et al*., 2017). However, these processing methods, as well as the environments and natural microflora, influence the types and concentrations of microbial metabolites in the final food products (Adebayo-Oyetoro *et al*., 2013). Cassava processing processes in Nigeria typically result in a variety of culinary and feed products. The production of lafun may or may not involve peeling of the cassava roots before washing, fermenting in water (either in a flowing stream or sttionary water) for softening, bagging/dewatering, drying,

and milling (Ogunnike *et al*., 2015). The production of fufu flour is similar, except that, after fermentation, the mash is wet-sieved before sedimentation, dewatering, and final drying. Lafun and fufu flours are categorized as dried fermented flours, while tapioca is an unfermented product produced by toasting the extracted wet starch (Otegbayo *et al*., 2013). The roasting of fermented cassava mash to make garri is similar to this process, and similar utensils are used.

2.3.9.1 *Importance of processing cassava roots*

Although cassava cultivation requires slight labor, post-harvest cassava processing is the most difficult of all root crops because it requires rapid handling and detoxification to make it edible (Root and Tuber Improvement Program, 2019). One of the major issues in the utilization of cassava is the high perishability of the tubers. Deterioration by biochemical changes and microbial infestation starts within 2-3 days after uprooting. Long distances between production areas and processing sites are often a problem leading to considerable post-harvest losses (Aditya *et al.,* 2017). Since there are no effective commercial storage methods available, it is necessary to process cassava into dry shelf-stable forms by reducing moisture content and thus lowering bulk and transportation costs (Parmar *et al.*, 2019).

As cassava contains toxic compounds, it requires special processing procedures that will eliminate or reduce the levels of cyanogenic glucosides, making the product safe for human consumption. Peeling reduces significantly the toxicity. Grating breaks down the internal structure of the root, releasing linamarase that will decrease the cyanoglycoside content by about 95% by hydrolyzing the glucosides into HCN (Parmar *et al.*, 2019). Since HCN is soluble in water, its amount is reduced by traditional detoxification methods such as boiling, soaking or de-watering (Dziedzoave *et al.*, 2016). Because HCN is

volatile, some of it will escape into the air during drying or roasting. During fermentation, glycosides are nearly completely broken down in fermented cassava flours like garri. As a result, cassava products are safe if properly prepared (RTIP, 2019).

Processing also increases the value of cassava by improving palatability and facilitating marketing of more acceptable hygienic quality products. Cassava, often considered as a low value root crop, is transformed into convenient foods to meet the increased demand from the urban population (Parmar *et al.*, 2019). Cassava processing is a widespread and labor-intensive job that is typically carried out by women in small-scale processing operations. Peeling, grating, drying, sifting, roasting, and fermenting are all time-consuming unit processes since they are frequently done by hand. Nonetheless, over the last three decades, the development of motorized cassava processing technology has significantly decreased drudgery and increased processing throughput. Mechanical graters, cassava chippers, screw presses, sieving machines, mills, and mechanical dryers are currently quite common in Ghana's cassava processing business. Small-scale cassava processors, on the other hand, frequently lack the funds to invest in equipment and maintain the business successful (Ministry of Food and Agriculture, 2015).

2.3.9.2 *Cassava flour production*

The processing of cassava roots into cassava flour is depicted in (**Figure 2.8)**. Healthy cassava roots with no bruises and cracking should be harvested 10-12 months after planting and processed within 24 hours. Grating, is generally, carried out by a motorized cassava grater that disintegrates cassava tissue, which facilitates later steps such as pressing and drying due to an increased surface area. De-watering or pressing is the removal of internal liquid from the roots by means of a screw press and is important to reduce toxicity (Kamaljit and Preeti, 2017).

The cassava mash is packed into a clean jute sack, allowing excess water to drain until the mash is crumbly. By limiting the pressing time, off-color and odor from fermentation are minimized (less than one hour). The particle size of cassava crumbles is reduced during disintegration. A hot air mechanical drier or a solar dryer can be used for drying. A hammer or disc-attrition mill is used to grind dry cassava mush into flour. To get smooth flour with consistent particle size, the flour is sieved via a motorized flour sifter equipped with a 250 m screen. Finally, the flour is wrapped in polypropylene bags to prevent moisture absorption during storage (Shittu *et al.*, 2018; Nwabueze and Anoruoh 2019).

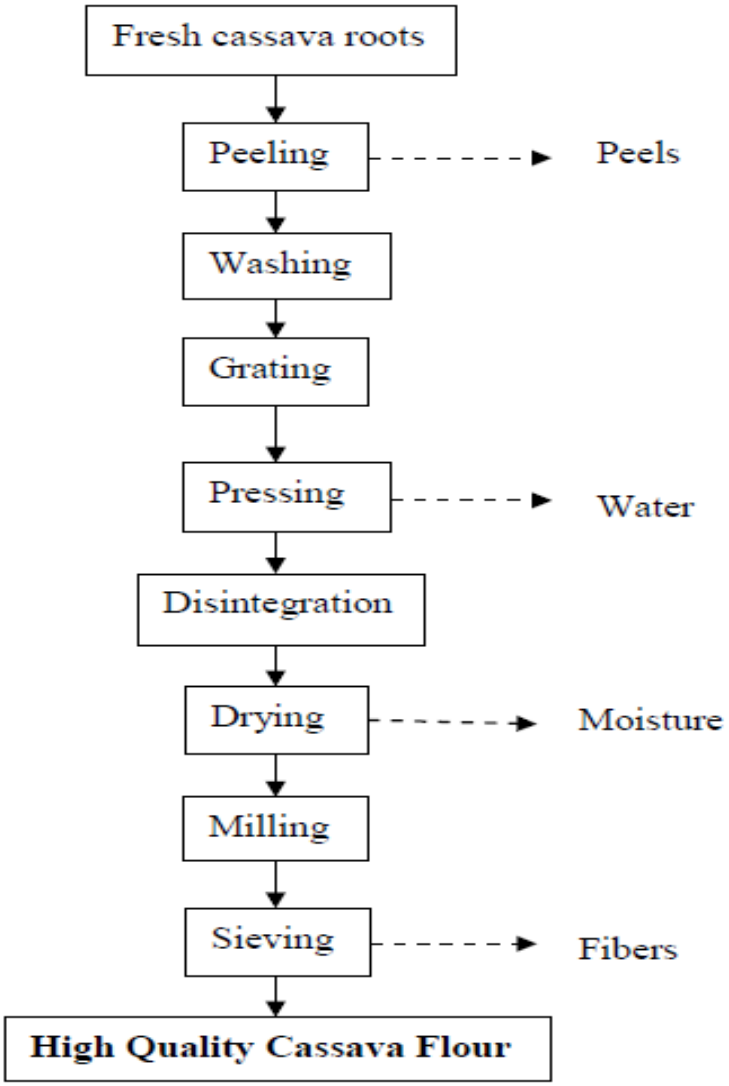


Fig. 2.8: Cassava flour preparation

Another fast method involves harvesting and sorting of good roots, peeling and washing manually, grating (usually done with a motorized cassava grater), dewatering (with screw or hydraulic press), pulverizing, drying (solar or oven drying), fine milling, sifting the milled flour with a motorized flour sifter having 250 µm sieve size and then, packaging (Jekayinfa and Olajide 2017). Flour extraction from cassava tuber depends on reduction of moisture. Cassava drying aims at reducing its water content to less than 15% (Shittu *et al.,* 2018). Temperature, ventilation, humidity, and tumble frequency are all factors that impact cassava drying.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was conducted between November 2020 and February 2021 in selected five Nigerian AEZs where cassava flour (Lafun) is produced and consumed: Sudan Savanna **(SS)** (Kano, Katsina, Kebbi, Jigawa and Sokoto State); Northern Guinea Savanna (NGS) (Bauchi, Gombe and Kaduna State); Southern Guinea Savanna **(**SGS**)** (Niger and Adamawa State); Derived Savanna (DS) (Taraba, Plateau, Kwara, Ekiti, Kogi, Osun, Oyo, Benue, Ogun, Enugu, Nasarawa State and FCT-Abuja) and Humid Forest (HF) (Edo, Ondo, Cross-River, Ebonyi, Anambra, Imo, Akwa-Ibom, Abia, Rivers, Bayelsa, Delta, and Lagos State).

The Sudan Savanna (SS) zone lies between latitudes 12°2' and 13°8' N and longitudes 3°9'and 13°9'E with a unimodal annual rainfall averaging between 650 and 1,000 mm and maximum temperatures varying from 30 to 40 °C (Udoh, *et al*., 2000). The Northern Guinea Savanna (NGS) zone lies within latitudes 9°10'and 11°59'N and longitudes 3°19' and 13°37'E and has a unimodal rainfall distribution averaging between 900 and 1,000 mm annually and maximum temperatures varying from 28 to 40 °C (Atehnkeng, *et al*., 2008). The Southern Guinea Savannah zone lies within latitudes 8°4'and 11°3'N and longitudes 2°41'and 13°33'E, with a bimodal rainfall averaging between 1,000 and 1,300 mm per year and maximum temperatures varying from 26 to 38 °C. The Derived Savanna (DS) lies within latitudes 6°8' and 9°30' N and longitudes 2°40'and 12°15' E and has a bimodal rainfall distribution averaging between 1,300 mm and 1,500 mm annually and maximum temperatures varying from 25 to 35 °C (Atehnkeng *et al*., 2008). The humid forest (HF) zone lies within latitudes 6°4' and 7°5' N and longitudes 3°5' and 8°8'E and

has a bimodal annual rainfall averaging between 1,300 and 2,000 mm and maximum temperatures ranging from 26–28 °C.

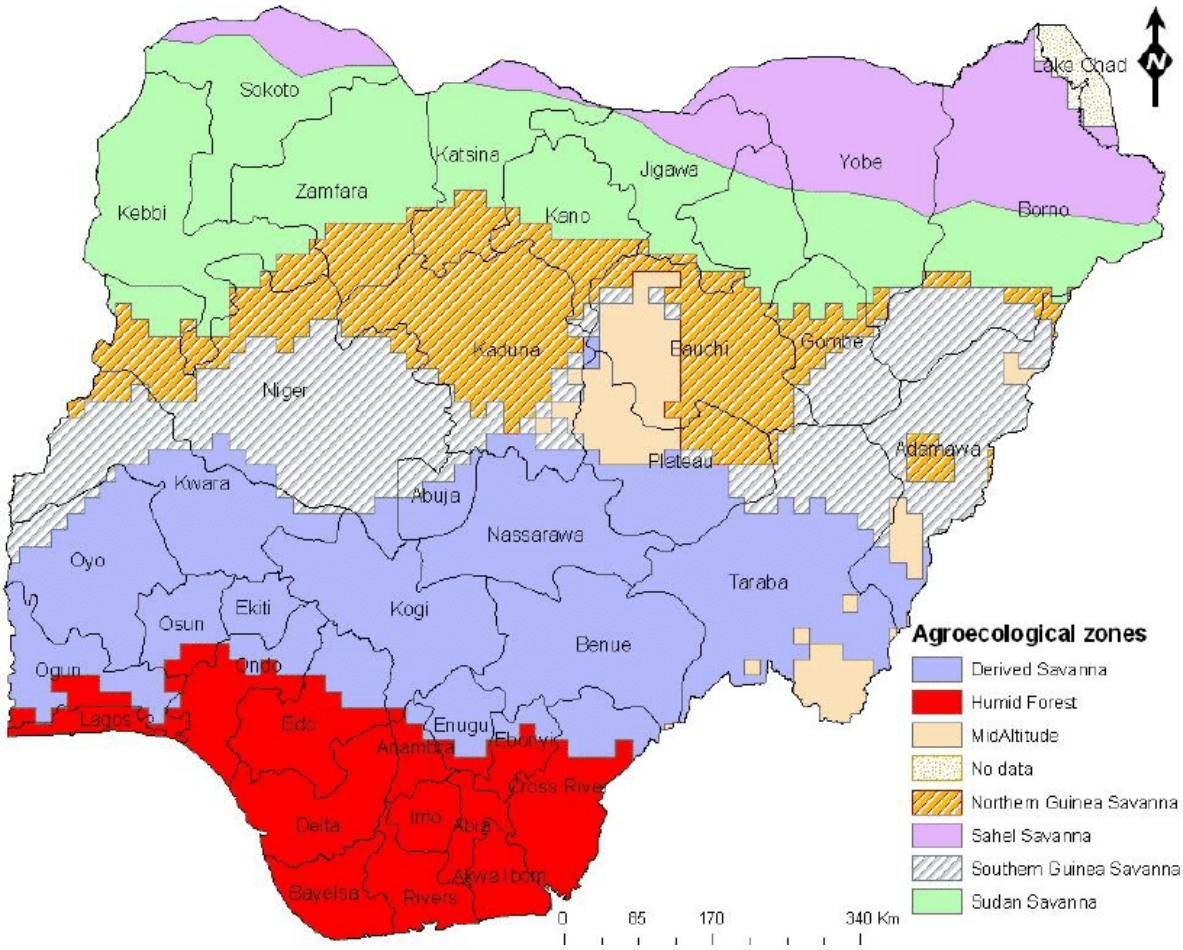


Fig. 3.1. Agro-Ecological Zones in Nigeria in relation to average rainfall

3.2 Sampling and Sample Preparation

Sampling and sample preparation prior to aflatoxin analysis were carried out as described by Adetunji *et al*. (2014) and the modified EC (2002) method, respectively in order to reduce variability. A total of 130 lafun samples (3 kg each) were collected across the five AEZs: The samples were hand-mixed and allowed to pass through a mesh screen. Sub-samples of 500 g were taken from each lot and sieved with 1-mm mesh. Sub-samples of 50 g were further taken from the lots into zip-lock envelopes forming 64 composite samples which were labeled appropriately and transported to the microbiological and

mycotoxin laboratory of the National Agency for Food and drug administration and control (NAFDAC) Oshodi, Lagos. Samples stored at −20 °C prior to analyses.

3.3 Chemical and Reagents

Phosphate buffered saline solution (pH 7.4), Potato Dextrose Agar, Extraction solvent (Acetonitrile/water solution 8:2, v/v), Methanol (Technical grade, distilled), Water, Anhydrous $MgSO_4$, NaCl (Sodium chloride), HPLC mobile phase solvent (Water/acetonitrile/methanol HPLC grade solution 6:2:3, v/v/v), Sodium chloride, HPLC aflatoxin standard solutions LLC.

3.4 Apparatus

Laboratory balance: (Readability 0.1 g), Analytical balance: (Readability 0.1 mg), Pipettes: (10mL), Fluorescence detector: Wavelengths 360 nm excitation filter × 420 nm, HPLC column: (4.6 mm × 25 cm), Glass microfiber filter paper: (5 cm diameter, retention: 1.6 µm), 20 mL syringe, Vertical shaker: (Adjustable for max. solid-liquid agitation, Calibrated micro-litre syringe: 25 and 500 µL, Disposable filter unit: (Cellulose, 0.45 µm), Volumetric glassware: (2, 3, 10, and 20 mL (0.5% accuracy), Filter paper: (24 cm diameter, pre-folded, and 30 µm retention).

3.5 Microbiological Examination of Lafun Sample

Fungi isolation was carried out using plate dilution method as described by Vanderzannt and Splittstoesser (1997) with slight modification. The media used was prepared and incubated according to the manufacturer's instructions. Three grams proportion of each sample was aseptically taken (after thorough mixing) and weighed into a beaker containing 30ml of 0.1% sterile peptone water (w/v) and allowed to soak for 2-3 minutes with occasional stirring with a sterile glass rod. The serial dilution was subsequently prepared by transferring 1ml aliquot of the supernatant into 9ml of sterile peptone water

as diluent. Further serial dilution was carried out and thereafter, 1ml of appropriate dilution was aseptically plated on Potato Dextrose agar (PDA) with Chloramphenicol added to the media. The media was incubated at 25 ± 2 ^{o}C for 5-7 days. Following incubation, the fungal colonies were counted with a colony counter, and the number of colonies per gram of sample was counted, recorded, and expressed as colony forming units per gram, as shown in Equation 1.

$$\text{CFU/g} = \frac{Number\ of\ Colonies \times reciprocal\ of\ the\ dilution\ factor}{Volume\ (ml)\ platted} \quad (3.1)$$

The fungal isolates were identified using compound microscope based on examinations of the conider heads, phialides, conidiophores and presence and absence of foot cells or rhizoids using the appropriate identification keys and atlas in literature (Oranusi and Olarewaju, 2013). The summary of incidence rate and frequency of isolated *fungi* species from the lafun samples were calculated using Equation 2 and 3, respectively.

$$\text{Incidence Rate} = \frac{Number\ of\ Isolates}{Total\ number\ of\ Isolates} \times 100 \quad (3.2)$$

$$\%\ \text{Frequency} = \frac{Number\ of\ Isolates}{Total\ number\ of\ Samples} \times 100 \quad (3.3)$$

3.6 Analysis of Lafun Samples for Aflatoxin Contamination using HPLC

A total of 68 composite of lafun samples were analyzed for the presence of four prominent regulated aflatoxin: B1 (AFB1), AFB2, AFG1 and AFG2 by high performance liquid chromatography (HPLC) method. Five grams (5g) of each ground representative sample was weighed into a 50-ml polypropylene tube and extracted with 20 ml acetonitrile/water/acetic acid (79:20:1, v/v/v) for 90 min on a rotary shaker. To the extract, 10grams of Anhydrous $MgSO_4$ and 1g of NaCl was added and shaken for 1minutes. The solution was then centrifuged for 5 minutes at 4000rpm to further separate the solid from the liquid. The method of clean up via solid-phase extraction was carried out as described

by Shephard (2003). 6ml of the supernatant was transferred into a 15ml clean up tube containing 150mg of PSA and 600mg of $MgSO_4$. The mixture was shaken vigorously using vertical vortex shaker and centrifuged for 5 minutes and 4000 rpm. 1ml of the purified extracts were then evaporated to dryness at 40°c under nitrogen and reconstituted in 1 ml of methanol/water (80/20; v/v) solution. Recovery assays for the individual samples were greater or equal to 80% and the limit of detection for aflatoxins was ≤ 0.1 µg/g.

The Aflatoxins, were quantified using HPLC Modula system (Agilent, Waldbronn, Germany) with an Ultraviolent detection. The system was accomplished with column types C-18, pressure max per flow rate of 420 pa x 1 ml/ml for the aflatoxins, for the separation and quantification of the selected Aflatoxins and standards. Twenty microlitre (20 µl) was injected as the volume used for the prepared samples with different mobile phase for individual aflatoxin: that is, water: methanol: acetonitrile (60:20:20).

3.7 Method Validation for HPLC

The methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs lay down by (European Commission Regulation, 2010) No 401/2006 of 23 February 2006 was adopted. According to the commission, the method was validated in terms of linearity (quantification), apparent recovery (% recovery) and sensitivity (limit of detection (LOD) and limit of quantification (LOQ)) using blank matrices of the lafun samples (401/Udoh *et al.,* 2000; Abia, *et al*., 2013)). External calibration curves were established based on the serial dilution of the aflatoxin standard solution. Linearity was determined by injecting aflatoxin standards at three different concentrations into the HPLC column. Calibration curve between the different concentrations and correlation co-efficient (R^2) indicated good linearity with R^2 values ranging from 0.9018-0.9998 for the different aflatoxin standards.

Table 3.1: Calibration Parameters for HPLC Analysis

Analytes	Calibration level (µg/kg)	Percentage recovery (%)	r^2	Equation of straight line
AFB_1	2.5, 5.0, 10.0	88	0.9018	y = 2634x
AFB_2	5.0, 10.0, 20.0	92	0.9851	y = 24120x
AFG_1	2.5, 5.0, 10.0	78	0.9761	y = 62824x
AFG_2	2.5, 5.0, 10.0	92	0.9882	y = 41585x

Keys: AFG_1- Aflatoxin G_1, AFG_2- Aflatoxin G_2, AFB_1- Aflatoxin B_1, AFB_2- Aflatoxin B_2.

Percentage recoveries of the analytes were carried out by spiking three different samples (5 g of each) that were least contaminated with the analyte standard (100 µl of standard concentration). The spiked samples were left overnight in a fume cupboard at room temperature for evaporation of the solvent to establish equilibrium between the sample matrix and the toxins. The aflatoxins from spiked samples was extracted by mycotoxin extraction method as described above. From each spiked sample, 20 µl of the extract was injected into the HPLC. The corresponding peak areas of the spiked samples were used for estimation of the apparent recovery by comparison with a standard of the same concentration prepared by dilution in pure solvent. The percentage recoveries were estimated using the formula presented in Equation 4 and the calibration parameters were presented in Table 1.

$$\% \text{ Recovery} = \frac{Peak\ Area\ of\ Spiked\ samples}{Peak\ Area\ of\ Liquid\ Standards} \times 100 \quad (3.4)$$

The sensitivity parameters (i.e. LOD and LOQ) for mycotoxins in the lafun samples were calculated from the signal to noise ratios (S/N) of the respective multiple reaction monitoring (MRM) chromatograms derived from the analysis of the spiked samples: LOD =3×S/N and LOD =10 ×S/N, respectively.

3.8 Exposure Assessment and their Potential Risk Characterization

3.8.1 Data collection

A simple questionnaire was administered to address the risk characterization of human exposure to Aflatoxins present in lafun sold in the various markets that are consumed in the five AEZs in Nigeria, a total of 150 respondents were required to fill the questionnaire in order to determine the exposure rate and risk associated with aflatoxin contamination. Also, the survey was age- and gender- weighted and they represent adult population groups between the ages of 20-60 years. In this regard, the adult population groups were successfully interviewed through a quota sampling and completed the dietary intake questionnaires within 15-30 minutes. A portable scale was used to determine the weight of each respondent from the studied region. The weight of forms in which lafun is being consumed was also taken in grams. The questionnaire assessed whether the respondents consumed lafun or not, and explore the extent to which they consume their products (if they were consumed on a daily basis or not at all).

3.8.2 Aflatoxins dietary intake

The estimated daily intake (EDI), and percentage tolerable daily intake (% TDI) values will be estimated for the staples. The method used by Rodrigues *et al*. (2011) and approved by JECFA was adopted in this study. The "Estimated daily intake" which estimates the amount of toxin that can be ingested daily (μg/kg bw/day) can be obtained by using the formula presented in Equation 5.

$$\text{Estimated daily intake (EDI)} = \frac{\text{Contamination level} \times \text{Consumption rate}}{\text{Body weight (kg/persons)}} \qquad (3.5)$$

Where "Contamination level" refers to the average toxin level found in a certain foodstuff (μg/Kg/day) and "Consumption rate" is the amount of the foodstuffs ingested on daily basis (gram/day).

However, the formula presented in the equation above was implemented by multiplying the average level of each aflatoxin present in the lafun samples with the average lafun consumption in Nigeria: 426.3g/person/day (0.4167 kg/person/day) "as estimated from the questionnaire" and then divided by mean body weight of 63.03, 64.29 and 61.58 kg for adult male, adult female and total population groups, respectively.

3.8.3. Determination of burden of aflatoxins-attributable to hepatocellular carcinoma incidence among lafun consumer

The JECFA estimated cancer potency values for aflatoxins were adopted in determining the annual burden and HCC incidence attributable to aflatoxins exposure in lafun. The values which corresponded to 0.3 cases of cancer per 100,000 population annually, for each ng/kg bw/day, among populations infected with hepatitis B virus (HBsAg+), and 30 times lesser (0.01 cases of cancer per 100000 population per ng/kg bw/day) among people not infected (EFSA, "European Food Safety Authority", 2007) were employed for this estimation. The HBsAg+ prevalence rate used was 13.6 % in Nigeria based on previous studies, and 86.4 % was extrapolated for HBsAg- groups (Roy *et al.,* 2013). Similarly, a recent report by National HIV/AIDs Indicator and Impact Survey (NAIIS) highlighted that HBV prevalence in Nigeria was 8.1 % and affirmed Nigeria populations to be approximately 190 million (Sapsford *et al.,* 2016). Hence, the annual HCC cases per 100,000 for $HBsAg^{+ve}$ and $HBsAg^{-ve}$ individuals, as well as that used to estimate the annual HCC cases based on populations that are $HBsAg^{+ve}$ and $HBsAg^{-ve}$ are represented in Equation 6, 7, and 8, respectively.

Annual HCC Cases/100,000 for $HBsAg^{+}$ individual = Aflatoxins EDI ×Potency Factor (0.3) Eq. 6

Annual HCC Cases/100,000 for $HBsAg^{-}$ individual = Aflatoxins EDI ×Potency Factor (0.01) Eq. 7

Annual HCC Cases = $\frac{Aflatoxins\ EDI \times Potency\ factor\ (0.3\ or\ 0.01)}{100{,}000} \times N(\text{HBsAg}^{+}\ \text{or}\ \text{HBsAg}^{-})$ Eq. 8

Where N, represents prevalence rate multiply by the total population of the individual in each of the five AEZs.

3.8.4 Determination of HCC risk

Based on the prevalence of $HBsAg^{+}$ (13.6 % or 8.1 %) of individual in Nigeria total population, the risk for liver cancer was estimated for different population groups consuming lafun in each of the five AEZs using the relation presented in Equation 9 and 10, respectively.

Cancer potency = $0.3 \times a nnual\ HCC\ cases\ (\text{HBsAg}^{+}) + 0.01 \times Annual\ HCC\ cases\ (\text{HBsAg}^{-})$ Eq. 9

HCC Population Risk = EDI × Cancer Potency Eq. 10

3.8.5 Cancer incidence attributable to dietary aflatoxins from consumption of lafun

This was computed as reported by Liu and Wu (2019) by dividing the estimated liver cancer risk per 100,000 population by the incidence rate of liver cancer in Nigeria (6.5 deaths/100,00 population) estimated for Nigeria by Global Burden of Disease Project and multiplying by 100% (WHO, 2004).

3.8.6 Disability adjusted life year (DALY) lost

DALY is an epidemiological measure of disease burden expressed in number of healthy life years lost due to death or disability caused by a disease. It is calculated by multiplying annual HCC cases (HBsAg-positive) per 100,000 populations by sex-specific HCC DALY estimate (13.05) for both male and female population (Abt Associates Incorporated, 2013).

3.9 Statistical Analysis

All data were analyzed by SPSS 26.0. One-way ANOVA was performed for the distribution of fungal species across the agro-ecological zones (AEZs). All means were tested for significance by the Duncan's Multiple Range Test at 95% confidence level.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Frequency and incidence rate of fungal species identified in Lafun samples from five AEZs in Nigeria

A total of 108 fungal isolates belonging to four genera: *Aspergillus* (n = 60), *Fusarium* (n = 19), *Penicillium* (n = 18) and *Mucor. Spp* (n = 11) were isolated from the lafun samples (Figure 4.1 & Table 4.1). The percentage population of *Aspergillus* was found to be (55.56%), while that of *Fusarium, Penicillium* and *Mucor. Spp* were found to be (17.59%), (16.67%) and (10.19%) respectively. The population of *Aspergillus* was higher than the population of the other fungal genera in lafun sample from all the AEZs (Figure 4.1). *Fusarium* (17.59%) was the second in the population frequency followed by *Penicllium* spp (16.67%) and least frequency in the hierarchy was *Mucor. Spp* (10.19%). Among the *Aspergillus* species isolated, *A. flavus, A. niger* and *A. fumigatus*, as well as *Penicillium,* and *Fusarium* species were aflatoxin-producing species and were the most abundant species in all the AEZs.

The total incident of *A. flavus* (40%) was the highest, followed by *A. parasiticus* (30%) among the *Aspergillus* species isolated, while among the *Fusarium* species, *F. graminearum* had the highest total incidence of (57.89%), and *P. verrucosum* had the highest incidence of (66.67%) among the *Penicillium Spp.* The incidence of *A. flavus* in the lafun sample from the HF (37.5%) and the DS (33.33%) zones were significantly ($p<0.05$) higher than the lafun samples from other AEZS. Following *A. flavus* in hierarchical succession was the *A. parasiticus* -clade whose had (50%) incidence in the DS and HF zones. *A. niger* has (0.00%) in the lafun samples from DS, HF and SS zones (Table 4.1). *F. culmorum* was recorded to have the highest incidence (50%%; $p<0.05$)

present in the lafun sample from the HF zone amongst the *Fusarium* isolates in AEZs. *P. notatum* was recorded to be having incidence of (0.00%;) in the NGS, SGS and SS zones respectively, and *Muco. spp*. has the highest incidence of (54.54%; $p<0.05$) in the DS and (0.00%) incidence in the SGS zones. The mycological analysis of the lafun samples showed that none of the samples were free from fungal infestation, yet lafun samples from the NGS had the least fungal infestation among all the samples in from AEZs in Nigeria.

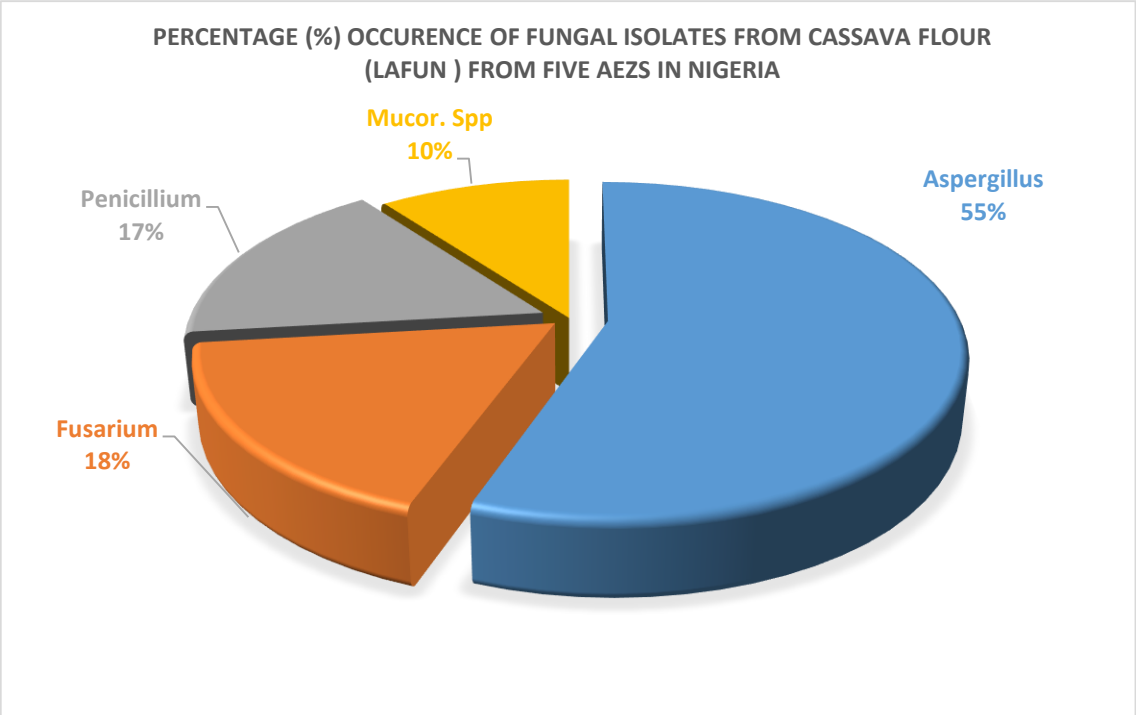
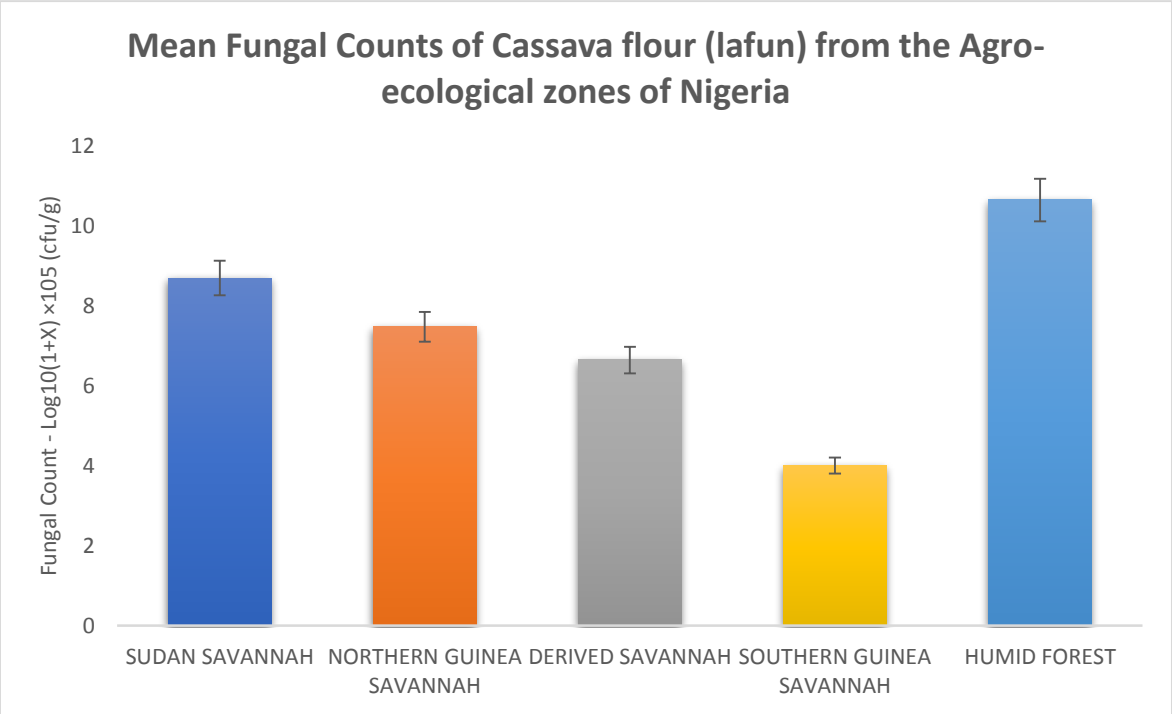


Figure 4.1: Percentage occurrence of fungal isolates of Cassava flour (lafun) from the Agro-ecological zones of Nigeria

4.1.2 Colony forming units (CFU) of fungal species found in lafun samples from the five Agroecological zones in Nigeria.

The rate of microbial contamination evaluated by the colony forming unit per gram (CFU/g) determined in thirty-two composites samples from the five agroecological zones in Nigeria where cassava serve as the major staple shows that HF has the highest average fungal load. The CFU contents in lafun across the five agro-ecological zones is presented in decreasing order as follows: HF zone (10.64±0.1 x10^4cfu/g) > SS zone (8.69±2.0

x10^4cfu/g) > NGS zone (7.47±1.1 x10^4cfu/g) > DS zone (6.64±0.21 x10^4cfu/g) > SGS zone (4.0±0.11 x10^4cfu/g) (Figure 4.2). The fungal count in all the AEZs shows slight variations amongst the groups of microorganisms within each state and from one state to another. The result shows significantly ($p<0.05$) in the Colony forming units (CFU) between and among each state in the AEZs. Based on the categorization of Gimeno (2002), samples can be categorized as good provided that the colony count is less than (3 x 10^4 CFU/g), regular (count range between 3x10^4 to 7 x 10^4 CFU/g) and bad (greater than 7 x 10^4 CFU/g). Therefore, the lafun samples from DS and SGS were found to be



regular, while samples from NDS, SS and HF were found to be bad in terms of microbial count.

Figure 4.2: Mean Fungal Counts of Cassava flour (lafun) from the Agro-ecological zones of Nigeria

4.1.3 Incidence and Level of Aflatoxins (ng/g) in Lafun Samples from all the Agro-Ecological zones in Nigeria.

The occurrence and levels of total aflatoxins (AFB_1 + AFB_2 + AFG_1 + AFG_2) based on agro-ecological zones are presented in Table 4.2. The concentrations of total aflatoxins in

the Lafun samples sold in the market is in relation to average rainfall pattern/state. In the various AEZs, the Lafun samples were contaminated with high concentration of aflatoxin G1 (AFG1). In particular, Gombe state with an annual rainfall of 66.84 mm (2.63 in) had the highest aflatoxin G1 (AFG1) contamination of (90.72±90.7^{a} ng/g) and a significantly low aflatoxin B1 (AFB1) contamination levels of (0.70±0.67^{a} ng/g), and also a reasonably high amount of AFG2 (109.16±108.9^{a}) was recorded in Kaduna state both of the NGS zone. The SS zone recorded the highest (15.64±0.45^{d}) average level of aflatoxin B1 (AFB1) contamination among all the AEZs, with Kano having the highest contamination level of (41.11±31.28^{a}) among all the states in Nigeria. The NGS zone (2.63±0.07^{c} ng/g) had the highest average aflatoxin B2 (AFB2) contamination level. It is also worthy of note that Jigawa, Enugu and Cross River had (0.00±0.00 ng/g); had no record of aflatoxin B12 (AFB1) contamination in the Lafun samples from these states.

The average total aflatoxin concentration (AFT) in lafun across the five agro-ecological zones is presented in decreasing order as follows: NGS zone (96.67±0.06^{e} ng/g), > DS zone (44.57±0.02^{d} ng/g) > SS zone (33.19±0.47^{c} ng/g) > HF zone (30.60±0.43^{b} ng/g) > SGS zone (20.57±0.47^{a} ng/g) (Figure 4.3). Aflatoxin B1 was found in Lafun samples from all the AEZs, and the average concentration ranges from 2.08±0.01^{a} ng/g in the (NGS) to 15.64±0.45^{d} ng/g in the (SS) zones, while that of Aflatoxin B2 ranges from 1.57±0.01^{a} ng/g in the (SGS) to 2.63±0.07^{c} µg/g in the (NGS) zones. The highest concentration of Aflatoxin G1 was recorded in the DS (22.53±0.01^{d} ng/g), and least in Lafun sample from SS (8.40±0.02^{a} ng/g) zones. As aforementioned, the highest concentration of Aflatoxin G2 was found in Lafun samples from NGS zone (42.94±0.04^{e} ng/g), the least concentration of (3.27±0.01^{a} ng/g) was recorded from SGS zone.

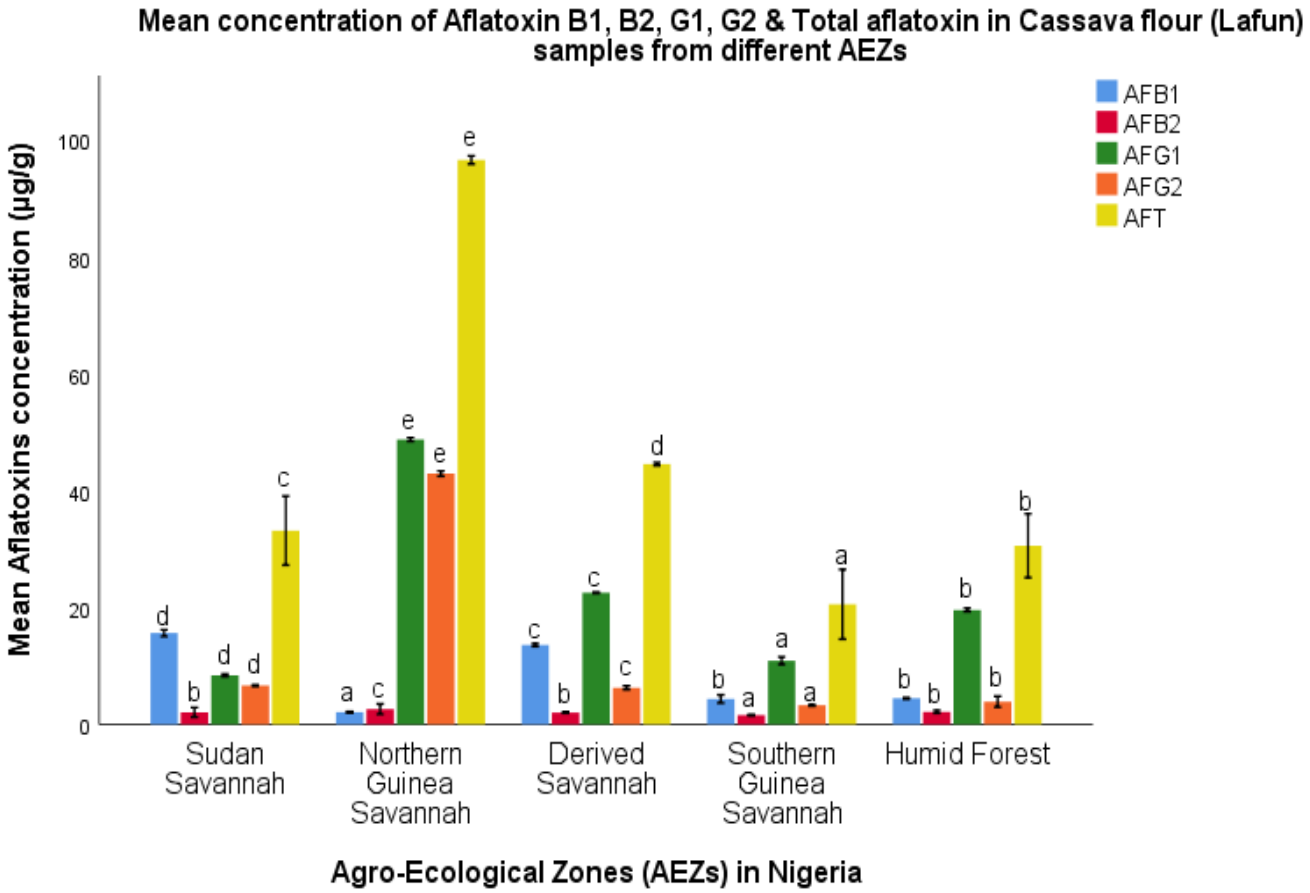


Figure 4.3: Mean Concentration of AF B1, B2, G1 & G2 in Cassava flour (lafun) from the Agro-ecological zones of Nigeria

Table 4.1: Distribution of Isolated Fungal species in Lafun Samples from different AEZs with Incidence Rate in Parenthesis.

Fungi Species	**Sample Location/Zones**					**Total** Incidence (n=64)	**% Occurrence of fungal species in AEZ***				
	NGS (n = 6)	SGS (n =4)	DS (n = 24)	HF (n = 22)	SS (n = 8)		NGS (n = 6)	SGS (n =4)	DS (n = 24)	HF (n = 22)	SS (n = 8)
A. flavus	2(22.22)	2(22.22)	8(20.51)	9(27.27)	3(16.67)	**24**	3.125	3.125	12.5	14.06	4.69
A. fumigatus	2(22.22)	1(11.11)	5(12.82)	4(12.12)	2(11.11)	**14**	3.125	1.56	7.81	6.25	3.125
A. parasiticus	1(11.11)	0(0.00)	7(17.95)	7(21.21)	3(16.67)	**18**	1.56	0.00	10.94	10.94	4.69
A. niger	2(22.22)	2(22.22)	0(0.00)	0(0.00)	0(0.00)	**04**	3.125	3.125	0.00	0.00	0.00
F. graminearum	0(0.00)	1(11.11)	3(7.69)	4(12.12)	3(16.67)	**11**	0.00	1.56	4.69	6.25	4.69
F. culmorum	0(0.00)	1(11.11)	1(2.56)	4(12.12)	2(11.11)	**08**	0.00	1.56	1.56	6.25	3.125
P. notatum	0(0.00)	0(0.00)	4(10.26)	2(6.06)	0(0.00)	**06**	0.00	0.00	6.25	3.125	0.00
P. verrucosum	0(0.00)	2(22.22)	5(12.82)	2(6.06)	3(16.67)	**12**	0.00	3.125	7.81	3.125	4.69
Mucor. Spp.	2(22.22)	0(0.00)	6(15.39)	1(3.03)	2(11.11)	**11**	3.125	0.00	9.38	1.56	6.38
Total	**09**	**09**	**39**	**33**	**18**	**108**	**14.06**	**14.06**	**60.94**	**51.56**	**31.39**

*****AEZ- Agro-ecological zones: (NGS)-Northern Guinea Savanna, (SGS)-Southern Guinea Savanna, (DS)-Derived Savanna, (HF)-Humid Forest and (SS)-Sudan Savanna.

Table 4.2: Incidence and Level of Aflatoxins (µg/g) in lafun Samples from the Agro-Ecological zones in Nigeria.

Zone	AFB1		AFB2		AFG1		AFG2		AFT	
	N (n) % Cont.	**Mean(±)SEM (Range) EU limit (No. of Cont. sample above EU limit)**	**N (n) % Cont.**	**Mean(±)SEM (Range) EU limit (No. of Cont. sample above EU limit)**	**N (n) % Cont.**	**Mean(±)SEM (Range) EU limit (No. of Cont. sample above EU limit)**	**N (n) % Cont.**	**Mean(±)SEM (Range) EU limit (No. of Cont. sample above EU limit)**	**N (n) % Cont.**	**Mean(±)SEM (Range) EU limit (No. 0of Cont. sample above EU limit)**
NGS	6 (5) 83.3	2.08±0.01[a] (0-6.81) 2 (2)	6 (5) 83.3	2.63±0.07[c] (0-8.19) Na (Na)	6 (4) 66.7	48.79±0.03[e] (0-181.44) Na (Na)	6 (3) 50.0	42.94±0.04[e] (0-218.05) Na (Na)	6 (6) 100	96.67±0.06[e] (59.69-134.61) 4 (3)
SGS	4 (3) 75.0	4.36±0.05[b] (0-8.71) 2 (3)	4 (2) 50.0	1.57±0.01[a] (0-4.77) Na (Na)	4 (2) 50.0	10.91±0.05[b] (0-38.06) Na (Na)	4 (2) 50.0	3.27±0.01[a] (0-10.30) Na (Na)	4 (4) 100	20.57±0.47[a] (10.39-29.82) 4 (2)
DS	24 (19) 76.2	13.60±0.02[c] (0-55.15) 2 (17)	24 (18) 75.0	2.01±0.01[b] (0-9.21) Na (Na)	24 (16) 66.7	22.53±0.01[d] (0-125.30) Na (Na)	24 (14) 58.3	6.28±0.02[c] (0-24.25) Na (Na)	24 (24) 100	44.57±0.02[d] (0.45-106.14) 4 (11)
HF	22 (16) 72.72	4.46±0.01[b] (0-36.26) 2 (9)	22 (15) 68.18	2.15±0.02[b] (0-10.26) Na (Na)	22 (19) 86.36	19.59±0.02[c] (0-165.13) Na (Na)	22 (13) 59.09	3.90±0.07[b] (0-22.43) Na (Na)	22 (22) 100	30.60±0.43[b] (1.24-107.55) 4 (10)
SS	8 (6) 75.0	15.64±0.45[d] (0-72.38) 2 (5)	8 (6) 75.0	2.07±0.06[b] (0-5.29) Na (Na)	8 (4) 50	8.40±0.02[a] (0-40.03) Na (Na)	8 (5) 62.5	6.65±0.01[d] (0-19.81) Na (Na)	8 (8) 100	33.19±0.47[c] (11.11-74.64) 4 (4)
Total sample	**64 (49) 76.56**	**40.14±0.54 (0-72.38) 2 (36)**	**64 (46) 71.88**	**10.43±0.17 (0-10.26) Na (Na)**	**64 (45) 70.31**	**110.22±0.13 (0-181.44) Na (Na)**	**64 (37) 57.81**	**63.04±0.15 (0-218.05) Na (Na)**	**64 (64) 100**	**225.6±1.45 (0-19.74) 4 (30)**

KEYS: Mean = Mean concentration; SEM = Standard Error of Means; AFB1 = Aflatoxin B1; AFB2 = Aflatoxin B2; AFG1 = Aflatoxin G1; AFG2 = Aflatoxin G2; AFT = Total Aflatoxins: (NGS)-Northern Guinea Savanna, (SGS)-Southern Guinea Savanna, (DS)-Derived Savanna, (HF)-Humid Forest and (SS)-Sudan Savanna; EU limit= European Union Maximum limits; N = Number of Samples;; n = Number of Positive Samples; % Cont. = Percentage Contamination; No. of Cont. sample above EU limit= Number of contaminated samples above EU limits. Values are in ± mean S.E. (S.E = Standard error of Mean) Values between experimental treatments Within Groups bearing the same superscript are not significantly different at the 5% level ($P<0.05$).

Table 4.3: Estimated annual burden of Hepato-Cellular Carcinoma (HCC) cases and risk of HCC/year attributable to aflatoxin exposure from Lafun consumers in the Agro-Ecological zones in Nigeria.

Mycotoxins	**Mean Conc. ($x10^3$)**	**Estimated daily Intake (EDI)**	**Estimated Annual HCC (Per 100,000)**		**Annual HCC cases (HBsAg Prevalence = 13.6%) ($x10^3$)**		**HCC Risk/year (13.6%) ($x10^5$)**	**Annual HCC cases (HBsAg Prevalence = 8.1%) ($x10^3$)**		**HCC Risk/year (8.1%) ($x10^5$)**
	(ng/kg)	(ng/kg. bw/day)	HBsAg +Ve	HBsAg – Ve	HBsAg +Ve	HBsAg -Ve		HBsAg +Ve	HBsAg -Ve	
		Male **(TP)** Female	Male **(TP)** Female	Male **(TP)** Female	Male **(TP)** Female	Male **(TP)** Female		Male **(TP)** Female	Male **(TP)** Female	
AFB_1	8.01	54.19	16.26	0.54	4.20	0.89	0.71	2.50	0.95	0.42
		(55.47)	**(16.64)**	**(0.55)**	**(4.30)**	**(0.91)**	**(0.74)**	**(2.56)**	**(0.97)**	**(0.44)**
		56.81	17.04	0.57	4.40	0.93	0.78	2.62	0.99	0.46
AFB_2	2.12	14.37	4.31	0.14	1.11	0.24	0.05	0.66	0.25	0.03
		(14.70)	**(4.41)**	**(0.15)**	**(1.14)**	**(0.24)**	**(0.05)**	**(0.68)**	**(0.26)**	**(0.03)**
		15.06	4.52	0.15	1.17	0.25	0.05	0.70	0.26	0.03
AFG_1	22.05	149.14	44.74	1.49	11.56	2.45	5.35	6.89	2.60	3.18
		(152.65)	**(45.80)**	**(1.53)**	**(11.83)**	**(2.51)**	**(5.60)**	**(7.05)**	**(2.67)**	**(3.34)**
		156.33	46.90	1.56	12.12	2.57	5.87	7.22	2.73	3.50
AFG_2	12.62	85.38	25.61	0.85	6.62	1.40	1.75	3.94	1.49	1.04
		(87.39)	**(26.22)**	**(0.87)**	**(6.77)**	**(1.43)**	**(1.84)**	**(4.03)**	**(1.53)**	**(1.09)**
		89.49	26.85	0.89	6.94	1.47	1.92	4.13	1.56	1.15
AFT	44.81	303.07	90.92	3.03	23.50	4.98	22.07	13.99	5.29	13.15
		(310.21)	**(93.06)**	**(3.10)**	**(24.05)**	**(5.09)**	**(23.13)**	**(14.32)**	**(5.42)**	**(13.77)**
		317.69	95.31	3.18	24.63	5.22	24.25	14.67	5.55	14.45

Keys: AFB_1 = Aflatoxin B_1; AFB_2 = Aflatoxin B_2; AFG_1 = Aflatoxin G_1; AFG_2 = Aflatoxin G_2; AFT = Total aflatoxins; EDI = Estimated daily intake; HCC = Hepato-Cellular Carcinoma; HBsAg = Hepatitis B virus; TP = Total population

Table 4.4: Risk assessment of aflatoxin exposure in Nigerian Cassava Flour (Lafun)

	AFT		**AFG2**		**AFG1**		**AFB2**		**AFB1**				
AEZ	Mean Conc. ($x10^3$) (ng/kg)	Estimated daily Intake (EDI) (ng/kg body weight/day)	Mean Conc. ($x10^3$) (ng/kg)	Estimated daily Intake (EDI) (ng/kg body weight/day)	Mean Conc. ($x10^3$) (ng/kg)	Estimated daily Intake (EDI) (ng/kg body weight/day)	Mean Conc. ($x10^3$) (ng/kg)	Estimated daily Intake (EDI) (ng/kg body weight/day)	Mean Conc. ($x10^3$) (ng/kg)	Estimated daily Intake (EDI) (ng/kg body weight/day)	Estimated liver cancer risk (cases/100, 000 population/year)	Cancer incidence attributable to dietary aflatoxin (%)	DALY
		Male **(TP)** Female		Male **(TP)** Female		Male **(TP)** Female		Male **(TP)** Female		Male **(TP)** Female	Male **(TP)** Female	Male **(TP)** Female	Male **(TP)** Female
SS	32.72	221.30 **(226.51)** 216.96	6.65	44.97 **(46.03)** 44.09	8.39	56.73 **(58.07)** 55.62	2.00	13.54 **(13.85)** 13.27	15.68	106.06 **(108.56)** 103.98	31.82 **(32.57)** 31.19	489.54 **(501.08)** 434.62	415.25 **(425.04)** 407.03
NGS	96.61	653.41 **(668.79)** 640.60	42.91	290.23 **(297.07)** 284.54	48.81	330.14 **(337.92)** 323.67	2.78	18.83 **(19.28)** 18.46	2.10	14.20 **(14.53)** 13.92	4.26 **(4.36)** 4.18	65.54 **(67.08)** 64.31	55.59 **(56.90)** 54.55
DS	44.55	301.31 **(308.41)** 295.41	6.32	42.74 **(43.75)** 41.91	22.53	152.35 **(155.93)** 149.36	2.08	14.06 **(14.39)** 13.79	13.63	92.16 **(94.33)** 90.35	27.65 **(28.30)** 27.11	425.39 **(435.39)** 417.08	360.83 **(369.32)** 353.79
SGS	20.17	135.98 **(139.18)** 133.31	3.26	22.05 **(22.57)** 21.62	10.96	74.13 **(75.87)** 72.67	1.58	10.70 **(10.95)** 10.49	4.30	29.10 **(29.78)** 28.53	8.73 **(8.93)** 8.56	134.31 **(137.39)** 131.69	113.93 **(116.54)** 111.71
HF	26.72	180.70 **(184.95)** 177.15	3.98	26.89 **(27.52)** 26.36	19.57	132.34 **(135.46)** 129.75	2.17	14.70 **(15.04)** 14.41	4.45	30.13 **(30.83)** 29.53	9.04 **(9.25)** 8.86	139.08 **(142.31)** 136.31	117.97 **(120.71)** 115.62
National	224.05	1515.4 **(1551.1)** 1588.5	63.12	426.88 **(436.94)** 447.47	110.25	745.69 **(763.25)** 781.65	10.62	71.83 **(73.52)** 75.29	40.06	270.97 **(277.35)** 284.04	131.19 **(134.28)** 128.62	2,018.3 **(2,065.9)** 1,978.8	1,712.0 **(1,752.4)** 1,678.5

Keys: *SS*- Sudan Savanna, NGS- Northern Guinea Savanna, SGS- Southern Guinea Savanna, DS- derived Savanna, HF- humid forest, TP- Total population, AFB1- Aflatoxin B1; AFB2- Aflatoxin B2; AFG1- Aflatoxin G1; AFG2- Aflatoxin G2; AFT- Total aflatoxins; EDI- Estimated daily intake

4.2 Discussion of Results

4.2.1 Fungal occurrence (load and incidence) in lafun and their implications

As a result of the huge risks connected with consuming contaminated foods, food safety remains a global concern. Local cassava flour (lafun) sourced from major markets in Nigeria's climatically diverse agro-ecological zones was examined for mycological profile and aflatoxin concentration in this study. The selected samples were grown on suitable conditions using standard mycological procedures to isolate/characterize fungus and evaluate aflatoxin concentration using commercial HPLC methods. The results of fungal counts on the cassava flour samples indicated a very diversified and variable pattern of fungal infection. With values representing mean of fungal counts from three replicates, the total fungal count ranged from $4.0x10^4$ cfu/g to $10.64x10^4$ cfu/g in the cassava flour (lafun) across the zones. The cassava flour was most contaminated in lafun sample at Bayelsa ($20.75x10^4$ cfu/g), Kebbi ($20.25x10^4$ cfu/g), all having no significant difference ($p>0.05$) across the zones. Okunlola and Folorunso (2015) discovered in their study that the higher fungal counts observed in the Humid forest states may be attributable to the higher occurrence of rainfall in these zones compared to the Southern Guinea and Derived savannah zones, which may have encouraged proliferation of fungi associated with stored foods, and the highly significant difference in counts for local cassava flour may be due to differences in local processing of the pellets into flour, as well as differences in environmental factors and contamination from equipment (Essono *et al*., 2019).

The fungi isolated from all the food products analyzed across the five agro-ecological of zones of Nigeria were identified as *Aspergillus Flavus, Aspergillus niger, Aspergillus parasiticus, Aspergillus fumigatus, Penicillium notatum, Penicillium verrucosum, Fusarium graminearum, Fusarium culmorum* and *Mucor spp*. *Aspergillus* sp showed a

unique pattern of distribution with a total incidence of (55.6%) across the zones and having highest occurrence in Bauchi (NGS zone), being present in all the cassava products analyzed. The incidence distribution of A. flavus analyzed across the agroecological zones was (40%) % in lafun sample and the organism occurred in almost all samples. *Fusarium* had percentage occurrence as (17.59%) with highest incidence from SS zone, being isolated from all the cassava products, though was completely absent with (0.00%) occurrence from lafun in the in Gombe, Kaduna and Bauchi in the NGS zone. *Penicillium* had percentage occurrence as (16.67%) consistently had a low distribution across the zones but also absent with (0.00%) occurrence from lafun in the NGS zone while Mucor spp. occurred as an uncommon contaminant across the zones though NGS (Adamawa and Niger) was the only zone without any occurrence of this organism.

The distribution of *Aspergillus* species, has been reported to significantly influence aflatoxin contamination of food products and these were observed to be more prevalent in the southern zones of Nigeria (Atehnkeng *et al*., 2018a). In this work, the occurrence of *Aspergillus flavus* in the cassava flour analyzed does not however imply a corresponding occurrence of aflatoxin contamination, though circumstances that favor mold growth may also favor aflatoxin production but mold growth may occur with little or no mycotoxin production (Muthomi *et al*., 2017). The specific strains of the *Aspergillus* species especially *A. flavus* are more important in influencing the level of aflatoxins, rather than counts or general fungal diversity. The S strains as compared to the L strains, produce high amount of toxins hence their presence may signify possible high rate of aflatoxin contamination. The predominance of L strains of A. flavus from Aspergillus species in soil and maize grain samples from Nigeria has been reported in the work of Atehnkeng *et al*. (2018b).

4.2.2 Aflatoxin contamination in lafun and their implications

Aflatoxins are internationally recognized as highly toxic carcinogens that contaminate crops worldwide (Perrone *et al*., 2014). In this research, the local cassava flour (lafun) was assayed for the total aflatoxin content and concentration of four aflatoxin types (AFBI, AFB2, AFG1 and AFG2) from five agro-ecological zones in Nigeria with values illustrated in Table 4.2. The results showed that all (100%) the flour samples were contaminated with aflatoxin. The lafun samples were observed to be more susceptible to aflatoxin G1 contamination having (22.05 ng/g) and an Estimated daily Intake (EDI) of (152.65 ng/kg. bw/day) than other aflatoxins, probably due to the crude processing and lack of Good Manufacturing Practices (GMP) during the conversion of cassava to local flour. The least aflatoxin contamination was with B2 from the cassava flour having (2.12 ng/g) and an Estimated daily Intake (EDI) of (14.70 ng/kg. bw/day). The levels of total aflatoxin B2 in the flour sample was below the maximum allowable limits (4 ng/kg) specified by the European Commission (ASEAN, 2011), which is also currently being used by the National Agency for Food and Drug Administration and Control (NAFDAC), in Nigeria. This observation agrees with the findings from other studies (Adebayo-Tayo *et al*., 2006; Romagnoli *et al*., 2007; Russell and Peterson, 2007). Ediage *et al*. (2011) observed that 8 ng/kg of aflatoxin B2 was present in cassava flour from the Republic of Benin. The results suggest that processed cassava products in Nigeria are safe with respect to the regulated aflatoxin. However, levels of total aflatoxins (44.81 ng/g) in this study above the allowable limits level found in the present study have also been reported in some foodstuffs (Zinedine *et al*., 2016).

The level of aflatoxin B1 found in the lafun of the present study was higher compared to the values (4 ng/kg) reported by Ediage *et al*. (2011) for cassava flour from the Republic of Benin. Many survey studies carried out in Nigeria have revealed that mycotoxin

contamination of food is a nation-wide problem. Ibeh *et al*. (1991). Ezekiel *et al*. (2018), and Adetunji *et al*. (2019) showed that garri, yam flour, cassava flour, rice and bean, melon; foodstuffs which are consumed across the country had aflatoxin contamination of 30%, 50%, 40%, 10%, and 20% respectively. Highly significant difference ($p<0.05$) in aflatoxin contamination was recorded in the various cassava flour across the zones which may have been due to climatic, processing and storage variations in the zones (Kaaya & Eboku, 2016). Rainfall is an important climatic factor that has been reported to influence fungal proliferation and increase aflatoxin content of food products (Cotty and Jaime, 2017). The mean annual rainfall in Nigeria agro-ecological zones showed an ordering from highest to the least to be HF, DS, SGS, NGS and SS with about 53% of total rainfall in Nigeria accounted for by HF zone of the country (Okunlola and Folorunso, 2015). Aflatoxin content of the lafun analyzed were higher DS than the SGS but their increase was not statistically significant ($p<0.05$). This observations in this research was further supported by previous study by Okunlola and Folorunso, (2015), that higher fungal counts and aflatoxin contamination may be observed in the DS zones due to higher amount of rainfall probably as a result of increase in moisture content of the products, especially during storage (Pleadin *et al*., 2019). In Nigeria, the southern zones are known to store food products much longer than the northern zones which are the agricultural factories of the nation. Several efforts by national government of nations, non-governmental organizations and individuals, to eliminate its occurrence in food products have proved inadequate.

4.2.3 Exposure, risk assessment and annual burden and hcc/year attributable to lafun consumption in agro-ecological zones in Nigeria

The information derived from the questionnaire shows that the average daily consumption of lafun in form of the prepared dough for swallow in all the AEZs is 426.3 g/day. While

the mean weight of the sampled population was 61.58kg, the women weighed higher than their male counterparts with average weights of 64.29 and 63.03 kg respectively. The respondents who mostly had secondary school education or below were absolutely ignorant of the present of aflatoxins in the lafun.

Table 4.3 summarizes the exposure and risk characterization of the aflatoxins evaluated in adults within the AEZ study areas. In all cases AFG1 recorded the highest average Estimated daily Intake (EDI) of (152.65 ng/kg bw/day), followed by AFG2 (87.39 ng/kg bw/day) and the least EDI was recorded at (14.70 μg/kg bw/day) for AFB1 in the total population of the AEZs. According to American Cancer Society (2011), even EDI level as low as 0.001 μg/kg bw/day may induce liver cancer hence, the levels of AFT in food should be As Low As Reasonable Achievable (ALARA) (EFSA, "European Food Safety Authority", 2007). The risk of HCC was estimated based on two prevalence rates as presented in Table 4.3. The result shows the annual burden of HCC cases in AEZs, indicating different population groups that are susceptible to risk for cancer due to aflatoxins exposure from Lafun consumption. The liver cancer risk for AFB_1 (being the most potent of aflatoxins) was observed to be the highest among the overall population groups. Based on the results, an estimated annual HCC cases of 16.64 and 93.06 per 100,000 persons is anticipated due to consumption of AFB_1 and AFT respectively in Lafun consumption in Nigeria. At 13.6% HBsAg prevalence rate, an estimated 74, 000 and 2,313, 000 new HCC cases are likely to occur annually in the over 190 million population of Nigeria due to AFB_1 and AFT in the Lafun, but if the HBsAg prevalence is assumed to be 8.1%, the figures will be 44, 000 and 1,377, 000 cases respectively. The estimated HCC cases due to aflatoxins are higher in males than in females and obviously also higher in $HBsAg^+$ than in $HBsAg^-$ populations.

Table 4.4 shows the exposure risk estimate of Lafun consumers to Aflatoxins across the AEZs. The mean exposure estimates of the total population of Lafun consumers to total aflatoxins (AFT) significantly ($p < 0.05$) increased from the SGS zone (130.18 ng/kg bw/ day) to the NGS zone (688.79 ng/kg bw/day), while the mean exposure estimate for AFG2 was highest in the NGS zone (297.07 ng/kg bw/day) and significantly ($p < 0.05$) decreased downwards to the SGS zone (22.57 ng/kg bw/ day). The mean exposure estimate for AFG1 was highest in the NGS zone (337.92 ng/kg bw/day), and the exposure risk also significantly ($p < 0.05$) decreased to (58.07 ng/kg bw/day) the SS zone. AFB2 was also highest in the NGS zone (19.28 ng/kg bw/day) and significantly ($p < 0.05$) reduced to (10.95 ng/kg bw/ day) in the SGS zone. As with aflatoxin exposure, the NGS zone had the highest mean exposure estimate for AFG1, AFG2 and AFB2 than the all the zones. The table shows that consumers of Lafun in the NGS zone are exposed to high risk of AFG1, AFG2 and AFB2 contamination in their diets. AFG1 contamination had the highest mean national exposure estimate of (763.25 ng/kg bw/ day) and AFB2 exposure of (73.52 ng/kg bw/ day) was detected least in mean national exposure estimate.

In addition, the central exposure estimates of Lafun consumers to dietary AFB1 (Table 4.4) ranged between (29.78–108.56 ng/kg bodyweight/day) with the least and the maximum exposure in the NGS and SS zones respectively. The range of the estimated national liver cancer risk attributable to aflatoxin AFB1 contamination of Lafun consumed in all the AEZs in Nigeria was between (4.36 and 32.57 cases/100,000 population/year) with a maximum case of (32.57 cases/100,000 population/year) in the SS zone and was followed by the DS zone (28.30 cases/100,000 population/year), respectively. The cancer incidence attributable to dietary aflatoxins AFB1 was also maximum in the SS zone (501.08 %) and was followed by the DS zone (435.39 %), respectively, while it was minimal in the NGS zone (68.08 %). More also, the national

cancer incidence attributable to dietary aflatoxins AFB1 was (2,065.9 %). Furthermore, the range of estimated healthy life years lost due to death or disability caused by ingestion of aflatoxins AFB1 (DALY) in the contaminated Lafun samples was between 56.90 and 425.04 with maximum value of (425.04) in the SS zone and was followed by DS zone (369.32), respectively. Finally, the estimated cancer in all the AEZs cases due to aflatoxins contaminations are higher in females than in males. Based on the results of this study, about 2,313, 000 cases of HCC annually are anticipated from intake of aflatoxins in lafun in Nigeria. Intake of such doses of aflatoxin could increase still-births and neonatal mortality, immunosuppression with increased susceptibly to infectious diseases such as pneumonia, stunted growth and HIV/AIDS (Smith *et al*., 2017; McMillan *et al.*, 2018).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study found that the NGS zones of Nigeria had greater fungal counts, the presence of potentially toxic *Aspergillus* species, and aflatoxin contamination in cassava flour (lafun) than other zones. Given the negative impact of aflatoxin on agriculture and other associated industries, as well as consumer health, special interventions targeting the unique nature of Nigeria's different agroecological zones are required to supplement ongoing control measures in the country in order to effectively reduce and eventually eliminate aflatoxin occurrence on indigenous food and feed. Data from this work shows that in Nigeria, only a tragic 43.75% of cassava products examined are below the EU standard. This has high economic consequences. Because of the frequency of consumption of lafun in Nigeria, the 56.25% of cassava flour having higher limit than EU standard, is expected to have serious health hazards due to consistent moderate exposures of consumers to the deadly toxin.

5.2 Recommendations

i. In light of the high rate of unwholesome cassava flour found in this work and considering the public health and economic implications of aflatoxins, there is the need to deploy effective and cost effective mycotoxin mitigation strategies along the food value chains, not for cassava flour alone but all agricultural produce that meet domestic and market values.

ii. Public enlightenment of farmers and traders on mycotoxins, and enforcement of regulatory limits on both local and imported products in order to reduce the hazards of mycotoxins are necessary.

iii. Researches to elucidate the presence and toxicological effects of other mycotoxins that were not accessed in this study should be carried out in order to also understand their public health risks.

iv. The outcome of such investigation will surely influence establishing maximum limits that will take cognizance of co-occurrence of mycotoxins.

v. It is also appropriate to conduct longitudinal follow up studies to determine the association between dietary mycotoxin exposure and health/disease outcomes in African communities.

vi. In addition, research should be carried out to discover proper organic and environmental friendly packaging that could help in the proper storage and preservation of cassava flour and other food product susceptible to mycotoxin infestation.

5.3 Contribution of Research to Knowledge

The study determined the presence of fungi and Aflatoxins in cassava flour produced and consumed in some selected agro-ecological zones of Nigeria. A total of 108 fungal isolates belonging to four genera: Aspergillus (n = 60), Fusarium (n = 19), Penicillium (n = 18) and Mucor. Spp (n = 11) were isolated from the lafun samples. The percentage population of Aspergillus was found to be (55.56%), while that of Fusarium, Penicillium and Mucor. Spp were found to be (17.59%), (16.67%) and (10.19%) respectively.The study indicated that 57.58 % of the total cassava flour samples that tested positive to AflatoxinB1 (AFB1) exceeded the 2 µg/kg maximum regulatory limit set by European Union.The average total aflatoxin concentration (AFT) in lafun across the five agro-ecological zones is presented in decreasing order as follows: NGS zone (96.67 ± 0.06^{e} ng/g), > DS zone (44.57 ± 0.02^{d} ng/g) > SS zone (33.19 ± 0.47^{c} ng/g) > HF zone (30.60 ± 0.43^{b} ng/g) > SGS zone (20.57 ± 0.47^{a} ng/g). Aflatoxin B1 was found in Lafun

samples from all the AEZs, and the average concentration ranges from 2.08 ± 0.01^{a} ng/g in the (NGS) to 15.64 ± 0.45^{d} ng/g in the (SS) zones, while that of Aflatoxin B2 ranges from 1.57 ± 0.01^{a} ng/g in the (SGS) to 2.63 ± 0.07^{c} μg/g in the (NGS) zones.

The research has also contributed in providing data for the health implication from the estimated dietary exposure of the study sample. A total of 2,313,000 hepatocellular carcinoma (HCC) cases annually are anticipated across the country with respect to the dietary exposure of the public to the contaminated study samples across various states in Nigeria. In light of the high rate of unwholesome cassava flour found in this work and considering the public health and economic implications of aflatoxins, this work thus provides basis for further research to be carried out to elucidate the presence and toxicological effects of other mycotoxins that were not accessed in this study in order to also understand their public health risks. The outcome of such investigation will surely influence establishing maximum limits that will take cognizance of co-occurrence of mycotoxins in cassava flour.

REFERENCES

Abdallah, O., Adil, C., Hanane, C., Abdelmajid, A., & Elhabib, A.A. (2019) Optimization and validation of a liquid chromatography/tandem mass spectrometry (LC-MS/MS) method for the determination of aflatoxins in maize, Heliyon, 5, 5, e01565, 2405-8440,

Abia, W. A., Warth, B., Sulyok, M., Krska, R., Tchana, A. N., Njobeh, P. B., & Moundipa, P. F. (2013). Determination of multi-mycotoxin occurrence in cereals, nuts and their products in Cameroon by liquid chromatography tandem mass spectrometry (LC-MS/MS). *Food Control*, 31(2), 438–453.

Abt Associates Incorporated. (2013). Country assessment for aflatoxin. Retrieved December13, 2013, from http://www.aflatoxinpartnership.org/en/Research_and_Information.aspx

Adebayo-Oyetoro, A. O., Oyewole, O. B., Obadina, A. O., & Omemu, M. A. (2013). Microbiological safety assessment of fermented cassava flour "lafun" available in Ogun and Oyo states of Nigeria. *International Journal of Food Science*.

Adebayo-Tayo, B. C., Onilude, A. A., Ogunjobi,, A. A., Gbolagade, J. S., & Oladapo, M. O. (2006). Detection of fungi and aflatoxin in shelved bush mango seeds (*Irvingia* sp.) stored for sale in Uyo, Nigeria. *African Journal of Biotechnology, 5*, 1729-1732.

Adetunji, M. C., Atanda, O. O., Ezekiel, C. N., Dipeolu, A. O., Uzochukwu, S. V. A., Oyedepo, J. & Chilaka, C. A. (2019) 'Distribution of mycotoxins and risk assessment of maize consumers in five agro-ecological zones of Nigeria', *European Food Research and Technology*, 239(2), 287–296.

Adetunji, M., Atanda, O., Ezekiel, C., Sulyok, M., Warth, B., Beltran, E. & Chilaka, C. (2014). Fungal and bacterial metabolites of stored maize (Zea mays, L.) from five agro-ecological zones of Nigeria. *Mycotoxin Research*, 5(3), 8-11.

Aditya, P., Barbara, S., & Oliver, H. (2017) Crops that feed the world: Production and improvement of cassava for food, feed, and industrial uses. *Food Security 9*, 907–927.

Ahlberg, S., Grace, D., Kiarie, G., Kirino, Y., & Lindahl, J. (2018) A Risk Assessment of Aflatoxin M1 Exposure in Low and Mid-Income Dairy Consumers in Kenya, *Toxins*, 10(348), 1–15.

Alene, A.D., Abdoulaye, T., Rusike, J., Labarta, R., Creamer, B., Río, M., Ceballos, H., & Becerra, L. A. (2018). Identifying crop research priorities based on potential economic and poverty reduction impacts: the case of cassava in Africa, Asia, and Latin America. *PLoS One*, 13(8), e0201803.

Allem, A.C. (2017), "The origins and taxonomy of cassava", Chapter 1, in: Hillocks, R.J., J.M. Thresh and A.C. Bellotti (eds.), *Cassava: Biology, Production and Utilization*, CABI, Wallingford, United Kingdom, 1-16.

Amare, M. G., & Keller, N. P. (2014). Molecular mechanisms of Aspergillus flavus secondary metabolism and development. *Fungal Genetics and Biology*, *66*, 11-18.

American Cancer Society. (2011). Global Cancer Facts and Figures. (2nd edition.). Atlanta: American Cancer Society.

Asiki, G., Seeley, J., & Srey, C. (2014). A pilot study to evaluate aflatoxin exposure in a rural Ugandan population. *Tropical Medicine and International Health*, 19: 592–599.

Association of South East Asian Nations (ASEAN). (2011). Report of the Scientific Committee of the Spanish Agency for Food Safety and Nutrition in relation to the effect on the Spanish population of the derogation of national regulation on maximum allowed limits for aflatoxins B1, B2, G1 & G2 in food (1-16).

Atehnkeng, J. O. S. E. P. H., Ojiambo, P. S., Ikotun, T., Sikora, R. A., Cotty, P. J., & Bandyopadhyay, R. (2008). Evaluation of atoxigenic isolates of Aspergillus flavus as potential biocontrol agents for aflatoxin in maize. *Food additives and Contaminants*, *25*(10), 1264-1271.

Atehnkeng, J., Ojiambo, P., Donner, M., Ikotun, T., Sikora, R., Cotty, P., & Bandyopadhyay, R. (2018a). Distribution and toxigenicity of Aspergillus species isolated from maize kernels from three agroecological zones in Nigeria. *International Journal of Food Microbiology*, 122, 74-84.

Atehnkeng, J., Ojiambo, P.S., Cotty, P.J., & Bandyopadhyay. (2018b). Field efficacy of a mixture of atoxigenic Aspergillus flavus Link:Fr vegetative compatibility groups in preventing aflatoxin contamination in maize (Zea mays L.) *Biological Control*, 72:62-70.

Awoyale,W., Abass, A.B., Ndavi, M., Maziya-Dixon, B., & Sulyok, M. (2017). Assessment of the potential industrial applications of commercial dried cassava products in Nigeria. Food Measures, 11, 598–609.

Bammler, T. K., Slone, D. H., & Eaton, D. L. (2015) 'Effects of dietary oltipraz and ethoxyquin on aflatoxin B1 biotransformation non-human primates.', *Toxicological sciences, an official journal of the Society of Toxicology*, 54(1), 30–41.

Bankole, S.A., & Adebanjo, A. (2018) 'Mycotoxins in food in West Africa: current situation and possibilities of controlling it', *African Journal of Biotechnology*, 2(9), 254–263.

Bbosa, G.S., Kitya, D., Odda, J., & Ogwal-Okeng, J. (2013) Aflatoxins metabolism, effects on epigenetic mechanisms and their role in carcinogenesis. *Research article*.

Bennett, J. W., & Klich, M. (2018). Mycotoxins. *Clinical Microbiology Reviews*, 16(3), 497-516.

Bosetti, C., Turati, F., & La Vecchia, C. (2014). Hepatocellular carcinoma epidemiology. *Best Practices in Research and Clinical Gastroenterology*, 28: 753– 770.

Bowman, J., Leslie, J., Wu, F., Kablan, A., & MacCartee, J. (2012). How mycotoxins impact agriculture, nutrition and development. *USAID Bureau for Food Security*.

Braithwaite, A., Smith, F. J., & Stock, R. (2018). *Chromatographic Methods*, Chapman and Hall.

Centers for Disease Control and Prevention (CDC) (2017). *CDC - Health Studies Program: Chemical Exposures - Aflatoxin, Centers for Disease Control and Prevention*. Available at: https://www.cdc.gov/nceh/hsb/chemi cals/aflatoxin.htm (Accessed: 14 January 2019)

Chandra, P. (2021). Aflatoxins: Food Safety, Human Health Hazards and Their Prevention. *In Aflatoxins—Occurrence, Detoxification, Determination and Health Risks*; IntechOpen: London, UK.

Cheek, M., Nic Lughadha, E., Kirk, P., Lindon, H., Carretero, J., Looney, B., ... & Niskanen, T. (2020). New scientific discoveries: Plants and fungi. *Plants, People, Planet*, *2*(5), 371-388.

Cotty P. J, & Jaime, G. R. (2017). Influences of climate in aflatoxin producing fungi and aflatoxins contamination. *International Journal of Food Microbiology* 119(1-2):109-115. 2017.

Darwish, W.S., Ikenaka, Y., Nakayama, S.M.M., & Ishizuka, M. (2014) An overview on mycotoxin contamination of foods in Africa. *Journal of Veterinary and Medical Science*, 76, 789-797.

Denning, D.W., Pleuvry, A., & Cole, D.C. (2013). Global burden of allergic bronchopulmonary aspergillosis with asthma and its complication chronic pulmonary aspergillosis in adults. *Medicine and Mycology Journal*, 51, 361–370.

Denton, F.T., Azogu, I.I., & Ukoll, M.K. (2019). Cassava Based Recipes for House Hold Utilization and Income generation. AIDU, Federal Department of Agriculture, Abuja, Nigeria.

Dhanasekaran, D., Shanmugapriya, S., Thajuddin, N., & Panneerselvam, A. (2021). Aflatoxins and aflatoxicosis in human and animals. In Aflatoxins: Biochemistry and Molecular Biology; Guevara-González, R.G., Ed.; BoD–Books on Demand: London, UK; 10, 221–254

Ding, N., Xing, F., Liu, X., Selvaraj, J. N., Wang, L., Zhao, Y., & Liu, Y. (2015). Variation in the fungal microbiome (mycobiome) and aflatoxin in stored in-shell peanuts at four different areas of China. *Frontiers in Microbiology*, 6, 1055.

Dragan, R.M., Marija, Š., & Tatjana B. (2010) Real and Perceived Risks for Mycotoxin Contamination in Foods and Feeds: Challenges for Food Safety Control. *Toxins*, 2(4), 572-592

Dziedzoave, N.T., Abass, A.B., Amoa-Awua, W.K.A., & Sablah M. (2016), *Quality Management Manual for Production of High Quality Cassava Flour*. International Institute of Tropical Agriculture.

EAC Policy (2018) Prevalence and mitigation of aflatoxins. Retrieved from https://www.wageningenacademic.com/doi/abs/10.3920/WMJ2018.2362

EC (European Commission) (2002) Commission decision of 24 July 2002 establishing guidance notes supplementing Annex II to Directive 2001/18/EC of the European Parliament and of the Council on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC

Edamisan, E. (2020). Cassava Production in Nigeria: Trends, Instability and Decomposition Analysis (1970–2017). *Instability and Decomposition Analysis (1970–2017)(July 3, 2020)*.

Ediage, E. N., Hell, K., and De Saeger, S. (2014). A comprehensive study to explore differences in mycotoxin patterns from agro-ecological regions through maize, peanut, and cassava products: A case study, Cameroon. *Journal of Agricultural and Food Chemistry*, 62(20).

Ediage, E.N., Di, Mavungu, J.D., Monbaliu, S., Van Peteghem, C., & De Saeger, S. (2011). A Validated Multianalyte LC-MS/MS Method for Quantification of 25 Mycotoxins in Cassava Flour, Peanut Cake and Maize Samples. Journal of Agricultural Food Chemistry, 59, 5173–5180.

EFSA, "European Food Safety Authority". (2007). Opinion of the scientific panel on contaminants in the food chain on a request from the commission related to the potential increase of consumer health risk by an increase of the existing maximum levels for aflatoxins in almonds, hazelnuts and pistachios. *EFSA Journal*, 446(1), 127.

Eke-Okoro, O.N., & Njoku, D.N. (2019). A review of cassava development in Nigeria from 1940-2010. *Journal of Agricultural and Biological Science*, 7(1):59-65.

Eshelli, M., Harvey, L., Edrada-Ebel, R., & McNeil, B. (2015). Metabolomics of the bio-degradation process of aflatoxin B1 by actinomycetes at an initial pH of 6.0. *Toxins*, 7(2), 439–456

Essers, A.J.A. (2015). Removal of Cyanogens from Cassava Roots: Studies on Domestic Sun-drying and Solid-substrate Fermentation in Rural Africa. PhD thesis, Wageningen Agricultural University, Netherlands.

Essono, G., Ayodele, M., Akoa, A., Foko, J., Filtenbong, O., & Olembe S. (2019). Aflatoxin producing *Aspergillus* spp. and aflatoxin levels in stored cassava chips as affected by processing practices. *Food Control* 20:648-654.

European Commission Regulation. (2010). EC. NO./65/2010 of 26 February 2010 amending regulation (EC). NO.1881/2016. Setting maximum levels for certain contaminants in foodstuffs as regards aflatoxins. *Official Journal of European Union*, 150, 8-12.

Ezekiel, C.N., Fapohunda, S.O., Olorunfemi,, M.F., Oyebanji,, A.O., & Obi, I (2018). *International Food Research Journal*. 20, 111–116.

Fallah, A. A., Fazlollahi, R., & Emami, A. (2016). Seasonal study of aflatoxin M1 contamination in milk of four dairy species in Yazd, Iran. *Food Control*, *68*, 77-82.

Felicia, W., John, D., Groopman, J., & Pestka, J. (2014). Public Health Impacts of Foodborne Mycotoxins *Annual Review of Food Science and Technology 5*:1, 351-372

Food and Agriculture Organization (FAO). (2018). "Corporate Document Repository," The impact of HIV/ AIDS on the agricultural sector, 2018,

Food and Agriculture Organization Corperate Statistical Database (FAOSTAT). (2019). Food and Agriculture Data. http://www.fao.org/faostat/en/#data/.

Francis, I., Edgar, M. A., Hairong, G., Sana, Tanvir, A., Gang, W., Chenxi, Z., Oyeyemi, D., Fuguo, X., Yongquan, Z., & Yang, L. (2020) *Updates on food and feed mycotoxin contamination and safety in Africa with special reference to Nigeria*. 245-260

Giannitti, F., Diab, S.S., Pacin, A.M., Barrandeguy, M., Larrere, C., Ortega, J., & Uzal, F.A. (2019) Equine leukoencephalomalacia (ELEM) due to fumonisins B1 and B2 in Argentina. *Pesqere Veterinary Brass*, 31, 407-412.

Gimeno, A. (2002). Los Hongos y las Micotoxinas en la Alimentación Animal; Conceptos, Problemas, Control Y Recomendaciones. Retrieved July 14, 2019, from http://www.engormix.com/MA-micotoxinas/articulos/criterios-calidad-micologica-t353/p0.htm.

Gong, Y. Y., Watson, S., & Routledge, M. N. (2016) 'Aflatoxin Exposure and Associated Human Health Effects, a Review of Epidemiological Studies', Food Safety. *Food Safety Commission, Cabinet Office, Government of Japan*, 4(1), 14–27.

Gong, Y.Y., Wilson, S., & Mwatha, J.K. (2019). Aflatoxin exposure may contribute to chronic hepatomegaly in Kenyan school children. *Environmental Health Perspective*, 120: 893–896.

Groopman, J.D., Egner, P.A., & Schulze, K.J. (2014). Aflatoxin exposure during the first 1000 days of life in rural South Asia assessed by aflatoxin B1-lysine albumin biomarkers. *Food Chemicals and Toxicology*, **74**: 184–189

Gruber-Dorninger, C., Novak, B., Nagl, V., & Berthiller, F. (2016). Emerging Mycotoxins: Beyond Traditionally Determined Food Contaminants. *Journal of Agriculture and Food Chemistry*.

Guengerich, F. P., Johnson, W. W., Shimada, T., Ueng, Y.F., Yamazaki, H., & Langou¨et, S. (2018). "Activation and detoxication of aflatoxin B1," *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 402(1-2), 121–128.

Gurav, N.P., & Medhe, S. (2018). 'Analysis of Aflatoxins B1, B2, G1 and G2 in Peanuts: Validation Study', *Analytical Chemistry: An Indian Journal*, 17(2), 126.

Han, C., Yu, T., Qin, W,, Liao, X., Huang, J., Liu, Z., Yu, L., Liu, X., Chen, Z., Yang, C., Wang, X., Mo, S., Zhu, G., Su, H., Li, J., Qin, X., Gui, Y., Mo, Z., Li, L., & Peng, T. (2020) Genome-wide association study of the TP53 R249S mutation in hepatocellular carcinoma with aflatoxin B1 exposure and infection with hepatitis B virus. Journal of *Gastrointestinal Oncology*. Dec;11(6):1333-1349.

Hawkins, L. K., Windham, G. L., & Williams, W. P. (2020). Effect of different postharvest drying temperatures on Aspergillus flavus survival and aflatoxin content in five maize hybrids. *Journal of Food Protection*, 68(7), 1521–1524.

Herzallah, S. M. (2019). "Determination of aflatoxins in eggs, milk, meat and meat products using HPLC fluorescent and UV detectors," *Food Chemistry*, 114(3), 1141–1146.

Ibeh, I.N., Uraih, N., & Ogonor J.I. (1991). *International Journal of Food Microbiology,* 1991, 14, 2, 171-4.

Igetei, R., Otegbayo, J.A., Ndububa, D.A., Lesi, O.A., Anumudu, C.I., Hainaut, P. & Gormally, E. (2018). Detection of p53 codon 249 mutation in Nigerian patients with hepatocellular carcinoma using novel evaluation of cellfree DNA. *Annual Hepatology*, 7(4):339- 344.

Ismaiel, A.A., & Papenbrock, J. (2015) Mycotoxins: producing fungi and mechanisms of phytotoxicity. *Agriculture,* 5, 492-537.

James, H.C. (2018). Cassava: New Potential for a Neglected Crop. International Agricultural Development Service (lADS) Development-Oriented Literature Series. Westview Press / Boulder and London.

Jekayinfa, S., & Olajide, J. (2017). Analysis of energy usage in the production of three selected cassava-based foods in Nigeria. *Journal of Food Engineering*, 82: 217–226.

Jeyaramraja, P. R., Meenakshi, S. N., & Woldesenbet, F. (2018). Relationship between drought and preharvest aflatoxin contamination in groundnut (Arachis hypogaea L.). *World Mycotoxin Journal*, 11(2), 187–199

Jimoh, K.O., & Kolapo. A.L. (2018). "Mycoflora and aflatoxin production in market samples of some selected Nigerian foodstuffs." *Research Journal of Microbiology*, 3: 169-174.

Johnson, W. W., Yamazaki, H., Shimada, T., Ueng, Y.F, & Guengerich, F. P. (2017). "Aflatoxin B1 8,9-epoxide hydrolysis in the presence of rat and human epoxide hydrolase," *Chemical Research in Toxicology*, 10(6,), 672–676.

José F. H., Natalia A. M., Dominik, H., Francisco, G. C., Laura, G., Ana, M., García-Campaña.(2018) Simple determination of aflatoxins in rice by ultra-high performance liquid chromatography coupled to chemical post-column derivatization and fluorescence detection. *Food Chemistry, 245*, 189-195.

Juan, C., Ritieni, A., & Mañes, J. (2017). Determination of trichothecenes and zearalenones in grain cereal, flour and bread by liquid chromatography tandem mass spectrometry. *Food Chemistry*, 134, 2389–2397.

Kaaya, A.N., & Eboku, D. (2016). Mould and aflatoxin contamination of dried cassava chips in eastern Uganda: association with traditional processing and storage practice. *Journal of Biological Sciences*, 10(8):718-729.

Kamaljit, K., & Preeti, A. (2017). Cassava as Potential Crop for the Food and Fermentation Industry: A review. *International Journal of Food Fermentation Technology*, 7(1): 1-12.

Karki, T., & Sinha, B. (2019). Mycotoxin contamination of foods and feeds in Nepal 2019. (eds) Semple RL, Frio AS. Hicks PA, Lozare JV. *Mycotoxin prevention and control in food grains*. Section 4.

Khlangwiset, P. (2021). Reducing the Risks of Aflatoxin through Public Health Interventions. Health, Graduate School of Public Health, University of Pittsburgh. PhD Dissertation.

Kimanya, M.E., Routledge, M.N., Mpolya, E., Ezekiel, C.N., Shirima, C.P., & Gong, Y.Y. (2021). Estimating the risk of aflatoxin-induced liver cancer in Tanzania based on biomarker data. *PLoS ONE*, 16, e0247281.

Kinyungu, S. W. (2019). Efficacy of pre-harvest Aspergillus flavus biocontrol treatment on reducing aflatoxin accumulation during drying. Doctoral dissertation, Purdue University Graduate School.

Kitya, D., Bbosa, G. S., & Mulogo, E. (2010). Aflatoxin levels in common foods of South Western Uganda: a risk factor to hepatocellular carcinoma. *European Journal of Cancer Care*, 10.1111/j.1365-2354..01087.x , 1-6.

Kok,W.T.,Van Neer, T. C.H. Traag,W.A., & Tuinstra, L. G. T. (2016). "Determination of aflatoxins in cattle feed by liquid chromatography and post-column derivatization with electrochemically generated bromine," *Journal of Chromatography A*, 367; 231–236, 2016.

Kumar, P., Mahato, D.K., Kamle, M., Mohanta, T.K., & Kang, S.G. (2017). Aflatoxins: A global concern for food safety, human health and their management. *Frontiers in Microbiology*, 7, 2170

Lamplugh, S. M., Hendrickse, R. G., Apeagyei, F., & Mwanmut, D. D. (2018) 'Aflatoxins in breast milk, neonatal cord blood, and serum of pregnant women.', *British medical journal (Clinical research ed.)*, 296(6627), 968.

Latif, S., & Muller, J. (2015). Potential of cassava leaves in human nutrition: A review. *Trends in Food Science & Technology*, *44*(2), 147-158.

Laura. A., Cristina, G., Gianfranco, G., Flavia, B., Cinzia, P., and Claudio, B. (2012) A Lateral Flow Immunoassay for the Rapid Detection of Ochratoxin A in Wine and Grape Must . *Agric. Food Chem.*, 60, 46, 11491–11497

Leger, R. J. S., Screen, S. E., & Shams-Pirzadeh, B. (2000). Lack of host specialization in Aspergillus flavus. *Applied and Environmental Microbiology*, 66(1), 320–324.

Leong, Y.H., Rosma, A., Latiff, A.A., & Izzah, A.N. (2022). Associations of serum aflatoxin B1-lysine adduct level with socio-demographic factors and aflatoxins intake from nuts and related nut products in Malaysia. *International Journal of Hygiene and Environmental Health*, 215: 368–372.

Levy, D. D., Groopman, J. D., Lim, S. E., Seidman, M. M. , & Kraemer, K. H. (2012). "Sequence specificity of aflatoxin B1-induced mutations in a plasmid replicated in xeroderma pigmentosum and DNA repair proficient human cells," *Cancer Research*, 52(20), 5668–5673.

Li, S., Cui, Y., Zhou, Y., Luo, Z., Liu, J., & Zhao, M. (2017). The industrial applications of cassava: current status, opportunities and prospects. *Journal of the Science of Food and Agriculture*, 97(8), 2282-2290.

Liu, J., Sun, L., Zhang, N., Zhang, J., Guo, J., Li, C., & Qi, D. (2016). Effects of nutrients in substrates of different grains on aflatoxin B1 production by Aspergillus flavus. *BioMed Research International*.

Liu, Y. & Wu, F. (2019) 'Global Burden of Aflatoxin-Induced Hepatocellular Carcinoma: A Risk Assessment', Environmental health perspectives. National Institute of Environmental *Health Science*, 118(6), 818–824.

Liu, Y., Chang, C.C., Marsh, G.M. & Wu, F. (2017). Population attributable risk of aflatoxin-related liver cancer: systematic review and metaanalysis. *European Journal of Cancer*, 48: 2125–2136.

Lizárraga-Paulín, E. G., Moreno-Martínez, E., & Miranda-Castro, S. P. (2022) 'Aflatoxins and their Impact on Human and Animal Health: An Emerging Problem', in Aflatoxins – Biochemistry and Molecular Biology. *InTech*, 255–282.

Llewellyn, G., McCay, J., Brown, R., Musgrove, D., Butterworth, L., Munson, A.E., & White, K.L., Jr. (1998) Immunological evaluation of the mycotoxin patulin in female B6C3F 1 mice. *Food Chemicals and Toxicology*, 36, 1107-1115.

Lulamba, E. T. (2016). Filter aids effectiveness on ochratoxin a removal during beer filtration (Doctoral dissertation, University of Johannesburg).

Ma, X., Wang, W., Chen, X., Xia, Y., Wu, S., Duan, N., & Wang, Z. (2014). Selection, identification, and application of Aflatoxin B1 aptamer. *European Food Research and Technology*, 238(6), 919-925.

Mahbobinejhad, Z., Aminian, H., Ebrahimi, L., & Vahdati, K. (2019). Reduction of aflatoxin production by exposing Aspergillus flavus to CO_2. *Journal of Crop Protection*, 8(4), 441–448.

Makau, C. M., Matofari, J. W., Muliro, P. S., & Bebe, B. O. (2016). Aflatoxin B1 and Deoxynivalenol contamination of dairy feeds and presence of Aflatoxin M1 contamination in milk from smallholder dairy systems in Nakuru, Kenya. *International journal of food contamination*, *3*(1), 1-10.

Makun, H. A., Adeniran, A. L., Mailafiya, C. S., Ayanda, S. I., Mudashiru, T. A., Ojukwu, J. U., and Salihu, A. D. (2013). Natural occurrence of ochratoxin A in some marketed Nigerian foods. *Food Control*, 31, 566e571.

Makun, H.A., Dutton, M.F., Njobeh, P.B., Mwanza, M., & Kabiru, A.Y. (2011) Natural multi-occurrence of mycotoxins in rice from Niger State, Nigeria. *Mycotoxin Research*, 27, 97- 104.

Manjula, K., Hell, K., Fandohan, P., Abass, A., and Bandyopadhyay, R. (2009). Aflatoxin and fumonisin contamination of cassava products and maize grain from markets in Tanzania and Republic of the Congo. *Toxin Reviews*, 28(2e3), 63e69.

Manjunath, K., & Mohana, D. C. (2018). Assessment of contamination levels and characterization of Aflatoxin B1 producing Aspergillusflavus strain from food and feed samples from local markets of South India. *International Journal of Life Sciences*, 6(1), 326–334.

Mannaa, M., & Kim, K. D. (2017). Influence of temperature and water activity on deleterious fungi and mycotoxin production during grain storage. *Mycobiology*, 45(4), 240–254.

Marroquín-Cardona, A. G., Johnson, N. M., Phillips, T. D., & Hayes, A. W. (2014). Mycotoxins in a changing global environment–a review. *Food and Chemical Toxicology*, 69, 220–230.

Mateo, E. M., Gómez, J. V., Gimeno-Adelantado, J. V., Romera, D., Mateo-Castro, R., & Jiménez, M. (2017). Assessment of azole fungicides as a tool to control growth of Aspergillus flavus and aflatoxin B1 and B2 production in maize. *Food Additives & Contaminants: Part A*, 34(6), 1039–1051

McCullough, A. K., & Lloyd, R. S. (2019) 'Mechanisms Underlying Aflatoxin associated Mutagenesis – Implications in Carcinogenesis', *DNA Repair*, 77, 76–86.

McMillan, A., Renaud, J. B., Burgess, K. M. N., Orimadegun, A. E., Akinyinka, O. O., Allen, S. J., Miller, J. D., Reid, G., & Sumarah, M. W. (2018) 'Aflatoxin exposure in Nigerian children with severe acute malnutrition', *Food and Chemical Toxicology, Pergamon*, 111, 356–362.

Ministry of Food and Agriculture (2015). *Cassava development in Ghana*. A review of cassava in Africa with country case studies on Nigeria, Ghana, the United Republic of Tanzania, Uganda and Benin. FAO, Rome. *Rome*, 104. License: CC BY-NC-SA 3.0 IGO.

Misihairabgwi J. M., Ezekiel C. N., Sulyok M., Shephard G. S., & Krska R. (2019) Mycotoxin contamination of foods in Southern Africa: A 10-year review (2007–2016), *Critical Reviews in Food Science and Nutrition, 59:1, 43-58*,

Mtunguja, M. K., Beckles, D. M., Laswai, H. S., Ndunguru, J. C., & Sinha, N. J. (2019). Opportunities to commercialize cassava production for poverty alleviation and improved food security in Tanzania. African Journal of Food, Agriculture, Nutrition and Development, 19(1), 13928-13946.

Mupunga, I., Mngqawa, P., & Katerere, D. R. (2017) 'Peanuts, aflatoxins and undernutrition in children in Sub- Saharan Africa', Nutrients. *Multidisciplinary Digital Publishing Institute (MDPI)*, 9(12), 1–12.

Murugavel, K.G., Naranatt, P.P, Shankar, E.M., Mathews, S., Raghuram, K., & Rajasambandam, P. (2017). Prevalence of aflatoxin B1 in liver biopsies of proven hepatocellular carcinoma in India determined by an in-house immunoperoxidase test. *Journal of Medical Microbiology*, 56(11): 1455-1459.

Muthomi, J.W., Mureithi, B.K., Chemining'wa, G.N., Gathumbi, J.K., & Mutit, E.W. (2017). *Aspergillus* species and aflatoxin B1 in soil, maize grain and flour samples from semi-arid and humid regions of Kenya. *International Journal of Agricultural Science* 2(1):22-34.

Niculita-Hirzel, H., Hantier, G., Storti, F., Plateel, G., & Roger, T. (2016) Frequent occupational exposure to Fusarium mycotoxins of workers in the swiss grain industry. *Toxins*, 8, 370.

Nwabueze, T.U., & Anoruoh, G.A. (2019). Evaluation of flour and extruded noodles from eight cassava mosaic disease (CMD)-resistant varieties. *Food Bioprocessing Technology*, 4: 80–91.

Nweke, F. U. (2015). Some fungal pathogens of yam (Dioscorea Spp.) in storage and the effects of their infection on the nutrient composition. *Journal of Biology, Agriculture and Healthcare,* 5(12), 153e157.

Nyaka, N. A. I. V., Kammegne, D. P., Ntsomboh, N. G., Mbenoun, M., Zok, S., and Fontem, D. (2015). Isolation and identification of some pathogenic fungi associated with cassava (Manihot esculenta Crantz) root rot disease in Cameroon. *African Journal of Agricultural Research,* 10(50), 4538e4542.

Nyirenda, D.B.; Chiwona-Karltun, L.; Chitundu, M.; Haggblade, S. & Brimer, L. (2021). Chemical safety of cassava products in regions adopting cassava production and processing—Experience from Southern Africa. *Food Chemistry and Toxicology*, 49, 607–612.

Odintsoya I.N., Pisareva L.F., & Khryapenkov, A.V. (2015) WORLDWIDE CANCER EPIDEMIOLOGY. *Siberian journal of oncology*.1(5):95-101.

Ogunnike, A.M., Adepoju, P.A., Longe, A.O., Elemo, G.N., & Oke, O.V. (2015). Effects of submerged and anaerobic fermentation on cassava flour (lafun). *African Journal of Biotechnology*, 14, 961–970.

Okunlola, O.A., & Folorunso K. (2015). Modelling Rainfall Series in the Geo-Political Zones of Nigeria. *Journal of Environment and Health Science* 5(2)5.

Olayinka, C. (2018) 'Nigeria loses 5, 000 lives, $ 155m yearly to afatoxin , says PACA', *The Guardian Nigeria*, 3 January, web page.

Oranusi, S. U., & Olarewaju, S. A. (2013). Mycoflora and Aflatoxin contamination of some foodstuffs. International Journal of Biotechnology and Allied Fields. *International Journal of Biotechnology and Allied Fields*, 1(1), 9-18.

Oresegun A., Fagbenro O. A., Ilona P., & Edah B (2016) Nutritional and anti-nutritional composition of cassava leaf protein concentrate from six cassava varieties for use in aqua feed, *Cogent Food & Agriculture, 2*:1,

Otegbayo, B.O., Samuel, F.O., & Alalade, T. (2013). Functional properties of soy-enriched tapioca. *African Journal of Biotechnology*, 12, 3583–3589.

Oyelami, O. A., Maxwell, S. M., Adelusola, K. A., Aladekoma, T. A., & Oyelese, A. O. (2017) 'Aflatoxins in the lungs of children with kwashiorkor and children with

miscellaneous diseases in Nigeria', *Journal of Toxicology and Environmental Health*, 51(6), 623–628.

Papadopoulou-Bouraoui, A., Stroka, J., & Anklam, E. (2002). "Comparison of two post-column derivatization systems, ultraviolet irradiation and electrochemical determination, for the liquid chromatographic determination of aflatoxins in food," *Journal of AOAC International*, 85(2), 411–416.

Parmar, A., Sturm, B., & Hensel, O. (2019) Crops that feed the world: Production and improvement of cassava for food, feed, and industrial uses. *Food Security. 9*, 907–927. https://doi.org/10.1007/s12571-017-0717-8

Perrone, G., Haidukowski, M., Stea, G., Epifani, F., Bandyopadhay, R., Leslie, J.F., & Logrieco A. (2014). Population structure and aflatoxin production by Aspergillus Sect. Flavi from maize in Nigeria and Ghana. *Food Microbiology* 41:52-59.

Piekkola, S., Turner, P.C., & Abdel-Hamid, M. (2017). Characterisation of aflatoxin and deoxynivalenol exposure among pregnant Egyptian women. *Food Additives and Contamination Part A, & Chemical Analytics and Control Expository Risk Assessment*, 29: 962–971.

Pleadin, J., Vulic, A., Persi, N., Skrivanko, M., Capek, B., & Cvetnic Z. (2019). Aflatoxin B1 occurrence in maize sampled from Croatian farms and feed factories during 2013. *Food Control* 40:286-291.

Qureshi, H., Hamid, S. S., Ali, S. S., Anwar, J., Siddiqui, A. A., & Khan, N. A. (2015). Cytotoxic effects of aflatoxin B1 on human brain microvascular endothelial cells of the blood-brain barrier. *Medical Mycology*, 53(4), 409–416.

Rahmani, A., Jinap, S., & Soleimany, F. (2019). "Qualitative and quantitative analysis of mycotoxins," *Comprehensive Reviews in Food Science and Food Safety*, 8(3), 202–251.

Rajarajan, P.N., Rajasekaran, K.M., & Devi, N.K. (2013). Aflatoxin contamination in agricultural commodities. *Indian Journal of Pharmaceutical and Biological Research*, 1(4), 148–151.

Ravindra, V. (2019). Cassava leaves as animal feed: potential and limitations. *Journal of Science, Food and Agriculture*, 61: 141–150.

Reverberi, M. (2019). Lipids in Aspergillus flavus-maize interaction. *Frontiers in Microbiology*, 5, 74

Rheeder, J. P., Van der W, L., Imrie, G., & Shephard, G. S. (2016). Fusarium species and fumonisins in subsistence maize in the former Transkei region, South Africa: a multi-year study in rural villages. *Food Additives & Contaminants: Part B*, *9*(3), 176-184.

Rodrigues, I., Handl, J., & Binder, E.M. (2011) Mycotoxin occurrence in commodities, feeds and feed ingredients sourced in the Middle East and Africa. *Food Additives and Contamination Part B, Surveilliance*, 4, 168-179.

Romagnoli, B., Meena, V., Gruppioni, N., & Bergamini, C. (2007). Aflatoxins in spices, aromatic herbs, herb teas and medicinal plants marketed in Italy. *Food Control, 18*, 697-701.

Root and Tuber Improvement Programme RTIP (2019), *Cassava processing in Ghana. An Information guide.* Ministry of Food and Agriculture, Ghana.

Roy, M., Harris, J., & Afreen, S. (2013). Aflatoxin contamination in food commodities in Bangladesh. *Food Additives and Contamination Part B*, 6: 17–23.

Rushing, B.R., & Selim, M.I. (2019). Aflatoxin B1: A review on metabolism, toxicity, occurrence in food, occupational exposure, and detoxification methods. *Food and Chemical Toxicology*, 124, 81–100.

Russell, R., & Peterson, M. (2007). Aflatoxin contamination in chilli from Pakistan. *Food Control, 18*, 817-820.

Sangare-Tigori, B., Moukha, S., Kouadio, H.J., Betbeder, A.M., Dano, D.S., & Creppy, E.E. (2016). Co-occurrence of aflatoxin B1, fumonisin B1, ochratoxin A and zearalenone in cereals and peanuts from Cote d'Ivoire. *Food Additives and Contamination*, 23, 1000-1007.

Sapsford, K. E., Taitt, C.R., & Fertig, S. (2016). "Indirect competitive immunoassay for detection of aflatoxin B1 in corn and nut products using the array biosensor," *Biosensors and Bioelectronics*, 21(12), 2298–2305.

Sarma, U.P., Bhetaria, P.J., & Devi, P. (2017) Aflatoxins: Implications on Health. *Indian Journal Clinical Biochemistry* 32, 124–133. https://doi.org/10.1007/s12291-017-0649-2

Schleicher, R.L., McCoy, L.F., Powers, C.D., Sternberg, M.R., Pfeiffer, C.M. (2018). Serum concentrations of an aflatoxin-albumin adduct in the National Health and Nutrition Examination Survey (NHANES) 1999-2000. *Clinica Chimica Acta*, 423: 46–50.

Shephard, G. S. (2003). Aflatoxin and food safety: recent African perspectives. *Journal of Toxicology: Toxin Reviews*, *22*(2-3), 267-286.

Shirima, C.P., Kimanya, M.E., & Kinabo, J.L. (2018). Dietary exposure to aflatoxin and fumonisin among Tanzanian children as determined using biomarkers of exposure. *Molecular Nutrition in Food Research*, 57: 1874–1881

Shittu, T.A., Dixon, A., Awonorin, S.O., Sanni, L.O., & Maziya-Dixon, B. (2018). Bread from composite cassava– wheat flour. II: Effect of cassava genotype and nitrogen fertilizer on bread quality. *Food Research International*, 41: 569-578.

Siciliano, I., Berta, F., Bosio, P., Gullino, M. L., & Garibaldi, A. (2017). Effect of different temperatures and CO2 levels on Alternaria toxins produced on the cultivated rocket, cabbage, and cauliflower. *World Mycotoxin Journal*, 10(1), 63–71.

Sirma, A.D., Lindahl, J.F., Makita, K., Senerwa, D., Mtimet, N., Kang'ethe, E.K., & Grace, D. (2018). The impacts of aflatoxin standards on health and nutrition in

sub-Saharan Africa: The case of Kenya, *Global Food Security, 18*, 57-61, 2211-9124,

Smith, L. E., Prendergast, A. J., Turner P. C., Humphret, J. H., & Stoltzfuz, R. J. (2017). Aflatoxin Exposure during Pregnancy, Maternal Anemia, and Adverse Birth Outcomes. *The American Journal of Tropical Medicine and Hygiene*, 96(4): 770-776.

Sneh S. B, Nitya S, Manoj K, Fatih O, Sukhvinder S. P., & Monica T. (2021) Recent developments in applications of lactic acid bacteria against mycotoxin production and fungal contamination, *Food Bioscience, 44*, 101444, 2212-4292,

Stephanie, L. C., Jacob, P. W., Justin, B. R., Amy, M., Stephen, R. J. D. M, Gregor R., & Mark W. S, (2021) Improved methods for biomarker analysis of the big five mycotoxins enables reliable exposure characterization in a population of childbearing age women in Rwanda, *Food and Chemical Toxicology, 147*, 111854, 0278-6915,

Streit, E., Schatzmayr, G., Tassis, P., Tzika, E., Marin, D., Taranu, I., Tabuc, C., Nicolau, A., Aprodu, I., Puel, O., & Oswald, I.P. (2017) Current situation of mycotoxin contamination and co-occurrence in animal feed-focus on Europe. *Toxins*, 4, 788-809.

Sulyok, M., Beed, F., Boni, S., Abass, A., Mukunzi, A., & Krska R. (2017) Quantitation of multiple mycotoxins and cyanogenic glucosides in cassava samples from Tanzania and Rwanda by an LC-MS/MSbased multi-toxin method. *Food Additives and Contamination part* A, *Chemical Analytics Control and Expository Risk Assessment*, 32(4): 488-502.

Sulyok, M.; Beed, F.; Boni, S.; Abass, A.; Mukunzi, A. & Krska, R. (2015). Quantitation of multiple mycotoxins and cyanogenic glucosides in cassava samples from Tanzania and Rwanda by an LC-MS/MS-based multi-toxin method. *Food Additives and Contaminations, Part A*, 32, 488–502.

Thomas, A. E. (2018). Toxicity of aflatoxins from selected consumables in Lagos (Nigeria). *Electronic Journal of Environmental, Agricultural and Food Chemistry*, 4(6), 1111-1116.

Turner, P. C., & Snyder, J. A. (2021). Development and limitations of exposure biomarkers to dietary contaminants mycotoxins. Toxins, 13(5), 314.

Udoh, J. M., Cardwell, K. F., & Ikotun, T. (2000). Storage structures and aflatoxin content of maize in five agroecological zones of Nigeria. *Journal of Stored Products Research*, *36*(2), 187-201.

UNICEF/WHO/World Bank (2019). Levels and trends in child malnutrition. *UNICEF/WHO/World Bank Group Joint Child Malnutrition Estimates*.

United Nations Aids (UNAIDS) (2017) *Nigeria | UNAIDS*. Available at: http://www.unaids.org/en/regionscoun tries/countries/nigeria (Accessed: 23 April 2019).

United Nations Aids (UNAIDS) (2019) New survey results indicate that Nigeria has an HIV prevalence of 1.4% - Nigeria|ReliefWeb. Available at: https://reliefweb.int/report/nigeria/n ew-survey-results-indicate-nigeria-hashiv-prevalence-14 (Accessed: 23 April 2019).

Van Oirschot, Q.E.A., O'Brian, G.M., Dufour, D., El-Sharkawy, M.A., & Mesa, E. (2020). The effect of pre-harvest pruning of cassava upon root deterioration and quality characteristics. *Journal of Science, Food and Agriculture*, 80: 1866–1873.

Vanderzannt, C., & Splittstoesser, D. (1997). Compedium of methods for the microbiological examination of foods. (p. 596). Washington D.C.: 3rd ed. American public health association.

Vondracek, M. Xi, Z., & Larsson, P. (2021). "Cytochrome P450 expression and related metabolism in human buccal mucosa," *Carcinogenesis*, 22(3), 481–488.

Wang, C., Sun, L., & Zhao, Q. (2019). A simple aptamer molecular beacon assay for rapid detection of aflatoxin B1. *Chinese Chemical Letters*, 30(5), 1017–1020.

Watson, S. (2018). 'Impaired growth in rural Gambian infants exposed to aflatoxin: a prospective cohort study', BMC Public Health. BioMed Central, 18(1), 1247.

Wen, J., Kong, W., Hu, Y., Wang, J., & Yang, M. (2014) Multi-mycotoxins analysis in ginger and related products by UHPLC-FLR detection and LC-MS/MS confirmation. *Food Control*, 43, 82-87.

Westby, A. (2012) 'Cassava utilisation, storage and small-scale processing', in Hillocks, R., Tresh, J. and Bellotti, A. (Eds.): *Cassava: Biology, Production and Utilization*, 281–300, CABI Publishing, Wallingford, UK.

Wild, C. P., & Turner, P. C. (2017). "The toxicology of aflatoxins as a basis for public health decisions," *Mutagenesis*, 17(6), 471–481.

World Health Organization & Food and Agriculture Organization of the United Nations. (2018). FAO/WHO Guide for Application of Risk Analysis Principles and Procedures during Food Safety Emergencies, Food and Agriculture Organization of the United Nations, Rome.

World Health Organization (WHO). (2004). The global burden of disease.

Wu, H.C. (2019). Aflatoxin B1 exposure, hepatitis B virus infection, and hepatocellular carcinoma in Taiwan. *Cancer Epidemic and Biomedical Previews*, 18: 846–853.

Xianjiang, L., Wen M., Zhiyong, M., Qinghe, Z., & Hongmei, L. (2021). The Occurrence and Contamination Level of Ochratoxin A in Plant and Animal-Derived Food Commodities. *Molecules 2021*, 26(22), 6928.

Yoshinari, T., Noda, Y., Yoda, K., Sezaki, H., Nagasawa, H., & Sakuda, S. (2019). Inhibitory activity of blasticidin a, a strong aflatoxin production inhibitor, on protein synthesis of yeast: Selective inhibition of aflatoxin production by protein synthesis inhibitors. *The Journal of Antibiotics*, 63(6), 309–314.

Yu, J., Chang, P.K., Ehrlich, K.C., Cary, J.W., Bhatnagar, D., Cleveland, T.E., Payne, G.A., Linz, J.E., Woloshuk, C.P., & Bennett, J.W. (2019). Clustered pathway genes in aflatoxin biosynthesis. *Applied and Environmental Microbiology*, 70: 1253-1262.

Zain, M. E. (2011). Impact of mycotoxins on humans and animals. *Journal of Saudi chemical society*, *15*(2), 129-144.

Zhang, J., Zheng, N., Liu, F., Li, S., Li., & Wang, J. I. (2015). Aflatoxin B1 and aflatoxin M1 induced cytotoxicity and DNA damage in differentiated and undifferentiated Caco-2 cells. *Food and Chemical Toxicology*, 83, 54–60.

Zinedine, A., Brera, C., Elakhdari, C., Catano, C., Debegnachi, F., Angelini, S., & Miraglia, M. (2016). Natural occurrence of mycotoxins in cereals and spices commercialized in Morocco. *Food Control*, *17*, 868-874.

APPENDIX

HPLC Chromatographs

Clarity VA - Chromatography SW
DataApex
www.dataapex.com

Chromatogram Info:

File Name	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Data\4L_24_03_2021 6_31_16 PM_0801.prm	File Created	: 24/03/2021 6:31:16 PM
Origin	: Acquired, Acquisition started 24/03/2021 6:19:15 PM	Acquired Date	: 24/03/2021 6:31:16 PM
Project	: MYCOTOXIN	By	: Toyin

Printed Version Info:

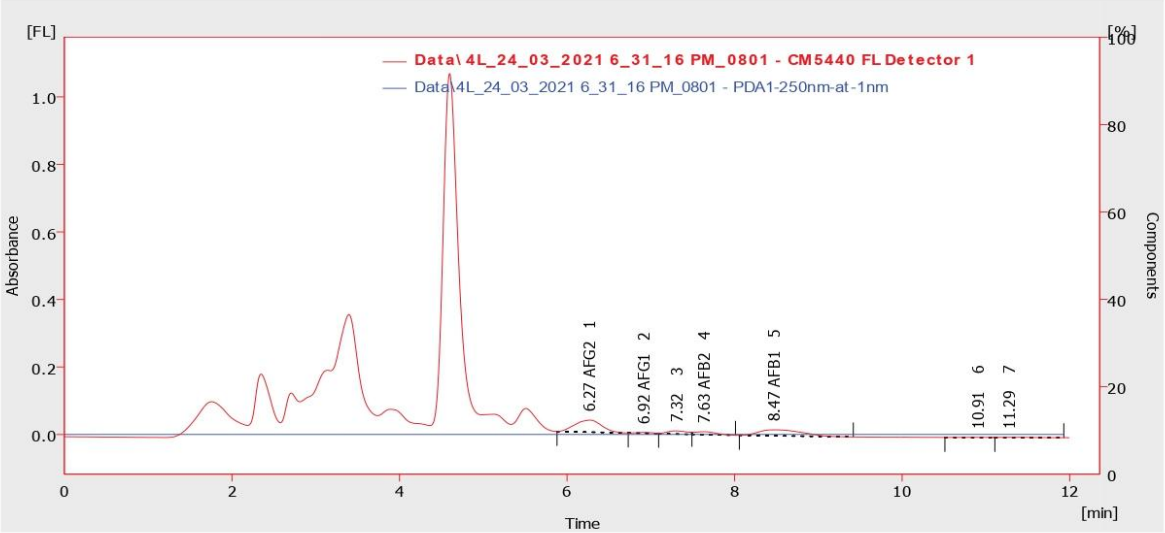
Printed Version	: 20/09/2021 5:02:28 PM, IA: 8.0 Rev.0 Recent (Linked Calibration)	Printed Date	: 05/10/2021 11:55:28 AM
Report Style	: C:\ClarityVA\DataFiles\WORK1\Data\Common\Chromatogram.sty	By	: Toyin
Calibration File	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Calib\2020 TOTAL AFLATOXINS\2020 DECEMBER\2021 TOTAL AFLATOXINS\2021 SEPTEMBER\200921.cal		

Analysis User Variables:

AnalysisUserVar1	: 0
AnalysisUserVar2	: 0
AnalysisUserVar3	: 0

Method User Variables:

MethodUserVar1	: 0
MethodUserVar2	: 0
MethodUserVar3	: 0



Result Table (ESTD - Data\4L_24_03_2021 6_31_16 PM_0801 - CM5440 FL Detector 1)

	Reten. Time [min]	Area [mFL.s]	Response	Amount [ng/g]	Amount% [%]	Compound Name
1	6.270	792.122	792.122	15.536	54.0	AFG2
2	6.923	37.888	37.888	1.737	6.0	AFG1
4	7.633	148.968	148.968	0.718	2.5	AFB2
5	8.473	638.248	638.248	10.792	37.5	AFB1
	Total	1617.226		28.783	100.0	

Clarity VA - Chromatography SW
DataApex
www.dataapex.com

Chromatogram Info:

File Name	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Data\4L_24_03_2021 6_45_43 PM_0812.prm	File Created	: 24/03/2021 6:45:43 PM
Origin	: Acquired, Acquisition started 24/03/2021 6:33:42 PM	Acquired Date	: 24/03/2021 6:45:43 PM
Project	: MYCOTOXIN	By	: Toyin

Printed Version Info:

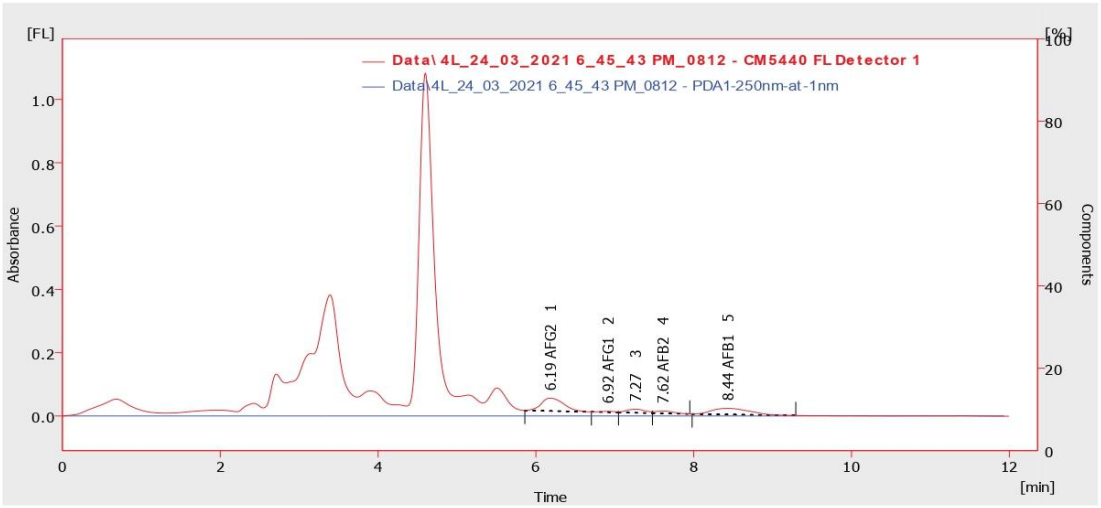
Printed Version	: 20/09/2021 5:02:55 PM, IA: 8.0 Rev.0 Recent (Linked Calibration)	Printed Date	: 05/10/2021 11:56:10 AM
Report Style	: C:\ClarityVA\DataFiles\WORK1\Data\Common\Chromatogram.sty	By	: Toyin
Calibration File	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Calib\2020 TOTAL AFLATOXINS\2020 DECEMBER\2021 TOTAL AFLATOXINS\2021 SEPTEMBER\200921.cal		

Analysis User Variables:

AnalysisUserVar1	: 0
AnalysisUserVar2	: 0
AnalysisUserVar3	: 0

Method User Variables:

MethodUserVar1	: 0
MethodUserVar2	: 0
MethodUserVar3	: 0



Result Table (ESTD - Data\4L_24_03_2021 6_45_43 PM_0812 - CM5440 FL Detector 1)

	Reten. Time [min]	Area [mFL.s]	Response	Amount [ng/g]	Amount% [%]	Compound Name
1	6.187	801.974	801.974	15.730	54.8	AFG2
2	6.917	35.236	35.236	1.616	5.6	AFG1
4	7.617	119.413	119.413	0.576	2.0	AFB2
5	8.437	637.170	637.170	10.773	37.5	AFB1
	Total	1593.793		28.694	100.0	

Clarity VA - Chromatography SW
DataApex
www.dataapex.com

Chromatogram Info:

File Name	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Data\4L_24_03_2021 7_00_09 PM_0823.prm	File Created	: 24/03/2021 7:00:09 PM
Origin	: Acquired, Acquisition started 24/03/2021 6:48:08 PM	Acquired Date	: 24/03/2021 7:00:09 PM
Project	: MYCOTOXIN	By	: Toyin

Printed Version Info:

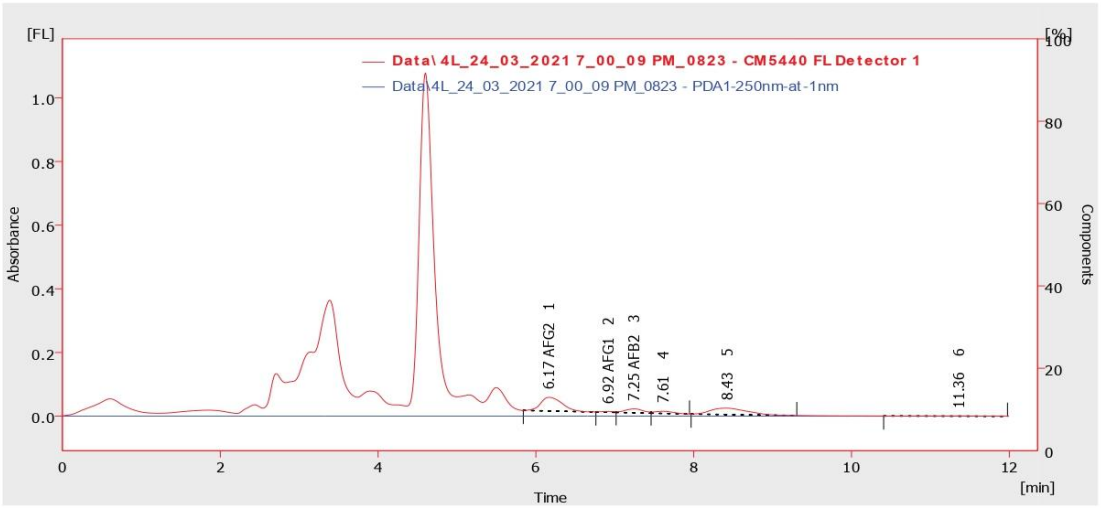
Printed Version	: 20/09/2021 7:43:31 AM, IA: 8.0 Rev.0 Recent (Linked Calibration)	Printed Date	: 05/10/2021 11:56:43 AM
Report Style	: C:\ClarityVA\DataFiles\WORK1\Data\Common\Chromatogram.sty	By	: Toyin
Calibration File	: C:\ClarityVA\DataFiles\WORK1\Calib\2020 AFLATOXIN CAL STD\SEPTEMBER AFLATOXIN\14092020\15092020 AFTOTAL new.cal		

Analysis User Variables:

AnalysisUserVar1	: 0
AnalysisUserVar2	: 0
AnalysisUserVar3	: 0

Method User Variables:

MethodUserVar1	: 0
MethodUserVar2	: 0
MethodUserVar3	: 0



Result Table (ESTD - Data\4L_24_03_2021 7_00_09 PM_0823 - CM5440 FL Detector 1)

	Reten. Time [min]	Area [mFL.s]	Response	Amount [ng/ml]	Amount% [%]	Compound Name
1	6.167	817.462	817.462	2.043	66.1	AFG2
2	6.917	29.828	29.828	0.851	27.5	AFG1
3	7.250	198.936	198.936	0.197	6.4	AFB2
	Total	1046.226		3.091	100.0	

Clarity VA - Chromatography SW
DataApex
www.dataapex.com

Chromatogram Info:

File Name	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Data\5L_24_03_2021 7_14_36 PM_0831.prm	File Created	: 24/03/2021 7:14:36 PM
Origin	: Acquired, Acquisition started 24/03/2021 7:02:35 PM	Acquired Date	: 24/03/2021 7:14:36 PM
Project	: MYCOTOXIN	By	: Toyin

Printed Version Info:

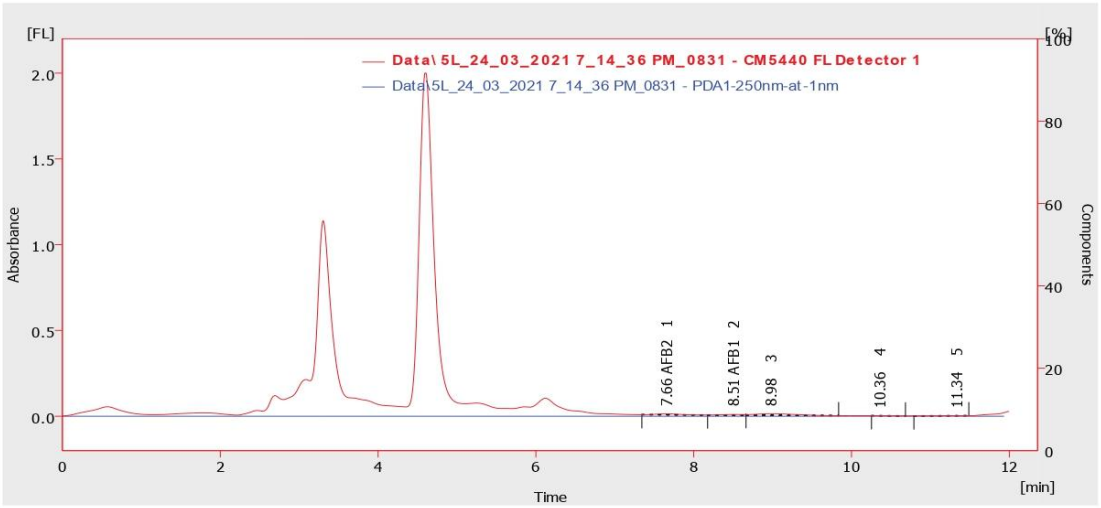
Printed Version	: 21/09/2021 8:29:08 AM, IA: 8.0 Rev.0 Recent (Linked Calibration)	Printed Date	: 05/10/2021 12:33:05 PM
Report Style	: C:\ClarityVA\DataFiles\WORK1\Data\Common\Chromatogram.sty	By	: Toyin
Calibration File	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Calib\2020 TOTAL AFLATOXINS\2020 DECEMBER\2021 TOTAL AFLATOXINS\2021 SEPTEMBER\200921.cal		

Analysis User Variables:

AnalysisUserVar1	: 0
AnalysisUserVar2	: 0
AnalysisUserVar3	: 0

Method User Variables:

MethodUserVar1	: 0
MethodUserVar2	: 0
MethodUserVar3	: 0



Result Table (ESTD - Data\5L_24_03_2021 7_14_36 PM_0831 - CM5440 FL Detector 1)

	Reten. Time [min]	Area [mFL.s]	Response	Amount [ng/g]	Amount% [%]	Compound Name
1	7.660	116.096	116.096	0.560	31.3	AFB2
2	8.513	72.676	72.676	1.229	68.7	AFB1
	Total	188.772		1.788	100.0	

Clarity VA - Chromatography SW
DataApex
www.dataapex.com

Chromatogram Info:

File Name	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Data\5L_24_03_2021 7_29_02 PM_0842.prm	File Created	: 24/03/2021 7:29:03 PM
Origin	: Acquired, Acquisition started 24/03/2021 7:17:02 PM	Acquired Date	: 24/03/2021 7:29:02 PM
Project	: MYCOTOXIN	By	: Toyin

Printed Version Info:

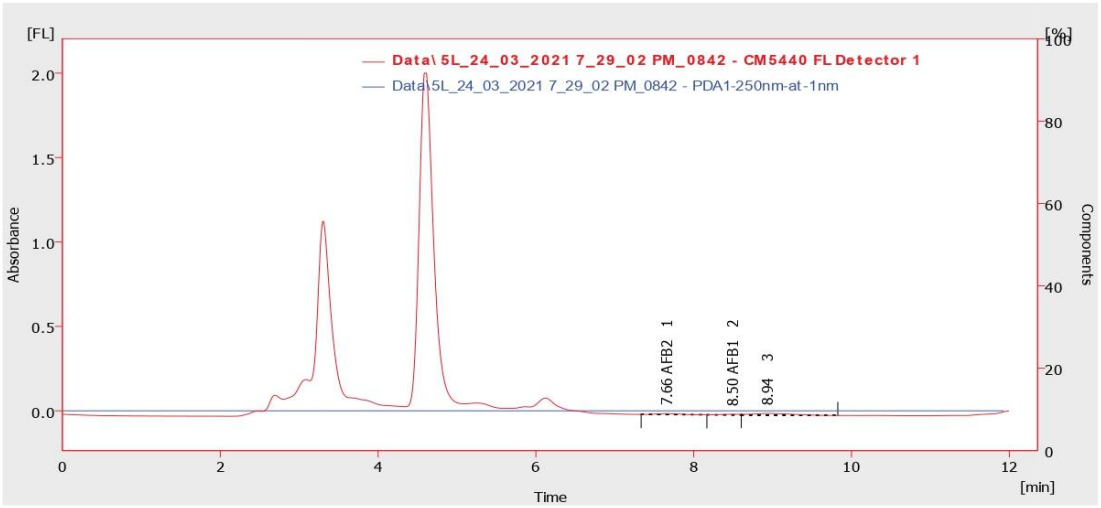
Printed Version	: 21/09/2021 8:29:27 AM, IA: 8.0 Rev.0 Recent (Linked Calibration)	Printed Date	: 05/10/2021 12:33:24 PM
Report Style	: C:\ClarityVA\DataFiles\WORK1\Data\Common\Chromatogram.sty	By	: Toyin
Calibration File	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Calib\2020 TOTAL AFLATOXINS\2020 DECEMBER\2021 TOTAL AFLATOXINS\2021 SEPTEMBER\200921.cal		

Analysis User Variables:

AnalysisUserVar1	: 0
AnalysisUserVar2	: 0
AnalysisUserVar3	: 0

Method User Variables:

MethodUserVar1	: 0
MethodUserVar2	: 0
MethodUserVar3	: 0



Result Table (ESTD - Data\5L_24_03_2021 7_29_02 PM_0842 - CM5440 FL Detector 1)

	Reten. Time [min]	Area [mFL.s]	Response	Amount [ng/g]	Amount% [%]	Compound Name
1	7.657	122.970	122.970	0.593	35.7	AFB2
2	8.500	63.016	63.016	1.065	64.3	AFB1
	Total	185.986		1.658	100.0	

Clarity VA - Chromatography SW
DataApex
www.dataapex.com

Chromatogram Info:

File Name	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Data\5L_24_03_2021 7_43_29 PM_0853.prm	File Created	: 24/03/2021 7:43:30 PM
Origin	: Acquired, Acquisition started 24/03/2021 7:31:29 PM	Acquired Date	: 24/03/2021 7:43:29 PM
Project	: MYCOTOXIN	By	: Toyin

Printed Version Info:

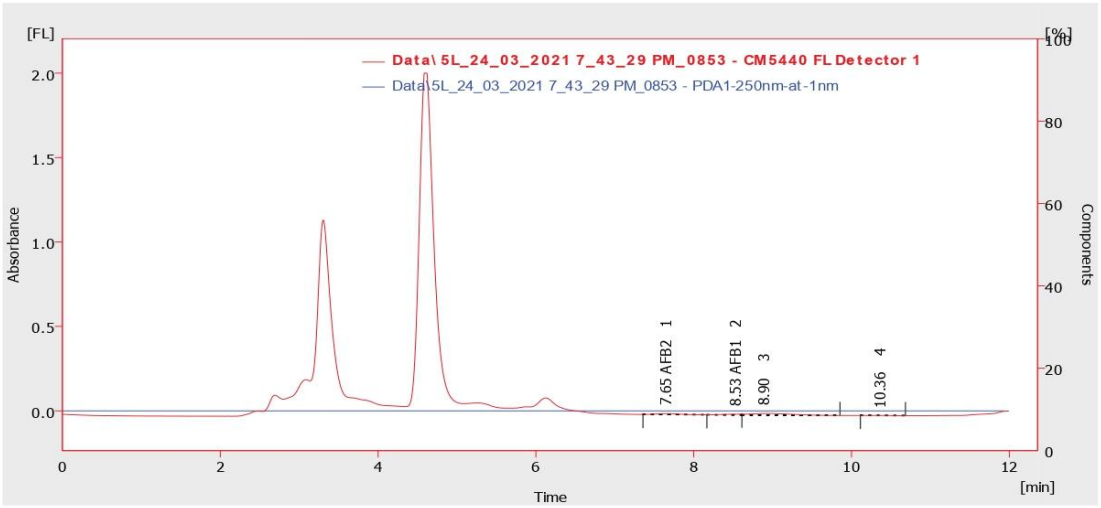
Printed Version	: 21/09/2021 8:30:07 AM, IA: 8.0 Rev.0 Recent (Linked Calibration)	Printed Date	: 05/10/2021 12:33:45 PM
Report Style	: C:\ClarityVA\DataFiles\WORK1\Data\Common\Chromatogram.sty	By	: Toyin
Calibration File	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Calib\2020 TOTAL AFLATOXINS\2020 DECEMBER\2021 TOTAL AFLATOXINS\2021 SEPTEMBER\200921.cal		

Analysis User Variables:

AnalysisUserVar1	: 0
AnalysisUserVar2	: 0
AnalysisUserVar3	: 0

Method User Variables:

MethodUserVar1	: 0
MethodUserVar2	: 0
MethodUserVar3	: 0



Result Table (ESTD - Data\5L_24_03_2021 7_43_29 PM_0853 - CM5440 FL Detector 1)

	Reten. Time [min]	Area [mFL.s]	Response	Amount [ng/g]	Amount% [%]	Compound Name
1	7.650	117.722	117.722	0.567	33.5	AFB2
2	8.530	66.626	66.626	1.127	66.5	AFB1
	Total	184.348		1.694	100.0	

Clarity VA - Chromatography SW
DataApex
www.dataapex.com

Chromatogram Info:

File Name	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Data\6L_24_03_2021 7_57_56 PM_0861.prm	File Created	: 24/03/2021 7:57:56 PM
Origin	: Acquired, Acquisition started 24/03/2021 7:45:55 PM	Acquired Date	: 24/03/2021 7:57:56 PM
Project	: MYCOTOXIN	By	: Toyin

Printed Version Info:

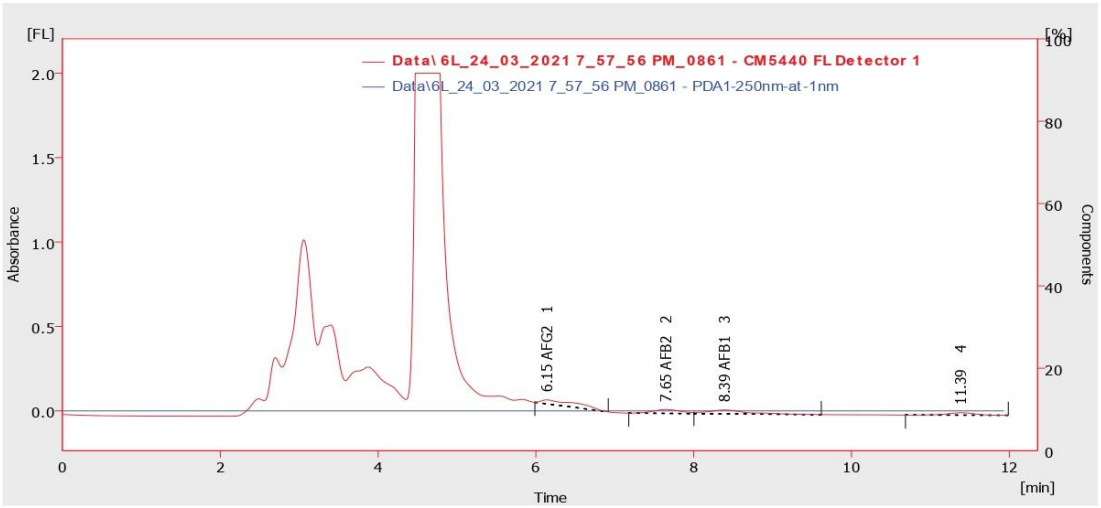
Printed Version	: 21/09/2021 9:03:51 AM, IA: 8.0 Rev.0 Recent (Linked Calibration)	Printed Date	: 05/10/2021 1:13:27 PM
Report Style	: C:\ClarityVA\DataFiles\WORK1\Data\Common\Chromatogram.sty	By	: Toyin
Calibration File	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Calib\2020 TOTAL AFLATOXINS\2020 DECEMBER\2021 TOTAL AFLATOXINS\2021 SEPTEMBER\200921.cal		

Analysis User Variables:

AnalysisUserVar1	: 0
AnalysisUserVar2	: 0
AnalysisUserVar3	: 0

Method User Variables:

MethodUserVar1	: 0
MethodUserVar2	: 0
MethodUserVar3	: 0



Result Table (ESTD - Data\6L_24_03_2021 7_57_56 PM_0861 - CM5440 FL Detector 1)

	Reten. Time [min]	Area [mFL.s]	Response	Amount [ng/g]	Amount% [%]	Compound Name
1	6.147	980.710	980.710	19.235	54.0	AFG2
2	7.650	572.330	572.330	2.759	7.8	AFB2
3	8.393	804.378	804.378	13.601	38.2	AFB1
	Total	2357.418		35.595	100.0	

Clarity VA - Chromatography SW
DataApex
www.dataapex.com

Chromatogram Info:

File Name	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Data\6L_24_03_2021 8_12_23 PM_0872.prm	File Created	: 24/03/2021 8:12:23 PM
Origin	: Acquired, Acquisition started 24/03/2021 8:00:22 PM	Acquired Date	: 24/03/2021 8:12:23 PM
Project	: MYCOTOXIN	By	: Toyin

Printed Version Info:

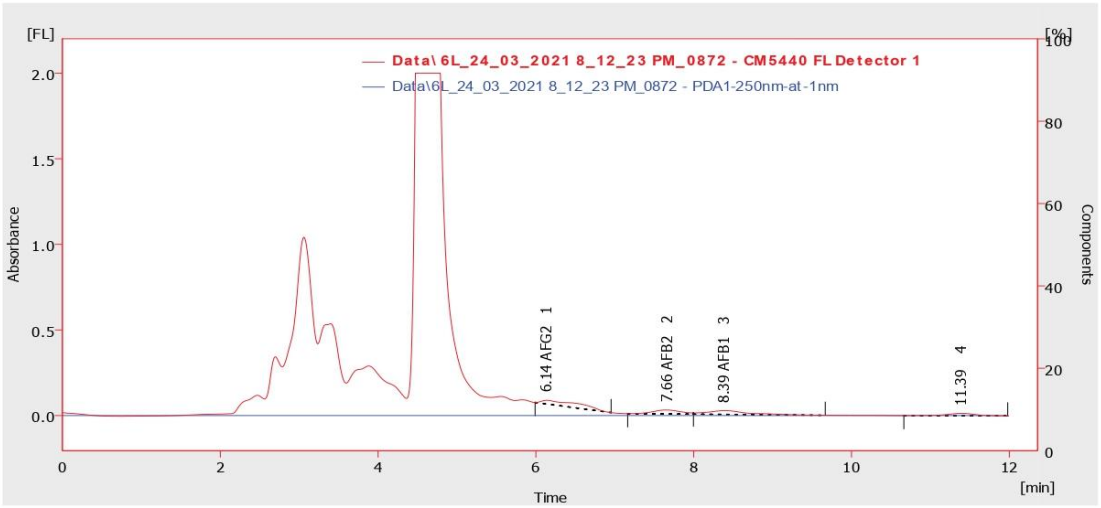
Printed Version	: 21/09/2021 9:04:11 AM, IA: 8.0 Rev.0 Recent (Linked Calibration)	Printed Date	: 05/10/2021 1:14:24 PM
Report Style	: C:\ClarityVA\DataFiles\WORK1\Data\Common\Chromatogram.sty	By	: Toyin
Calibration File	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Calib\2020 TOTAL AFLATOXINS\2020 DECEMBER\2021 TOTAL AFLATOXINS\2021 SEPTEMBER\200921.cal		

Analysis User Variables:

AnalysisUserVar1	: 0
AnalysisUserVar2	: 0
AnalysisUserVar3	: 0

Method User Variables:

MethodUserVar1	: 0
MethodUserVar2	: 0
MethodUserVar3	: 0



Result Table (ESTD - Data\6L_24_03_2021 8_12_23 PM_0872 - CM5440 FL Detector 1)

	Reten. Time [min]	Area [mFL.s]	Response	Amount [ng/g]	Amount% [%]	Compound Name
1	6.137	995.180	995.180	19.519	54.3	AFG2
2	7.657	576.781	576.781	2.780	7.7	AFB2
3	8.387	807.878	807.878	13.660	38.0	AFB1
	Total	2379.840		35.959	100.0	

Clarity VA - Chromatography SW
DataApex
www.dataapex.com

Chromatogram Info:

File Name	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Data\6L_24_03_2021 8_26_50 PM_0883.prm	File Created	: 24/03/2021 8:26:50 PM
Origin	: Acquired, Acquisition started 24/03/2021 8:14:49 PM	Acquired Date	: 24/03/2021 8:26:50 PM
Project	: MYCOTOXIN	By	: Toyin

Printed Version Info:

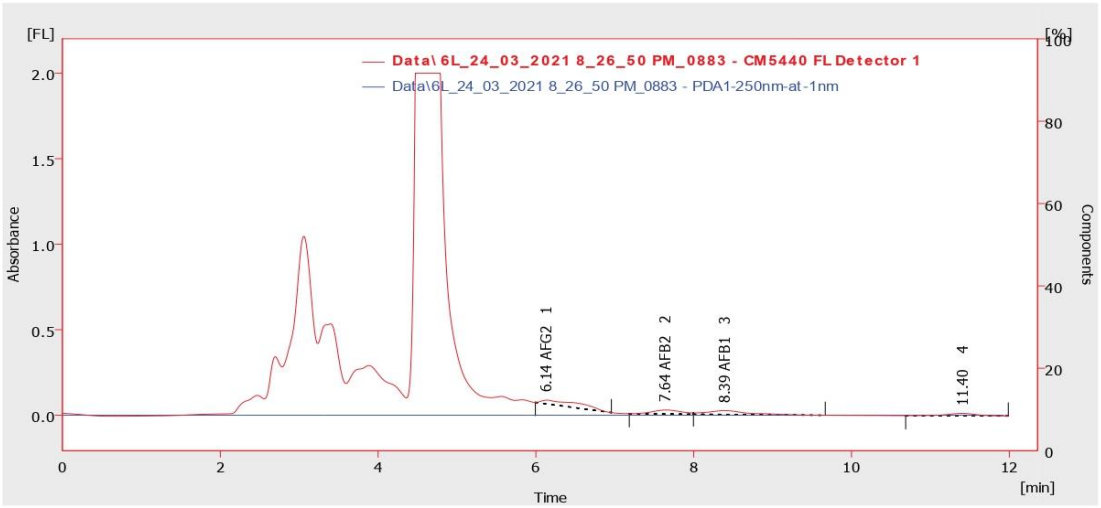
Printed Version	: 21/09/2021 9:04:34 AM, IA: 8.0 Rev.0 Recent (Linked Calibration)	Printed Date	: 05/10/2021 1:14:47 PM
Report Style	: C:\ClarityVA\DataFiles\WORK1\Data\Common\Chromatogram.sty	By	: Toyin
Calibration File	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Calib\2020 TOTAL AFLATOXINS\2020 DECEMBER\2021 TOTAL AFLATOXINS\2021 SEPTEMBER\200921.cal		

Analysis User Variables:

AnalysisUserVar1	: 0
AnalysisUserVar2	: 0
AnalysisUserVar3	: 0

Method User Variables:

MethodUserVar1	: 0
MethodUserVar2	: 0
MethodUserVar3	: 0



Result Table (ESTD - Data\6L_24_03_2021 8_26_50 PM_0883 - CM5440 FL Detector 1)

	Reten. Time [min]	Area [mFL.s]	Response	Amount [ng/g]	Amount% [%]	Compound Name
1	6.137	1053.795	1053.795	20.669	55.5	AFG2
2	7.640	581.624	581.624	2.804	7.5	AFB2
3	8.390	815.411	815.411	13.787	37.0	AFB1
	Total	2450.830		37.260	100.0	

Clarity VA - Chromatography SW
DataApex
www.dataapex.com

Chromatogram Info:

File Name	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Data\7L_24_03_2021 8_41_17 PM_0891.prm	File Created	: 24/03/2021 8:41:18 PM
Origin	: Acquired, Acquisition started 24/03/2021 8:29:17 PM	Acquired Date	: 24/03/2021 8:41:17 PM
Project	: MYCOTOXIN	By	: Toyin

Printed Version Info:

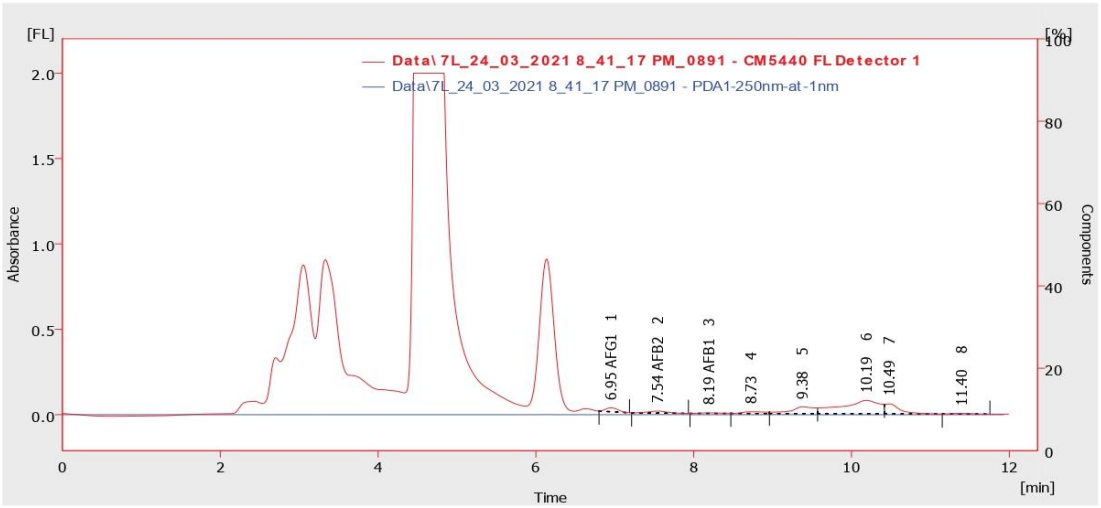
Printed Version	: 21/09/2021 9:08:38 AM, IA: 8.0 Rev.0 Recent (Linked Calibration)	Printed Date	: 05/10/2021 1:20:23 PM
Report Style	: C:\ClarityVA\DataFiles\WORK1\Data\Common\Chromatogram.sty	By	: Toyin
Calibration File	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Calib\2020 TOTAL AFLATOXINS\2020 DECEMBER\2021 TOTAL AFLATOXINS\2021 SEPTEMBER\200921.cal		

Analysis User Variables:

AnalysisUserVar1	: 0
AnalysisUserVar2	: 0
AnalysisUserVar3	: 0

Method User Variables:

MethodUserVar1	: 0
MethodUserVar2	: 0
MethodUserVar3	: 0



Result Table (ESTD - Data\7L_24_03_2021 8_41_17 PM_0891 - CM5440 FL Detector 1)

	Reten. Time [min]	Area [mFL.s]	Response	Amount [ng/g]	Amount% [%]	Compound Name
1	6.953	255.272	255.272	11.705	84.7	AFG1
2	7.543	204.627	204.627	0.986	7.1	AFB2
3	8.193	66.872	66.872	1.131	8.2	AFB1
	Total	526.771		13.822	100.0	

Clarity VA - Chromatography SW
DataApex
www.dataapex.com

Chromatogram Info:

File Name	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Data\40L_31_03_2021 6_26_17 PM_5971.prm	File Created	: 31/03/2021 6:26:17 PM
Origin	: Acquired, Acquisition started 31/03/2021 6:14:16 PM	Acquired Date	: 31/03/2021 6:26:17 PM
Project	: MYCOTOXIN	By	: Toyin

Printed Version Info:

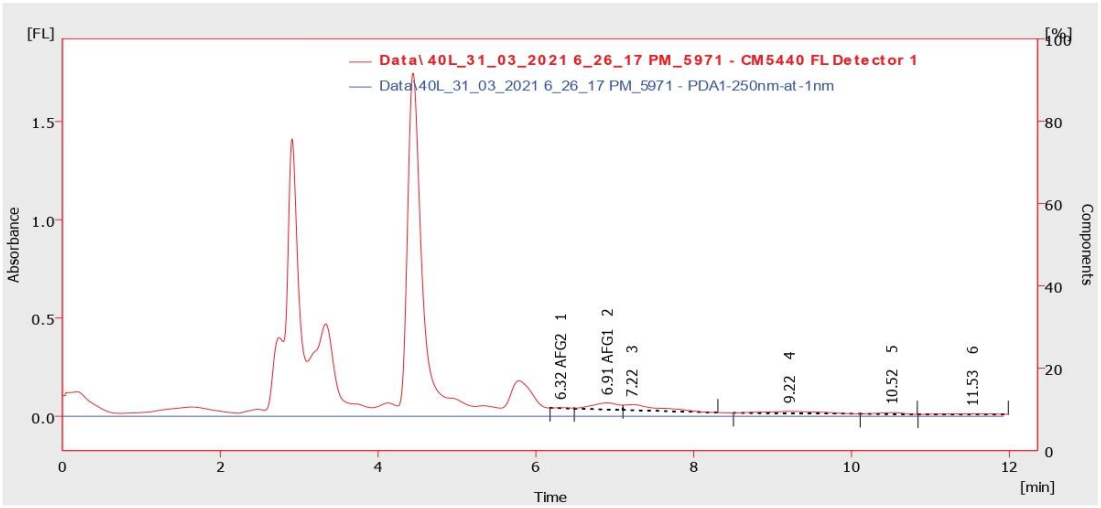
Printed Version	: 20/09/2021 3:23:42 PM, IA: 8.0 Rev.0 Recent (Linked Calibration)	Printed Date	: 05/10/2021 11:15:34 AM
Report Style	: C:\ClarityVA\DataFiles\WORK1\Data\Common\Chromatogram.sty	By	: Toyin
Calibration File	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Calib\2020 TOTAL AFLATOXINS\2020 DECEMBER\2021 TOTAL AFLATOXINS\2021 SEPTEMBER\200921.cal		

Analysis User Variables:

AnalysisUserVar1	: 0
AnalysisUserVar2	: 0
AnalysisUserVar3	: 0

Method User Variables:

MethodUserVar1	: 0
MethodUserVar2	: 0
MethodUserVar3	: 0



Result Table (ESTD - Data\40L_31_03_2021 6_26_17 PM_5971 - CM5440 FL Detector 1)

	Reten. Time [min]	Area [mFL.s]	Response	Amount [ng/g]	Amount% [%]	Compound Name
1	6.320	44.497	44.497	0.873	2.3	AFG2
2	6.913	792.198	792.198	36.324	97.7	AFG1
	Total	836.694		37.197	100.0	

Clarity VA - Chromatography SW
DataApex
www.dataapex.com

Chromatogram Info:

File Name	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Data\40L_31_03_2021 6_40_44 PM_5982.prm	File Created	: 31/03/2021 6:40:44 PM
Origin	: Acquired, Acquisition started 31/03/2021 6:28:43 PM	Acquired Date	: 31/03/2021 6:40:44 PM
Project	: MYCOTOXIN	By	: Toyin

Printed Version Info:

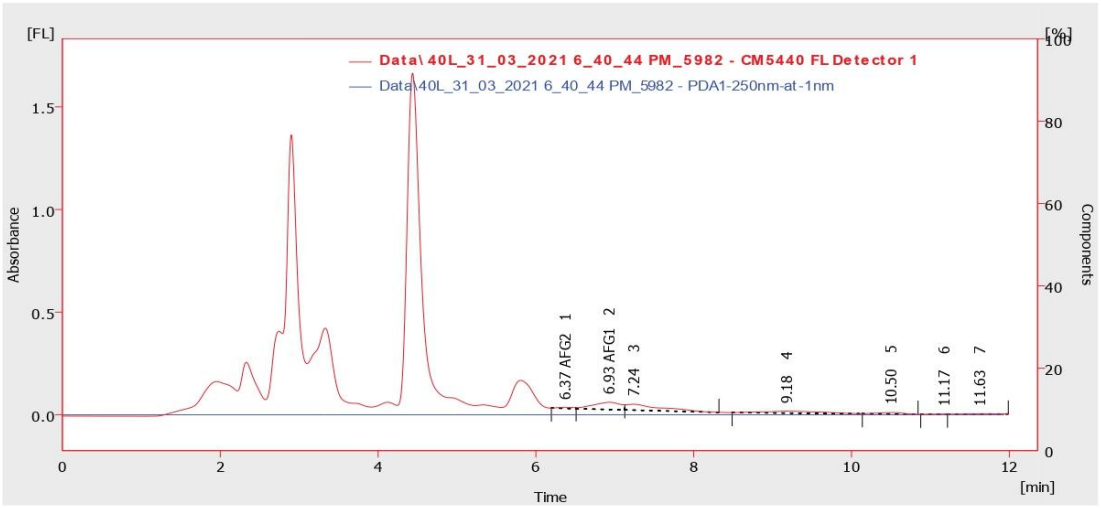
Printed Version	: 20/09/2021 3:23:57 PM, IA: 8.0 Rev.0 Recent (Linked Calibration)	Printed Date	: 05/10/2021 11:16:07 AM
Report Style	: C:\ClarityVA\DataFiles\WORK1\Data\Common\Chromatogram.sty	By	: Toyin
Calibration File	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Calib\2020 TOTAL AFLATOXINS\2020 DECEMBER\2021 TOTAL AFLATOXINS\2021 SEPTEMBER\200921.cal		

Analysis User Variables:

AnalysisUserVar1	: 0
AnalysisUserVar2	: 0
AnalysisUserVar3	: 0

Method User Variables:

MethodUserVar1	: 0
MethodUserVar2	: 0
MethodUserVar3	: 0



Result Table (ESTD - Data\40L_31_03_2021 6_40_44 PM_5982 - CM5440 FL Detector 1)

	Reten. Time [min]	Area [mFL.s]	Response	Amount [ng/g]	Amount% [%]	Compound Name
1	6.370	76.778	76.778	1.506	3.7	AFG2
2	6.933	853.827	853.827	39.150	96.3	AFG1
	Total	930.605		40.656	100.0	

Clarity VA - Chromatography SW
DataApex
www.dataapex.com

Chromatogram Info:

File Name	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Data\41L_31_03_2021 6_55_11 PM_5991.prm	File Created	: 31/03/2021 6:55:11 PM
Origin	: Acquired, Acquisition started 31/03/2021 6:43:11 PM	Acquired Date	: 31/03/2021 6:55:11 PM
Project	: MYCOTOXIN	By	: Toyin

Printed Version Info:

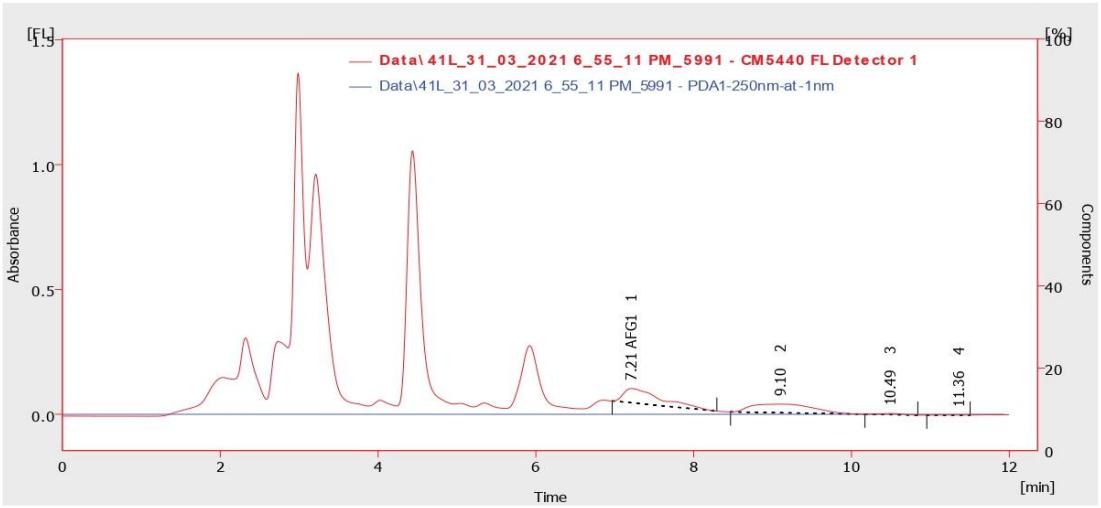
Printed Version	: 20/09/2021 3:25:18 PM, IA: 8.0 Rev.0 Recent (Linked Calibration)	Printed Date	: 05/10/2021 11:22:55 AM
Report Style	: C:\ClarityVA\DataFiles\WORK1\Data\Common\Chromatogram.sty	By	: Toyin
Calibration File	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Calib\2020 TOTAL AFLATOXINS\2020 DECEMBER\2021 TOTAL AFLATOXINS\2021 SEPTEMBER\200921.cal		

Analysis User Variables:

AnalysisUserVar1	: 0
AnalysisUserVar2	: 0
AnalysisUserVar3	: 0

Method User Variables:

MethodUserVar1	: 0
MethodUserVar2	: 0
MethodUserVar3	: 0



Result Table (ESTD - Data\41L_31_03_2021 6_55_11 PM_5991 - CM5440 FL Detector 1)

	Reten. Time [min]	Area [mFL.s]	Response	Amount [ng/g]	Amount% [%]	Compound Name
1	7.210	1935.657	1935.657	88.754	100.0	AFG1
	Total	1935.657		88.754	100.0	

Clarity VA - Chromatography SW
DataApex
www.dataapex.com

Chromatogram Info:

File Name	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Data\41L_31_03_2021 7_09_38 PM_6002.prm	File Created	: 31/03/2021 7:09:39 PM
Origin	: Acquired, Acquisition started 31/03/2021 6:57:38 PM	Acquired Date	: 31/03/2021 7:09:38 PM
Project	: MYCOTOXIN	By	: Toyin

Printed Version Info:

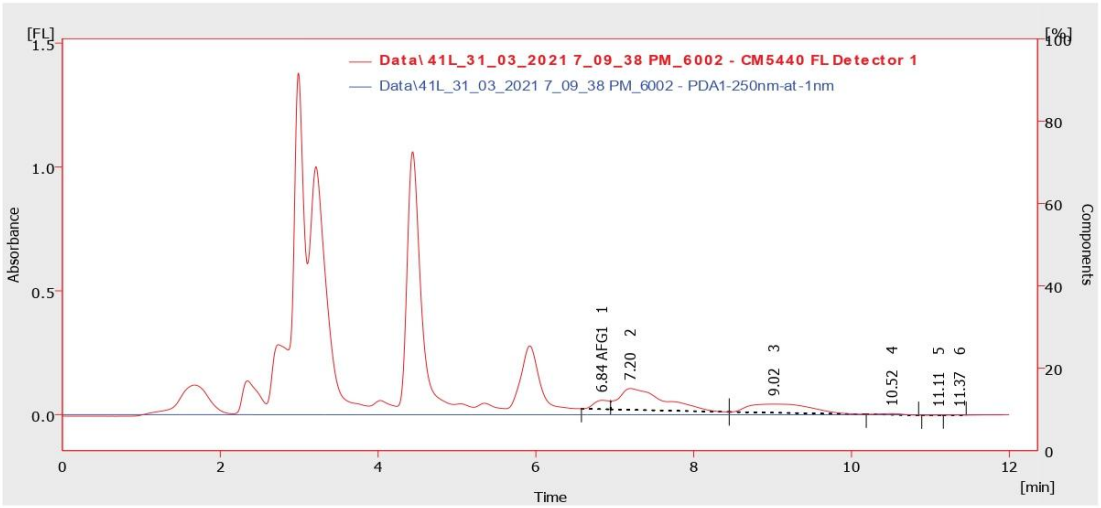
Printed Version	: 20/09/2021 9:15:11 AM, IA: 8.0 Rev.0 Recent (Linked Calibration)	Printed Date	: 05/10/2021 11:23:30 AM
Report Style	: C:\ClarityVA\DataFiles\WORK1\Data\Common\Chromatogram.sty	By	: Toyin
Calibration File	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Calib\2020 TOTAL AFLATOXINS\2020 DECEMBER\2021 TOTAL AFLATOXINS\2021 SEPTEMBER\200921.cal		

Analysis User Variables:

AnalysisUserVar1	: 0
AnalysisUserVar2	: 0
AnalysisUserVar3	: 0

Method User Variables:

MethodUserVar1	: 0
MethodUserVar2	: 0
MethodUserVar3	: 0



Result Table (ESTD - Data\41L_31_03_2021 7_09_38 PM_6002 - CM5440 FL Detector 1)

	Reten. Time [min]	Area [mFL.s]	Response	Amount [ng/g]	Amount% [%]	Compound Name
1	6.843	480.766	480.766	11.022	100.0	AFG1
	Total	480.766		11.022	100.0	

Clarity VA - Chromatography SW
DataApex
www.dataapex.com

Chromatogram Info:

File Name	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Data\42L_31_03_2021 7_24_06 PM_6011.prm	File Created	: 31/03/2021 7:24:06 PM
Origin	: Acquired, Acquisition started 31/03/2021 7:12:05 PM	Acquired Date	: 31/03/2021 7:24:06 PM
Project	: MYCOTOXIN	By	: Toyin

Printed Version Info:

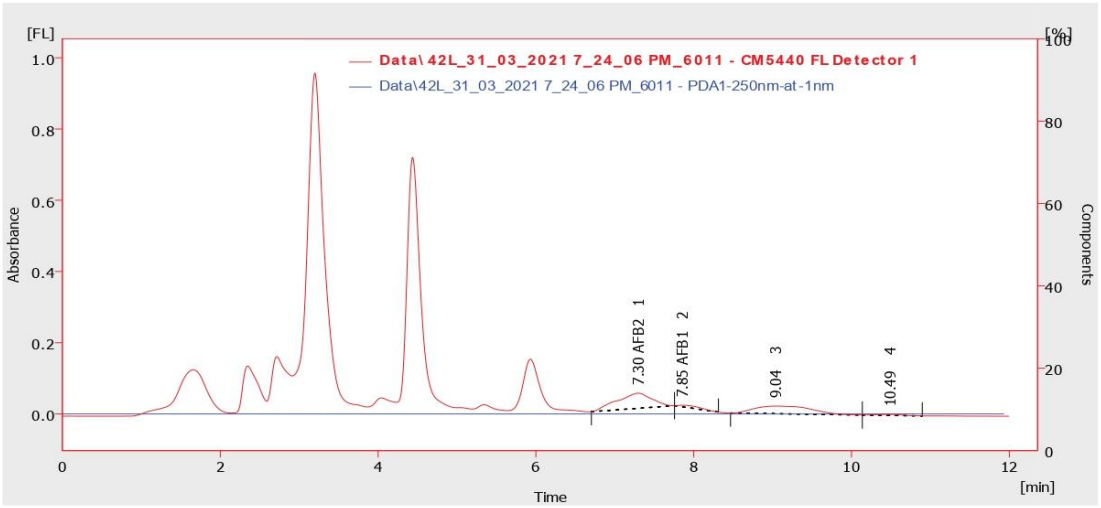
Printed Version	: 20/09/2021 4:09:38 PM, IA: 8.0 Rev.0 Recent (Linked Calibration)	Printed Date	: 05/10/2021 11:27:08 AM
Report Style	: C:\ClarityVA\DataFiles\WORK1\Data\Common\Chromatogram.sty	By	: Toyin
Calibration File	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Calib\2020 TOTAL AFLATOXINS\2020 DECEMBER\2021 TOTAL AFLATOXINS\2021 SEPTEMBER\200921.cal		

Analysis User Variables:

AnalysisUserVar1	: 0
AnalysisUserVar2	: 0
AnalysisUserVar3	: 0

Method User Variables:

MethodUserVar1	: 0
MethodUserVar2	: 0
MethodUserVar3	: 0



Result Table (ESTD - Data\42L_31_03_2021 7_24_06 PM_6011 - CM5440 FL Detector 1)

	Reten. Time [min]	Area [mFL.s]	Response	Amount [ng/g]	Amount% [%]	Compound Name
1	7.300	1317.181	1317.181	6.350	76.6	AFB2
2	7.853	115.015	115.015	1.945	23.4	AFB1
	Total	1432.196		8.294	100.0	

Clarity VA - Chromatography SW
DataApex
www.dataapex.com

Chromatogram Info:

File Name	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Data\42L_31_03_2021 7_38_32 PM_6022.prm	File Created	: 31/03/2021 7:38:32 PM
Origin	: Acquired, Acquisition started 31/03/2021 7:26:31 PM	Acquired Date	: 31/03/2021 7:38:32 PM
Project	: MYCOTOXIN	By	: Toyin

Printed Version Info:

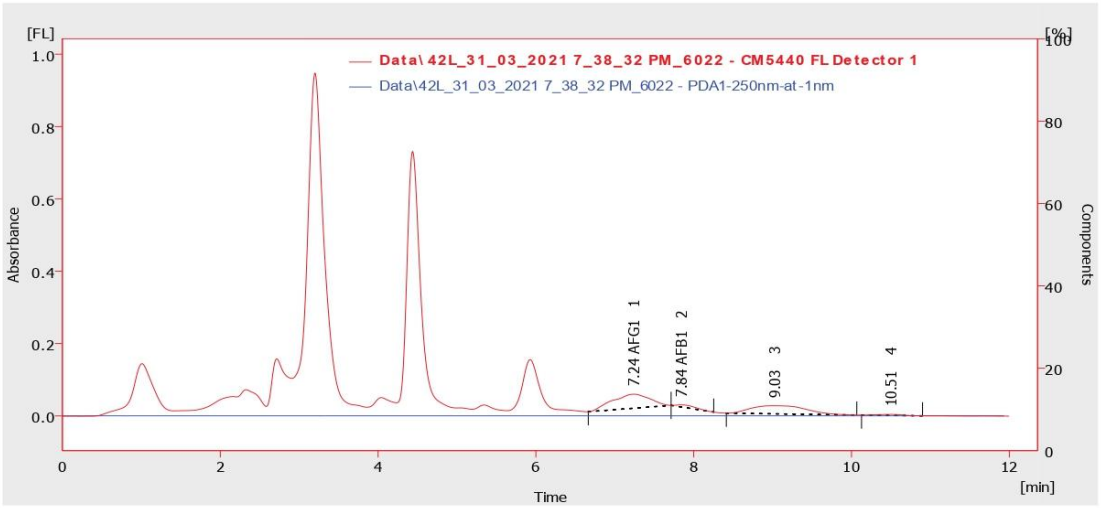
Printed Version	: 20/09/2021 4:10:20 PM, IA: 8.0 Rev.0 Recent (Linked Calibration)	Printed Date	: 05/10/2021 11:27:40 AM
Report Style	: C:\ClarityVA\DataFiles\WORK1\Data\Common\Chromatogram.sty	By	: Toyin
Calibration File	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Calib\2020 TOTAL AFLATOXINS\2020 DECEMBER\2021 TOTAL AFLATOXINS\2021 SEPTEMBER\200921.cal		

Analysis User Variables:

AnalysisUserVar1	: 0
AnalysisUserVar2	: 0
AnalysisUserVar3	: 0

Method User Variables:

MethodUserVar1	: 0
MethodUserVar2	: 0
MethodUserVar3	: 0



Result Table (ESTD - Data\42L_31_03_2021 7_38_32 PM_6022 - CM5440 FL Detector 1)

	Reten. Time [min]	Area [mFL.s]	Response	Amount [ng/g]	Amount% [%]	Compound Name
1	7.243	1293.860	1293.860	59.326	96.8	AFG1
2	7.843	117.047	117.047	1.979	3.2	AFB1
	Total	1410.907		61.305	100.0	

Clarity VA - Chromatography SW
DataApex
www.dataapex.com

Chromatogram Info:

File Name	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Data\43L_31_03_2021 7_53_00 PM_6031.prm	File Created	: 31/03/2021 7:53:00 PM
Origin	: Acquired, Acquisition started 31/03/2021 7:40:59 PM	Acquired Date	: 31/03/2021 7:53:00 PM
Project	: MYCOTOXIN	By	: Toyin

Printed Version Info:

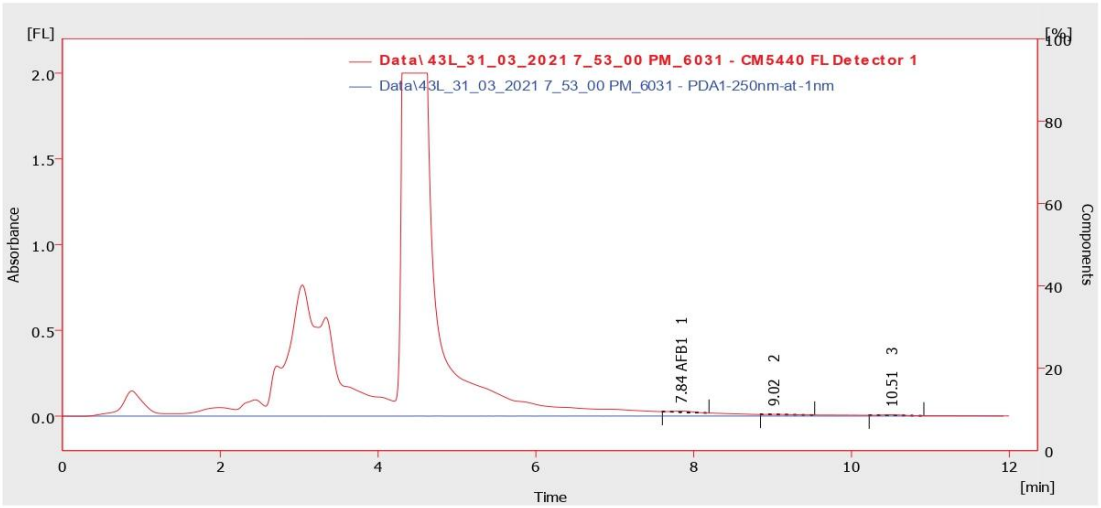
Printed Version	: 20/09/2021 4:32:46 PM, IA: 8.0 Rev.0 Recent (Linked Calibration)	Printed Date	: 05/10/2021 11:31:54 AM
Report Style	: C:\ClarityVA\DataFiles\WORK1\Data\Common\Chromatogram.sty	By	: Toyin
Calibration File	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Calib\2020 TOTAL AFLATOXINS\2020 DECEMBER\2021 TOTAL AFLATOXINS\2021 SEPTEMBER\200921.cal		

Analysis User Variables:

AnalysisUserVar1	: 0
AnalysisUserVar2	: 0
AnalysisUserVar3	: 0

Method User Variables:

MethodUserVar1	: 0
MethodUserVar2	: 0
MethodUserVar3	: 0



Result Table (ESTD - Data\43L_31_03_2021 7_53_00 PM_6031 - CM5440 FL Detector 1)

	Reten. Time [min]	Area [mFL.s]	Response	Amount [ng/g]	Amount% [%]	Compound Name
1	7.843	111.110	111.110	1.879	100.0	AFB1
	Total	111.110		1.879	100.0	

Clarity VA - Chromatography SW
DataApex
www.dataapex.com

Chromatogram Info:

File Name	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Data\43L_31_03_2021 8_07_27 PM_6042.prm	File Created	: 31/03/2021 8:07:27 PM
Origin	: Acquired, Acquisition started 31/03/2021 7:55:26 PM	Acquired Date	: 31/03/2021 8:07:27 PM
Project	: MYCOTOXIN	By	: Toyin

Printed Version Info:

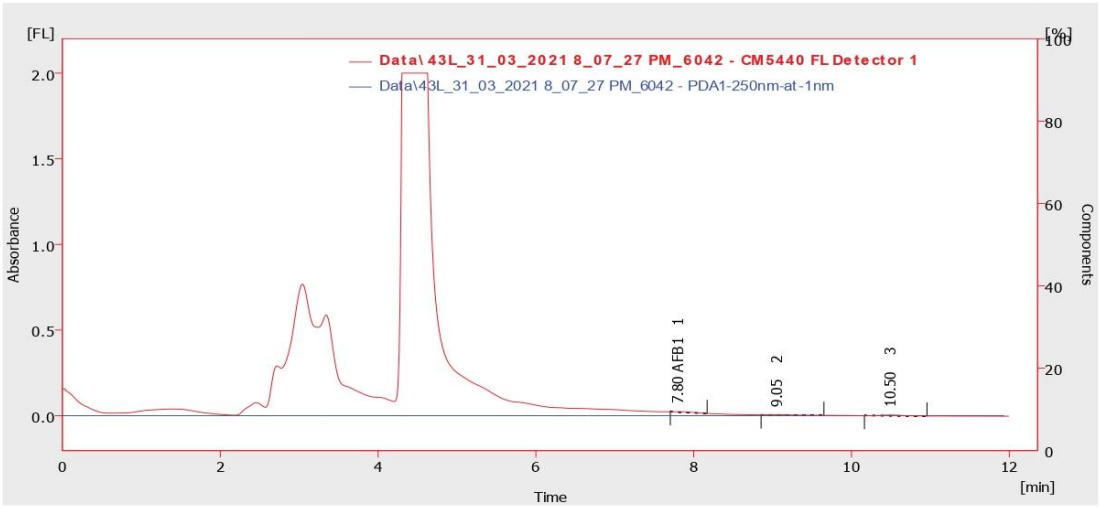
Printed Version	: 20/09/2021 4:33:24 PM, IA: 8.0 Rev.0 Recent (Linked Calibration)	Printed Date	: 05/10/2021 11:32:43 AM
Report Style	: C:\ClarityVA\DataFiles\WORK1\Data\Common\Chromatogram.sty	By	: Toyin
Calibration File	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Calib\2020 TOTAL AFLATOXINS\2020 DECEMBER\2021 TOTAL AFLATOXINS\2021 SEPTEMBER\200921.cal		

Analysis User Variables:

AnalysisUserVar1	: 0
AnalysisUserVar2	: 0
AnalysisUserVar3	: 0

Method User Variables:

MethodUserVar1	: 0
MethodUserVar2	: 0
MethodUserVar3	: 0



Result Table (ESTD - Data\43L_31_03_2021 8_07_27 PM_6042 - CM5440 FL Detector 1)

	Reten. Time [min]	Area [mFL.s]	Response	Amount [ng/g]	Amount% [%]	Compound Name
1	7.800	51.276	51.276	0.867	100.0	AFB1
	Total	51.276		0.867	100.0	

Clarity VA - Chromatography SW
DataApex
www.dataapex.com

Chromatogram Info:

File Name	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Data\44L_31_03_2021 8_21_54 PM_6051.prm	File Created	: 31/03/2021 8:21:54 PM
Origin	: Acquired, Acquisition started 31/03/2021 8:09:53 PM	Acquired Date	: 31/03/2021 8:21:54 PM
Project	: MYCOTOXIN	By	: Toyin

Printed Version Info:

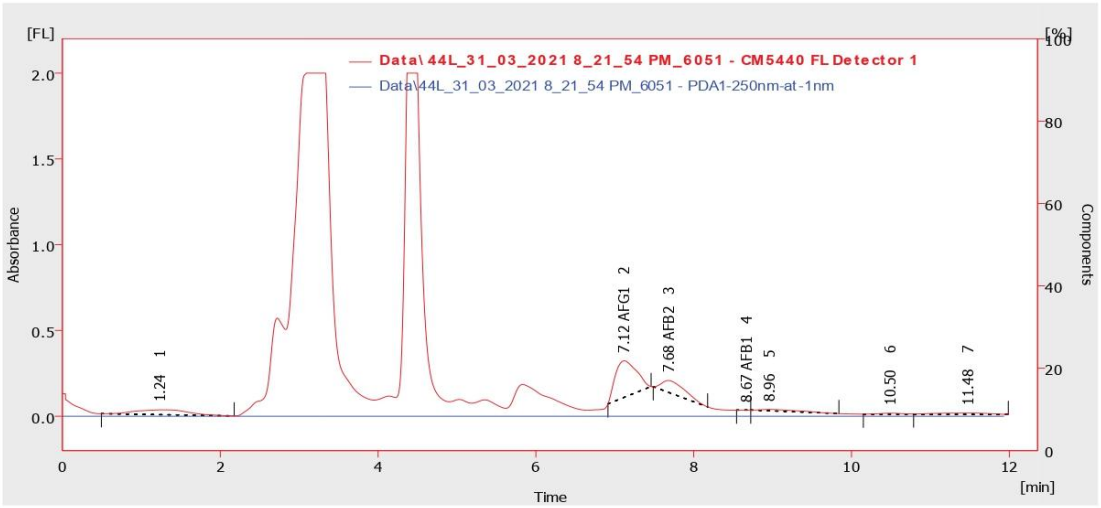
Printed Version	: 20/09/2021 4:37:59 PM, IA: 8.0 Rev.0 Recent (Linked Calibration)	Printed Date	: 05/10/2021 11:35:15 AM
Report Style	: C:\ClarityVA\DataFiles\WORK1\Data\Common\Chromatogram.sty	By	: Toyin
Calibration File	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Calib\2020 TOTAL AFLATOXINS\2020 DECEMBER\2021 TOTAL AFLATOXINS\2021 SEPTEMBER\200921.cal		

Analysis User Variables:

AnalysisUserVar1	: 0
AnalysisUserVar2	: 0
AnalysisUserVar3	: 0

Method User Variables:

MethodUserVar1	: 0
MethodUserVar2	: 0
MethodUserVar3	: 0



Result Table (ESTD - Data\44L_31_03_2021 8_21_54 PM_6051 - CM5440 FL Detector 1)

	Reten. Time [min]	Area [mFL.s]	Response	Amount [ng/g]	Amount% [%]	Compound Name
2	7.117	3958.867	3958.867	181.522	96.1	AFG1
3	7.683	1528.947	1528.947	7.370	3.9	AFB2
4	8.673	1.993	1.993	0.034	0.0	AFB1
	Total	5489.806		188.926	100.0	

Clarity VA - Chromatography SW
DataApex
www.dataapex.com

Chromatogram Info:

File Name	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Data\44L_31_03_2021 8_36_21 PM_6062.prm	File Created	: 31/03/2021 8:36:21 PM
Origin	: Acquired, Acquisition started 31/03/2021 8:24:21 PM	Acquired Date	: 31/03/2021 8:36:21 PM
Project	: MYCOTOXIN	By	: Toyin

Printed Version Info:

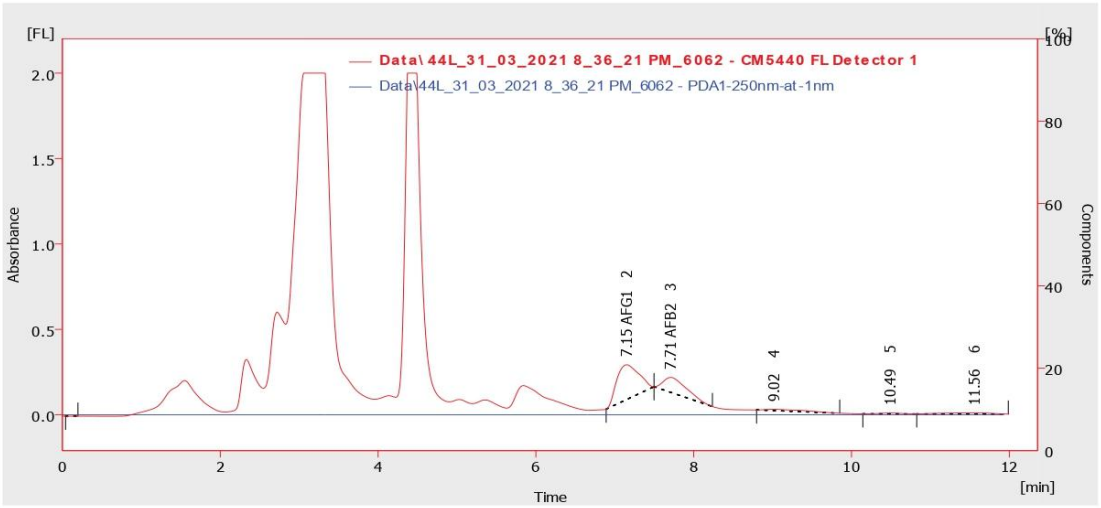
Printed Version	: 20/09/2021 4:38:40 PM, IA: 8.0 Rev.0 Recent (Linked Calibration)	Printed Date	: 05/10/2021 11:35:38 AM
Report Style	: C:\ClarityVA\DataFiles\WORK1\Data\Common\Chromatogram.sty	By	: Toyin
Calibration File	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Calib\2020 TOTAL AFLATOXINS\2020 DECEMBER\2021 TOTAL AFLATOXINS\2021 SEPTEMBER\200921.cal		

Analysis User Variables:

AnalysisUserVar1	: 0
AnalysisUserVar2	: 0
AnalysisUserVar3	: 0

Method User Variables:

MethodUserVar1	: 0
MethodUserVar2	: 0
MethodUserVar3	: 0



Result Table (ESTD - Data\44L_31_03_2021 8_36_21 PM_6062 - CM5440 FL Detector 1)

	Reten. Time [min]	Area [mFL.s]	Response	Amount [ng/g]	Amount% [%]	Compound Name
2	7.150	3955.464	3955.464	181.366	95.3	AFG1
3	7.707	1868.075	1868.075	9.005	4.7	AFB2
	Total	5823.539		190.372	100.0	

Clarity VA - Chromatography SW
DataApex
www.dataapex.com

Chromatogram Info:

File Name	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Data\45L_31_03_2021 8_50_48 PM_6071.prm	File Created	: 31/03/2021 8:50:48 PM
Origin	: Acquired, Acquisition started 31/03/2021 8:38:47 PM	Acquired Date	: 31/03/2021 8:50:48 PM
Project	: MYCOTOXIN	By	: Toyin

Printed Version Info:

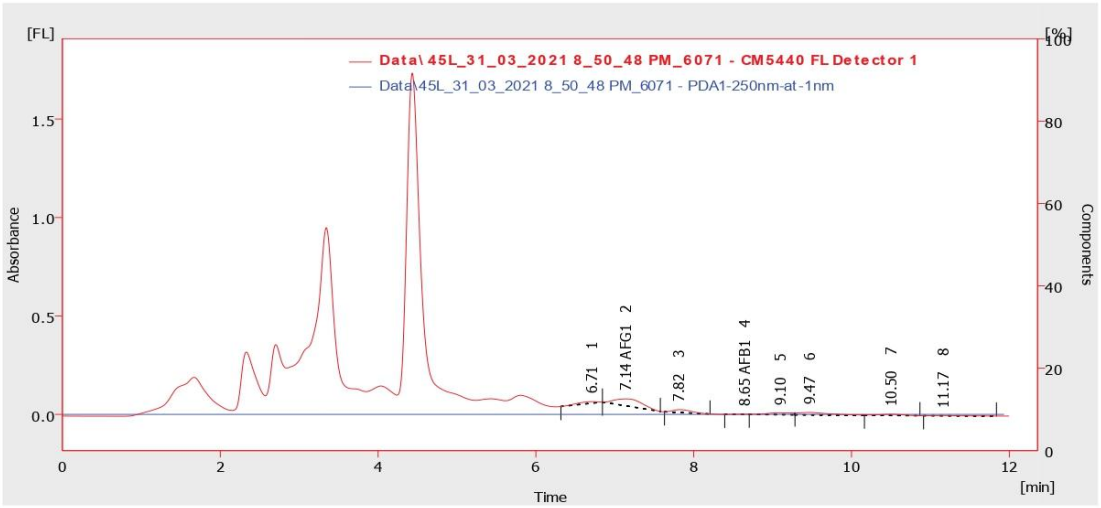
Printed Version	: 20/09/2021 4:42:14 PM, IA: 8.0 Rev.0 Recent (Linked Calibration)	Printed Date	: 05/10/2021 11:40:22 AM
Report Style	: C:\ClarityVA\DataFiles\WORK1\Data\Common\Chromatogram.sty	By	: Toyin
Calibration File	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Calib\2020 TOTAL AFLATOXINS\2020 DECEMBER\2021 TOTAL AFLATOXINS\2021 SEPTEMBER\200921.cal		

Analysis User Variables:

AnalysisUserVar1	: 0
AnalysisUserVar2	: 0
AnalysisUserVar3	: 0

Method User Variables:

MethodUserVar1	: 0
MethodUserVar2	: 0
MethodUserVar3	: 0



Result Table (ESTD - Data\45L_31_03_2021 8_50_48 PM_6071 - CM5440 FL Detector 1)

	Reten. Time [min]	Area [mFL.s]	Response	Amount [ng/g]	Amount% [%]	Compound Name
2	7.140	897.648	897.648	41.159	99.2	AFG1
4	8.650	19.348	19.348	0.327	0.8	AFB1
	Total	916.996		41.486	100.0	

Clarity VA - Chromatography SW
DataApex
www.dataapex.com

Chromatogram Info:

File Name	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Data\45L_31_03_2021 9_05_15 PM_6082.prm	File Created	: 31/03/2021 9:05:15 PM
Origin	: Acquired, Acquisition started 31/03/2021 8:53:15 PM	Acquired Date	: 31/03/2021 9:05:15 PM
Project	: MYCOTOXIN	By	: Toyin

Printed Version Info:

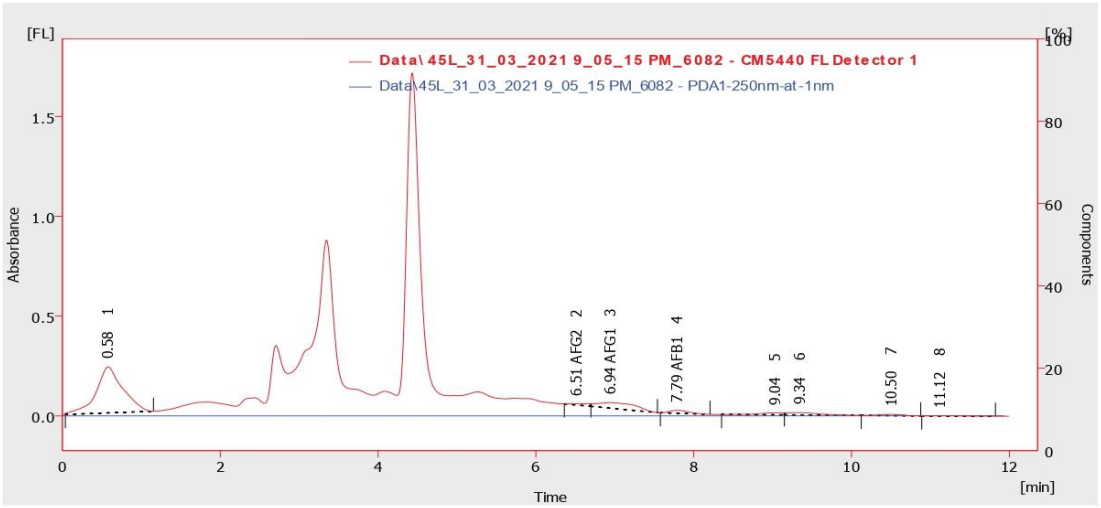
Printed Version	: 20/09/2021 4:42:41 PM, IA: 8.0 Rev.0 Recent (Linked Calibration)	Printed Date	: 05/10/2021 11:41:27 AM
Report Style	: C:\ClarityVA\DataFiles\WORK1\Data\Common\Chromatogram.sty	By	: Toyin
Calibration File	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Calib\2020 TOTAL AFLATOXINS\2020 DECEMBER\2021 TOTAL AFLATOXINS\2021 SEPTEMBER\200921.cal		

Analysis User Variables:

AnalysisUserVar1	: 0
AnalysisUserVar2	: 0
AnalysisUserVar3	: 0

Method User Variables:

MethodUserVar1	: 0
MethodUserVar2	: 0
MethodUserVar3	: 0



Result Table (ESTD - Data\45L_31_03_2021 9_05_15 PM_6082 - CM5440 FL Detector 1)

	Reten. Time [min]	Area [mFL.s]	Response	Amount [ng/g]	Amount% [%]	Compound Name
2	6.513	142.438	142.438	2.794	5.2	AFG2
3	6.943	1014.616	1014.616	46.522	86.9	AFG1
4	7.790	248.963	248.963	4.210	7.9	AFB1
	Total	1406.017		53.526	100.0	

Clarity VA - Chromatography SW
DataApex
www.dataapex.com

Chromatogram Info:

File Name	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Data\46L_31_03_2021 9_19_42 PM_6091.prm	File Created	: 31/03/2021 9:19:43 PM
Origin	: Acquired, Acquisition started 31/03/2021 9:07:42 PM	Acquired Date	: 31/03/2021 9:19:43 PM
Project	: MYCOTOXIN	By	: Toyin

Printed Version Info:

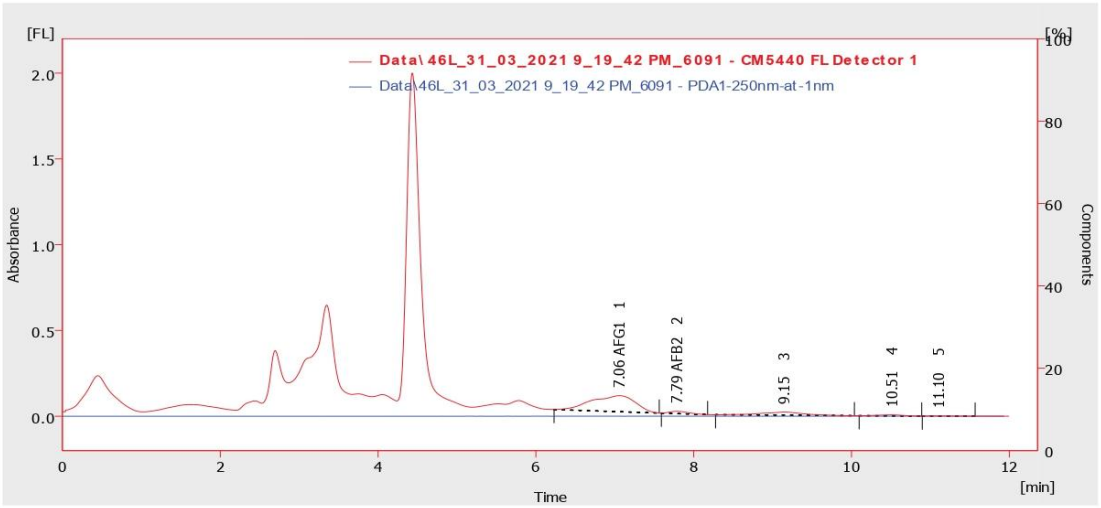
Printed Version	: 20/09/2021 4:45:26 PM, IA: 8.0 Rev.0 Recent (Linked Calibration)	Printed Date	: 05/10/2021 11:43:25 AM
Report Style	: C:\ClarityVA\DataFiles\WORK1\Data\Common\Chromatogram.sty	By	: Toyin
Calibration File	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Calib\2020 TOTAL AFLATOXINS\2020 DECEMBER\2021 TOTAL AFLATOXINS\2021 SEPTEMBER\200921.cal		

Analysis User Variables:

AnalysisUserVar1	: 0
AnalysisUserVar2	: 0
AnalysisUserVar3	: 0

Method User Variables:

MethodUserVar1	: 0
MethodUserVar2	: 0
MethodUserVar3	: 0



Result Table (ESTD - Data\46L_31_03_2021 9_19_42 PM_6091 - CM5440 FL Detector 1)

	Reten. Time [min]	Area [mFL.s]	Response	Amount [ng/g]	Amount% [%]	Compound Name
1	7.057	3607.581	3607.581	165.415	99.4	AFG1
2	7.787	223.991	223.991	1.080	0.6	AFB2
	Total	3831.572		166.495	100.0	

Clarity VA - Chromatography SW
DataApex
www.dataapex.com

Chromatogram Info:

File Name	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Data\46L_31_03_2021 9_34_09 PM_6102.prm	File Created	: 31/03/2021 9:34:09 PM
Origin	: Acquired, Acquisition started 31/03/2021 9:22:08 PM	Acquired Date	: 31/03/2021 9:34:09 PM
Project	: MYCOTOXIN	By	: Toyin

Printed Version Info:

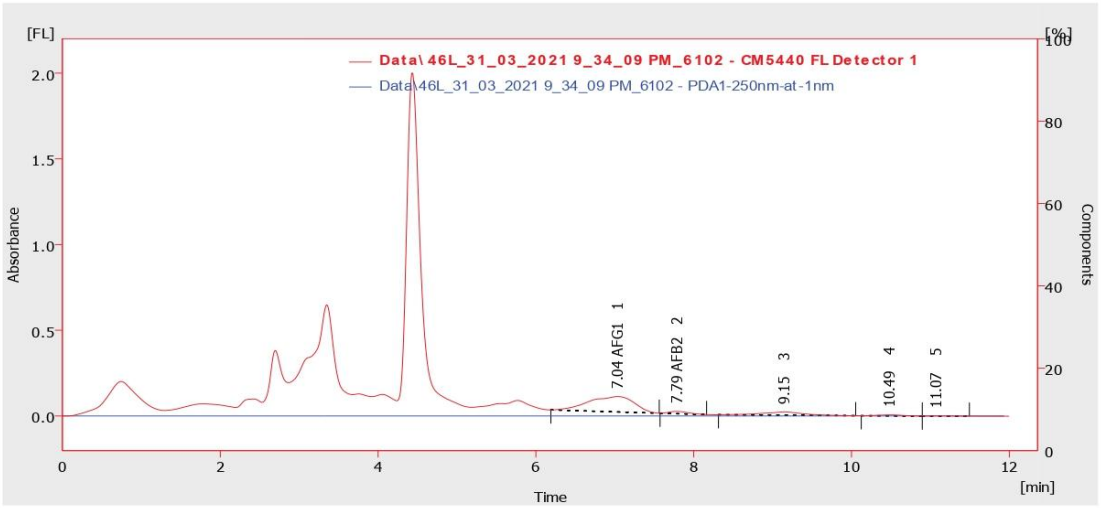
Printed Version	: 20/09/2021 4:45:46 PM, IA: 8.0 Rev.0 Recent (Linked Calibration)	Printed Date	: 05/10/2021 11:43:46 AM
Report Style	: C:\ClarityVA\DataFiles\WORK1\Data\Common\Chromatogram.sty	By	: Toyin
Calibration File	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Calib\2020 TOTAL AFLATOXINS\2020 DECEMBER\2021 TOTAL AFLATOXINS\2021 SEPTEMBER\200921.cal		

Analysis User Variables:

AnalysisUserVar1	: 0
AnalysisUserVar2	: 0
AnalysisUserVar3	: 0

Method User Variables:

MethodUserVar1	: 0
MethodUserVar2	: 0
MethodUserVar3	: 0



Result Table (ESTD - Data\46L_31_03_2021 9_34_09 PM_6102 - CM5440 FL Detector 1)

	Reten. Time [min]	Area [mFL.s]	Response	Amount [ng/g]	Amount% [%]	Compound Name
1	7.040	3594.972	3594.972	164.837	99.3	AFG1
2	7.787	223.942	223.942	1.080	0.7	AFB2
	Total	3818.914		165.916	100.0	

Clarity VA - Chromatography SW
DataApex
www.dataapex.com

Chromatogram Info:

File Name	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Data\47L_31_03_2021 9_48_35 PM_6111.prm	File Created	: 31/03/2021 9:48:35 PM
Origin	: Acquired, Acquisition started 31/03/2021 9:36:34 PM	Acquired Date	: 31/03/2021 9:48:35 PM
Project	: MYCOTOXIN	By	: Toyin

Printed Version Info:

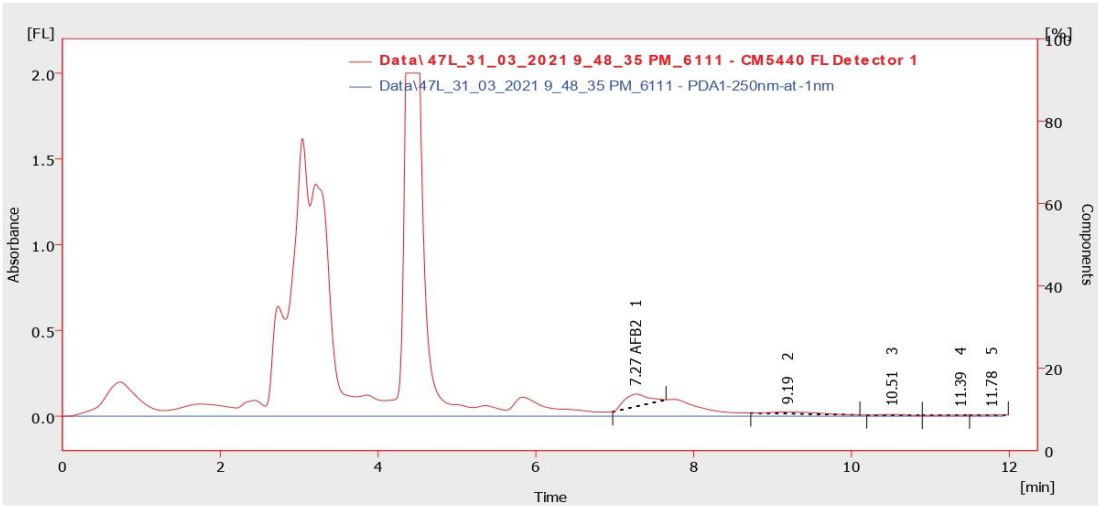
Printed Version	: 20/09/2021 4:48:06 PM, IA: 8.0 Rev.0 Recent (Linked Calibration)	Printed Date	: 05/10/2021 11:46:15 AM
Report Style	: C:\ClarityVA\DataFiles\WORK1\Data\Common\Chromatogram.sty	By	: Toyin
Calibration File	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Calib\2020 TOTAL AFLATOXINS\2020 DECEMBER\2021 TOTAL AFLATOXINS\2021 SEPTEMBER\200921.cal		

Analysis User Variables:

AnalysisUserVar1	: 0
AnalysisUserVar2	: 0
AnalysisUserVar3	: 0

Method User Variables:

MethodUserVar1	: 0
MethodUserVar2	: 0
MethodUserVar3	: 0



Result Table (ESTD - Data\47L_31_03_2021 9_48_35 PM_6111 - CM5440 FL Detector 1)

	Reten. Time [min]	Area [mFL.s]	Response	Amount [ng/g]	Amount% [%]	Compound Name
1	7.273	1523.414	1523.414	7.344	100.0	AFB2
	Total	1523.414		7.344	100.0	

Clarity VA - Chromatography SW
DataApex
www.dataapex.com

Chromatogram Info:

File Name	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Data\47L_31_03_2021 10_03_02 PM_6122.prm	File Created	: 31/03/2021 10:03:02 PM
Origin	: Acquired, Acquisition started 31/03/2021 9:51:01 PM	Acquired Date	: 31/03/2021 10:03:02 PM
Project	: MYCOTOXIN	By	: Toyin

Printed Version Info:

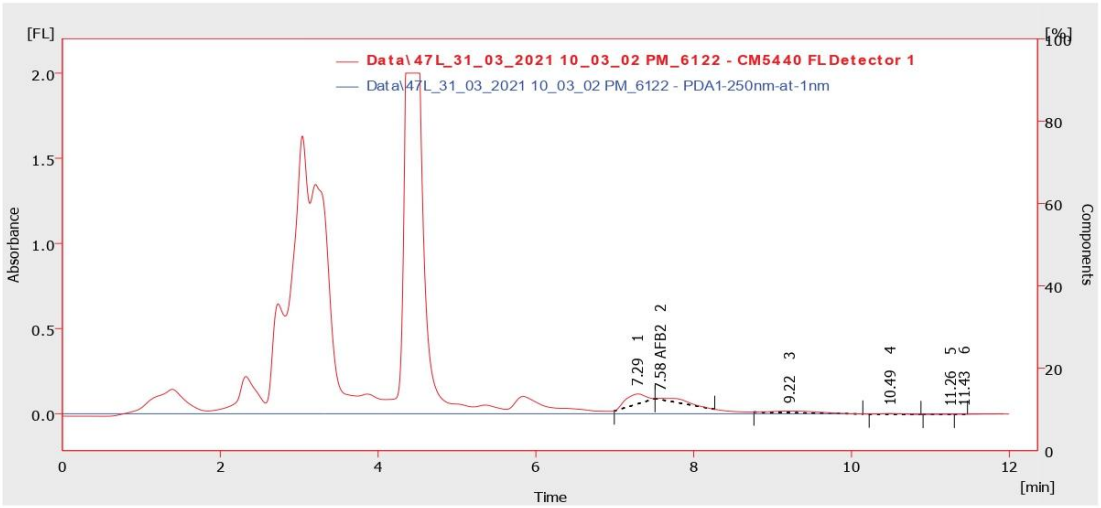
Printed Version	: 20/09/2021 4:47:37 PM, IA: 8.0 Rev.0 Recent (Linked Calibration)	Printed Date	: 05/10/2021 11:45:50 AM
Report Style	: C:\ClarityVA\DataFiles\WORK1\Data\Common\Chromatogram.sty	By	: Toyin
Calibration File	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Calib\2020 TOTAL AFLATOXINS\2020 DECEMBER\2021 TOTAL AFLATOXINS\2021 SEPTEMBER\200921.cal		

Analysis User Variables:

AnalysisUserVar1	: 0
AnalysisUserVar2	: 0
AnalysisUserVar3	: 0

Method User Variables:

MethodUserVar1	: 0
MethodUserVar2	: 0
MethodUserVar3	: 0



Result Table (ESTD - Data\47L_31_03_2021 10_03_02 PM_6122 - CM5440 FL Detector 1)

	Reten. Time [min]	Area [mFL.s]	Response	Amount [ng/g]	Amount% [%]	Compound Name
2	7.580	456.018	456.018	2.198	100.0	AFB2
	Total	456.018		2.198	100.0	

Clarity VA - Chromatography SW
DataApex
www.dataapex.com

Chromatogram Info:

File Name	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Data\48L_31_03_2021 10_17_28 PM_6131.prm	File Created	: 31/03/2021 10:17:28 PM
Origin	: Acquired, Acquisition started 31/03/2021 10:05:27 PM	Acquired Date	: 31/03/2021 10:17:28 PM
Project	: MYCOTOXIN	By	: Toyin

Printed Version Info:

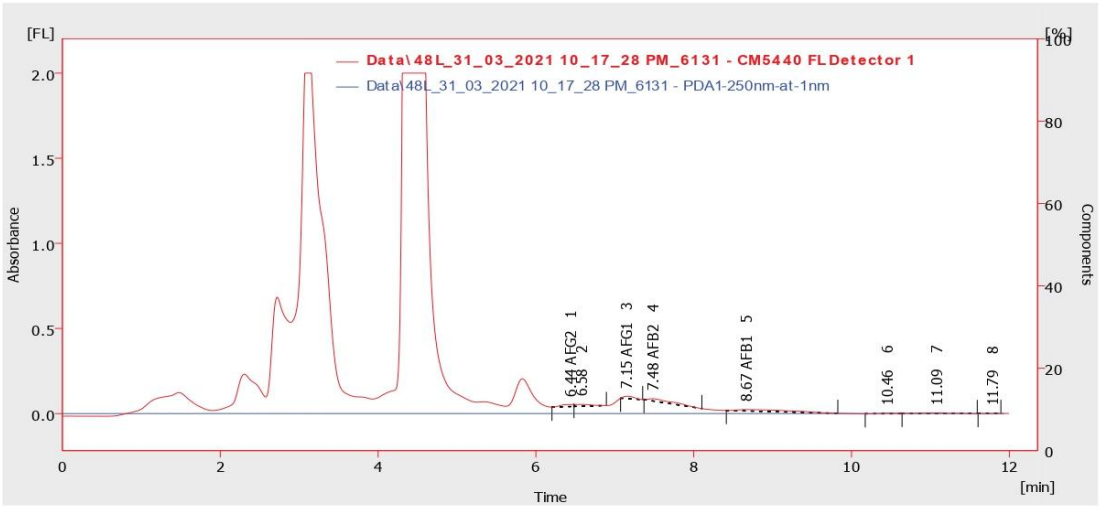
Printed Version	: 20/09/2021 4:50:20 PM, IA: 8.0 Rev.0 Recent (Linked Calibration)	Printed Date	: 05/10/2021 11:48:25 AM
Report Style	: C:\ClarityVA\DataFiles\WORK1\Data\Common\Chromatogram.sty	By	: Toyin
Calibration File	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Calib\2020 TOTAL AFLATOXINS\2020 DECEMBER\2021 TOTAL AFLATOXINS\2021 SEPTEMBER\200921.cal		

Analysis User Variables:

AnalysisUserVar1	: 0
AnalysisUserVar2	: 0
AnalysisUserVar3	: 0

Method User Variables:

MethodUserVar1	: 0
MethodUserVar2	: 0
MethodUserVar3	: 0



Result Table (ESTD - Data\48L_31_03_2021 10_17_28 PM_6131 - CM5440 FL Detector 1)

	Reten. Time [min]	Area [mFL.s]	Response	Amount [ng/g]	Amount% [%]	Compound Name
1	6.443	139.654	139.654	2.739	14.8	AFG2
3	7.153	135.578	135.578	6.217	33.6	AFG1
4	7.483	354.289	354.289	1.708	9.2	AFB2
5	8.667	463.778	463.778	7.842	42.4	AFB1
	Total	1093.298		18.505	100.0	

Clarity VA - Chromatography SW
DataApex
www.dataapex.com

Chromatogram Info:

File Name	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Data\48L_31_03_2021 10_31_55 PM_6142.prm	File Created	: 31/03/2021 10:31:55 PM
Origin	: Acquired, Acquisition started 31/03/2021 10:19:54 PM	Acquired Date	: 31/03/2021 10:31:55 PM
Project	: MYCOTOXIN	By	: Toyin

Printed Version Info:

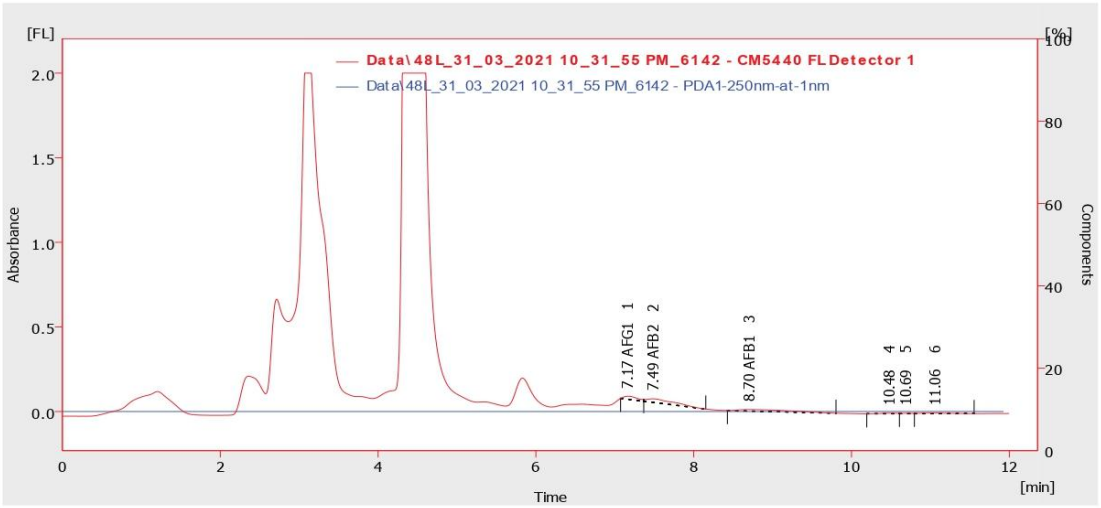
Printed Version	: 20/09/2021 9:08:55 AM, IA: 8.0 Rev.0 Recent (Linked Calibration)	Printed Date	: 05/10/2021 11:48:50 AM
Report Style	: C:\ClarityVA\DataFiles\WORK1\Data\Common\Chromatogram.sty	By	: Toyin
Calibration File	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Calib\2020 TOTAL AFLATOXINS\2020 DECEMBER\2021 TOTAL AFLATOXINS\2021 SEPTEMBER\200921.cal		

Analysis User Variables:

AnalysisUserVar1	: 0
AnalysisUserVar2	: 0
AnalysisUserVar3	: 0

Method User Variables:

MethodUserVar1	: 0
MethodUserVar2	: 0
MethodUserVar3	: 0



Result Table (ESTD - Data\48L_31_03_2021 10_31_55 PM_6142 - CM5440 FL Detector 1)

	Reten. Time [min]	Area [mFL.s]	Response	Amount [ng/g]	Amount% [%]	Compound Name
1	7.167	233.345	233.345	5.350	49.7	AFG1
2	7.487	584.460	584.460	1.409	13.1	AFB2
3	8.703	473.767	473.767	4.005	37.2	AFB1
	Total	1291.572		10.764	100.0	

Clarity VA - Chromatography SW
DataApex
www.dataapex.com

Chromatogram Info:

File Name	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Data\49L_31_03_2021 10_46_21 PM_6151.prm	File Created	: 31/03/2021 10:46:22 PM
Origin	: Acquired, Acquisition started 31/03/2021 10:34:21 PM	Acquired Date	: 31/03/2021 10:46:21 PM
Project	: MYCOTOXIN	By	: Toyin

Printed Version Info:

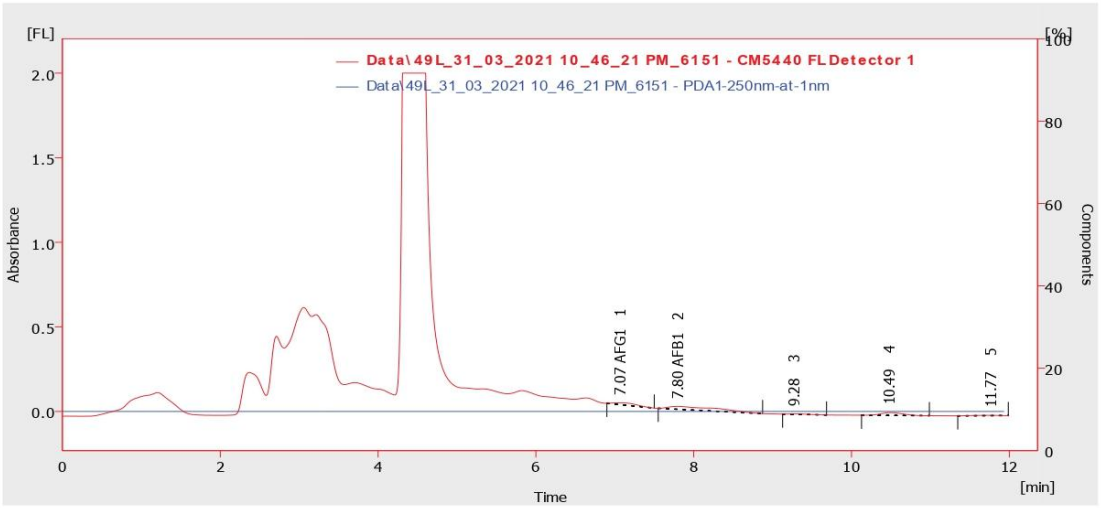
Printed Version	: 20/09/2021 4:56:18 PM, IA: 8.0 Rev.0 Recent (Linked Calibration)	Printed Date	: 05/10/2021 11:51:50 AM
Report Style	: C:\ClarityVA\DataFiles\WORK1\Data\Common\Chromatogram.sty	By	: Toyin
Calibration File	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Calib\2020 TOTAL AFLATOXINS\2020 DECEMBER\2021 TOTAL AFLATOXINS\2021 SEPTEMBER\200921.cal		

Analysis User Variables:

AnalysisUserVar1	: 0
AnalysisUserVar2	: 0
AnalysisUserVar3	: 0

Method User Variables:

MethodUserVar1	: 0
MethodUserVar2	: 0
MethodUserVar3	: 0



Result Table (ESTD - Data\49L_31_03_2021 10_46_21 PM_6151 - CM5440 FL Detector 1)

	Reten. Time [min]	Area [mFL.s]	Response	Amount [ng/g]	Amount% [%]	Compound Name
1	7.073	250.789	250.789	11.499	44.0	AFG1
2	7.800	864.266	864.266	14.613	56.0	AFB1
	Total	1115.054		26.112	100.0	

Clarity VA - Chromatography SW
DataApex
www.dataapex.com

Chromatogram Info:

File Name	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Data\65L_07_04_2021 5_44_26 PM_7161.prm	File Created	: 07/04/2021 5:44:26 PM
Origin	: Acquired, Acquisition started 07/04/2021 5:32:25 PM	Acquired Date	: 07/04/2021 5:44:26 PM
Project	: MYCOTOXIN	By	: Toyin

Printed Version Info:

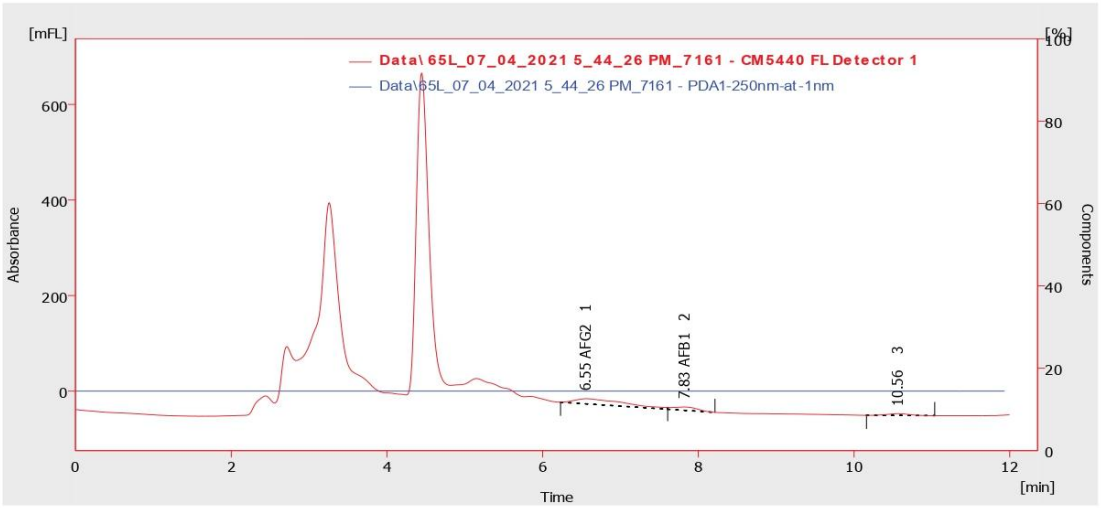
Printed Version	: 21/09/2021 8:55:23 AM, IA: 8.0 Rev.0 Recent (Linked Calibration)	Printed Date	: 05/10/2021 12:51:04 PM
Report Style	: C:\ClarityVA\DataFiles\WORK1\Data\Common\Chromatogram.sty	By	: Toyin
Calibration File	: C:\ClarityVA\DataFiles\WORK1\Data\MYCOTOXIN\Calib\2020 TOTAL AFLATOXINS\2020 DECEMBER\2021 TOTAL AFLATOXINS\2021 SEPTEMBER\200921.cal		

Analysis User Variables:

AnalysisUserVar1	: 0
AnalysisUserVar2	: 0
AnalysisUserVar3	: 0

Method User Variables:

MethodUserVar1	: 0
MethodUserVar2	: 0
MethodUserVar3	: 0



Result Table (ESTD - Data\65L_07_04_2021 5_44_26 PM_7161 - CM5440 FL Detector 1)

	Reten. Time [min]	Area [mFL.s]	Response	Amount [ng/g]	Amount% [%]	Compound Name
1	6.547	525.203	525.203	10.301	80.0	AFG2
2	7.827	152.446	152.446	2.578	20.0	AFB1
	Total	677.649		12.879	100.0	