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Analysis of Aflatoxin Level and Fungi Contamination From Foodstuffs Sold in Osogbo Metropolis, Nigeria

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ABSTRACT

Some of the food products sold in open markets in Nigeria were reported to have been contaminated by *Aspergillus* spp that releases aflatoxin which are toxic and carcinogenic. This study sought to provide baseline information on the levels of aflatoxin in the food commodities sold in the city by determining and analyzing aflatoxin produced by isolated fungi. One hundred and ninety nine samples consisting of 67 unprocessed samples of dried maize, 66 unprocessed samples of dried groundnut and 66 unprocessed samples of dried yam chip from Igbona, Akindeko, Ayegbaju and Oluode markets in Osogbo metropolis were cultured on Potato Dextrose Agar and later sub-cultured on *Aspergillus flavus parasiticus* Agar with chloramphenicol ($500mgL^{-1}$) supplement. Identification of isolates was carried out based on their morphological and microscopic characteristics. Aflatoxin was detected and quantified via Thin Layer Chromatography with detection limit of 1 part per billion. The highest incidence was recorded for A. *flavus* representing 52.4% followed by *A. parasiticus* (27.6%) and *A. brasiliensis* (20.0%). In addition, out of the nineteen food samples that yielded *Aspergillus* mould, 11(57.9 %) were contaminated with aflatoxins of varied concentrations ranging from 2 - ≥20 ppb, while 8 (42.1%) samples contained no aflatoxin above 1ppb. The levels of aflatoxin contamination of food products sampled were higher than set regulatory limit (≤20 ppb) in unprocessed food products by Standard Organization of Nigeria. This study's findings provided useful insights into the extent of aflatoxin contamination in the foodstuffs sold in Osogbo metropolis, Nigeria.
Keywords: *Aspergillus flavus,* Food safety, Mycotoxin, Staple foods

INTRODUCTION

Food is a fundamental component of human nutrition and an essential part of our daily lives. However, it can also be a source of contamination by various toxic substances, including mycotoxins produced by fungi. Aflatoxins, a type of mycotoxin produced by *Aspergillus* fungi, are potent carcinogenic and mutagenic agents that pose significant public health risks when present in foodstuffs. The consumption of food contaminated with aflatoxins can cause acute and chronic health problems, including liver damage, immune system suppression, and cancer (Khan *et al.*, 2024; Shepard, 2016).

Nigeria, like many other sub-Saharan African countries, is at a higher risk of aflatoxin contamination due to several factors, including inadequate food storage and processing practices, high humidity, and temperature that promote fungal growth (Chilenga *et al.*, 2024). Several studies have reported high levels of aflatoxin contamination in foodstuffs sold in Nigeria, including maize, peanuts, and cassava, among others (Chilenga *et al.*, 2024).

Due to the high toxicity and carcinogenic potential of aflatoxins, they are of high concern for the safety of food worldwide (Dhakal *et al.*, 2023). Aflatoxin contamination has been reported mostly in peanuts, cotton seed, corn, pea, sorghum, rice, pistachio, maize, oilseed rape, groundnut, spices, meat and meat products (Shabeer *et al.*, 2022). Aflatoxin is mainly produced by *Aspergillus flavus_Aspergillus parasiticus* as well as *Aspergillus nominus* (Gong *et al.*, 2024). These toxins are named for the fungus producing them, e.g. "A" from the genus name *Aspergillus*, "fla" from the species name *flavus* added to toxin to give the name aflatoxin (Sharma *et al.*, 2025). Contamination with aflatoxin has been reported mostly in peanuts, cotton seed, corn, pea, sorghum, rice, pistachio, maize, oilseed rape, groundnut, spices, meat and meat products, (Martinez-Miranda *et al.*, 2019). Based on chromatographic and fluorescence characteristics, more than 20 afflatoxins are known (Xiang *et al.*, 2021) but the major ones are aflatoxin B1 (AFB1), B2 (AFB2), G1 (AFG1) and G2 (AFG2), as well as M1 (AFM1) and M2 (AFM2) (Lerda, 2010). Aflatoxin B1 is the most carcinogenic and best studied of the compounds. AFM1 and AFM2 are hydroxylated forms of AFB1 and AFB2 (Alameri *et al.*, 2023). When AFB1 in contaminated feed or foodstuffs is ingested by domestic animals, such as dairy cows, the toxin undergoes liver biotransformation and is converted into aflatoxin M1 (AFM1), becoming the hydroxylated form of AFB1. AFM1 is excreted in milk, tissues and biological fluids of these animals (Min *et al.*, 2021) and in this form can be taken up by consumers.

The pathological condition resulting from this ingestion is called aflatoxicosis (Abrehame *et al.*, 2023). Acute toxicity, caused by ingestion of large amounts of aflatoxin from heavily contaminated food, causes decreased liver function and could lead to blood clotting disorder, jaundice, a decrease in serum proteins that are synthesized by the liver, edema, abdominal pain, vomiting and death of the affected person. In 2004, a case occurred in Kenya, in which there were 317 cases and 125 deaths reported due to consumption of aflatoxin contaminated maize (Omara *et al.*, 2020).

Beside its acute toxicity, aflatoxin has a high carcinogenic potential. It was estimated that about 25 200 – 155 000 people worldwide, 40% of which in Africa suffer from liver cancer induced by aflatoxin (Elhassan, 2023) and AFB1 was later categorized as a group 1 carcinogen for humans by the International Agency for Research on Cancer (IARC) (Rushing & Selim, 2019). Suleiman *et al.* (2021) in their survey for aflatoxin in various types of local Nigerian foods using TLC qualitatively detected aflatoxin in dried pepper. Factors affecting the incidence of mycotoxigenic fungi and mycotoxins in Nigeria include climatic condition, availability of nutrient for mould, soil types, farming system, pre harvest conditions, post-harvest handling and storage (Silva *et al.*, 2023).

The range of limits of Aflatoxin B1 in food is (1-20 µg/kg) in at least 29 EU countries. The maximum limit of contamination with aflatoxin in peanuts in Brazil and USA is 20µg/kg while Canada and the European Union have imposed a limit of 15 µg/kg (Sharma *et al.,* 2017). Aflatoxin has the highest mycotoxin regulations in Africa and Morocco had the most detailed mycotoxin regulations. Nigeria has adopted the European commission mycotoxin regulations which it uses primarily for export commodities, although these are applied in-country whenever there is a need to take a critical look especially on industrially processed food commodities (Aziz *et al.,* 2021).

The detection of AFs has been performed by the Association of Official Analytical Chemists (AOAC) official method in food and feed samples (Albert *et al.*, 2021). Among the most commonly employed methods are chromatographic methods like thin layer chromatography (TLC) (Madhavai, 2024), high performance liquid chromatography (HPLC) and liquid chromatography mass spectroscopy (LCMS) (Tao *et al.*, 2018), besides the enzyme-linked immunosorbent assay (ELISA) (Joia & Modaqeq, 2024). However, the drawbacks of these standard methods are that they are unsuitable for rapid and real-time applications in food and feed samples as they are tedious, time-consuming and require skilled personnel to operate. Therefore, rapid and robust methods like polymerase chain reaction (PCR) and non-destructive methods based on fluorescence/near-infrared spectroscopy (FS/NIRS) and hyperspectral imaging (HSI) have emerged for the quick and easy detection of AFs (Patel *et al.,* 2024).

Osogbo metropolis, the capital city of Osun state in Nigeria, has a population of approximately 1.5 million people and serves as a major hub for the distribution of foodstuffs to other parts of the state. However, limited studies have investigated the prevalence and levels of aflatoxin contamination in foodstuffs sold in Osogbo metropolis. Therefore, this study aimed to evaluate the level of aflatoxin and fungi contamination in foodstuffs sold in Osogbo metropolis, Nigeria. The study was carried out to detect contamination of some selected staple food in our locality and provide baseline information on the levels of aflatoxin in the food commodities sold in the city by determining and analyzing aflatoxin produced by isolated fungi and identify possible intervention measures to mitigate aflatoxin contamination in foodstuffs sold in Osogbo metropolis.

MATERIALS AND METHODS

Dried maize, dried groundnut and dried yam chips for this study were obtained from vendors in the selected markets. Samples were collected from four selected markets namely, Igbona, Akindeko, Oluode and Ayegbaju markets **(**Figure 1**)**. Sampling was carried out for two months from January 2017 – April 2017 and dried maize, dried yam chip and dried groundnut were randomly sampled. A total of one hundred and ninety nine (199) samples collected, consisting 67 unprocessed samples of dried maize, 66 unprocessed samples of dried groundnut and 66 unprocessed samples of dried yam chip, were labeled, packaged in sample collection bags, and taken to the laboratory.

The fungi isolation and identification were carried out in Research Laboratory of Medical Microbiology and Parasitology Department, Ladoke Akintola University of Technology, Osogbo, Nigeria. Aflatoxin analysis of the food items was carried out in Aflatoxin Laboratory of International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

Three grams (3g) of each food sample was surface disinfected by dipping them in 3 % Sodium hypochlorite and sterile distilled water. 2g of food samples were randomly plated on PDA and incubated at 27°C for 3-5 days. The resulting colony suspected to be *A.*

flavus, A. parasiticus, or *A. niger / brasiliensis* were later subcultured on Aspergillus Flavus Parasiticus Agar (AFPA-Oxoid) with chloramphenicol (500 mg L^{-1}) supplement. The dishes were incubated at 30°C for 3-5 days (Tuli *et al.*, 2024).

Colonial morphology observations were carried out as shown in Table 1 and were compared with reference cultures from plant pathology laboratory of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Thereafter, microscopic identification using lactophenol cotton blue was carried out (Bankole *et al.*, 2023).

RESULTS AND DISCUSSION

Frequency of *Aspergillus spp.* isolates present in selected foodstuffs

Out of 199 samples of food products subjected to fungal analyses, 105/199, representing 52.7%, *Aspergillus* species comprising (*A. flavus, A. parasiticus,* and *A. niger/brasiliensis*) were isolated. *Aspergillus flavus* predominantly contaminated all the food products with 52.4%, followed by *Aspergillus parasiticus* at 27.6% and *Aspergillus brasiliensis* at 20.0% (Table 2).

In addition, this study also showed that food products from Igbona market had the highest *Aspergillus* contamination, followed by Ayegbaju and Akindeko, while Oluode market had the least *Aspergillus*-contaminated food products, as shown in Figure 1. The prevalence of *A. flavus* contamination in Nigeria aligns with trends observed in other African countries, where aflatoxin contamination poses serious food safety and public health risks.

Recent studies show that aflatoxin contamination is widespread in Nigeria. Research by Adefunke et al. (2023) highlights the presence of aflatoxins in cereals, spices, and locally fermented foods due to poor storage conditions and lack of awareness among farmers and consumers.

Similarly, Singh et al. (2022) found high genetic diversity of *A. flavus* in Nigerian chili peppers, with 65% of isolates producing aflatoxins, emphasizing the need for better mitigation strategies.

Across Africa, similar trends have been documented. In Kenya, aflatoxin outbreaks have had severe public health impacts, with maize contamination leading to fatalities. A study by Temba et al. (2023) established a strong correlation between *A. flavus* biomass and aflatoxin contamination in Kenyan maize, stressing the importance of preharvest fungal control. In Niger, a recent study by Falade et al. (2022) reported alarmingly high aflatoxin levels in staple crops such as maize, groundnut, and sorghum, with some exceeding regulatory limits by up to 2,000 times.

In Egypt, *A. flavus* contamination in maize and rice remains a concern, with studies recommending stricter monitoring to prevent exposure. Similarly, research in South Africa has confirmed aflatoxin presence in homegrown maize, with contamination levels often surpassing food safety limits (Ortega-Beltran et al., 2021).

Efforts to mitigate aflatoxin contamination through biocontrol methods have shown promise. The *Aflasafe* biocontrol technology, which uses atoxigenic *A. flavus* strains to competitively inhibit aflatoxin-producing fungi, has been successfully implemented in Nigeria, significantly reducing contamination levels in maize and groundnut (Bandyopadhyay et al., 2019).

Thin Layer chromatography

Aflatoxin B1, B2 G1 and G2 were detected in most food products. The content of the total aflatoxins ranged from not detectable to 9631 ppb. Groundnut from Igbona sold by vendor 2 had the highest level of total aflatoxin contamination of (9631 ppb) i.e fungi isolated from groundnut produced more quantity of aflatoxins as compared to fungi isolated from maize and dried yam chip as shown in Table 3 and Figure 2.

Fungal analysis of the food products

Aspergillus species are major public health problem of modern mycology (Zakaria, 2024). They are known to cause *Aspergillus* infection and mycotoxicosis especially upon the consumption of food contaminated with *Aspergillus* species. Food commodities in Osogbo local markets were found to be frequently contaminated by *Aspergillus* species. Virtually all kinds of food commodities collected for this survey were colonized by *Aspergilli.*

Out of 105 *Aspergilli* isolated in this study, high incidences of *Aspergillus flavus* were found in all food products that were collected. The results of high incidences of *Aspergillus flavus* in this study agrees with what has been previously reported in Nigeria in post-harvest maize (Kinyungu *et al.,* 2019). The highest recorded incidence of *Aspergillus spp*. in maize in the study was 80.1%.

Data from maize sample analyzed (Table 1) showed contamination by *Aspergilli* with prevalence of *Aspergillus flavus* (53.7%), *Aspergillus niger/brasiliensis* (9.0%) and *Aspergillus parasiticus* (18.0%) respectively. The result from this study is in line with other reports with high incidences of these *Aspergillus spp* in maize seeds from different parts of Nigeria (Massomo, 2020). Report from other parts of Africa and the world also showed high incidences of *Aspergilli* in maize seeds at different stage of cultivation (Nji *et al.,* 2022) which are contributory causes of different forms of health implications in man (Ráduly *et al.*, 2020).

Groundnut samples screened for fungal contamination (Table 1) showed different species belonging to genera *Aspergillus spp* with *A. flavus* contaminating most of the samples with (21.2%) incidence, *A. niger/brasiliensis* (13.6%) and *A. parasiticus* (16.7%) respectively. Prevalence of *A. flavus* could be attributed to improper storage practices of groundnut. This result is in agreement with the report from (Asare Bediako *et al.,* 2019).

Data from dried yam chips analysis showed contamination by *Aspergilli* with the prevalence of *A. flavus* (7.6%), *A. niger/brasiliensis* (9.1%) and *A. parasiticus* (9.1%) respectively. In addition, the *Aspergillus spp* growth in dried yam chips had the least prevalence which may be due to long and persistent drying process.

According to a previous study, *Aspergillus* species were the major contaminants of human food in storage (Tesfaye *et al.*,, 2024). The relative frequencies of *Aspergillus* species may be higher because of their ability to grow at large range of temperatures, harvesting methods and storage conditions of food commodities, and also the ability of *Aspergillus* to colonize almost all food commodities.

The *Aspergilli* contamination of food products in this study may be highly attributed to the climatic condition of Osogbo which is warm (average annual temperature of 26.1°C) and humid (average annual humidity of 85.6%) most time of the year, which are favourable conditions for growth of aflatoxigenic fungi and toxin synthesis.

Aflatoxin Contents of the selected food products

In this study, nineteen of the food samples that yielded *Aspergillus* mould were further analyzed for aflatoxins (B1, B2, G1 and G2). Out of which, 11(57.9 %) were contaminated by aflatoxins of varied concentrations ranging from 2 - ≥20 parts per billion (ppb), they all contained total aflatoxin above maximum acceptable limit for the unprocessed dried maize, dried groundnut and dried yam chips, (2- ≥20ppb) while 8 (42.1%) samples contained no aflatoxin above 1ppb.

Maximum acceptable limit of (≤20 ppb) for total aflatoxin (B1, B2, G1, and G2) in the food products recommended by Standard Organization of Nigeria (SON) was used in comparison with aflatoxin concentration of food products in this work and all the food products had total aflatoxin (B1, B2, G1, and G2) at levels far higher than all known maximum acceptable limit. The maximum concentration of total aflatoxin in this study is 9631ppb (**Table 2**) with mean value of 1221ppb that is higher than 20 ppb recommended by SON. The same concentration was higher than 158 µg/kg reported by (Monger *et al.,* 2024) from Tanzania. It was also higher than 138 µg/kg reported by (Matsiko *et al.,* 2017) from Nigeria. The maximum total aflatoxin concentration recorded from this study may be linked to extremely low level of awareness of the farmers and vendors of food products about prevention of aflatoxin contamination. The results from the study further showed that groundnut had highest level of aflatoxin contamination with maximum total aflatoxin concentration of 9631ppb sold by vendor 2 in Igbona market as compared to aflatoxin contamination in maize and dried yam chip which is in accordance with the study done by (Magan & Aldred, 2007). The concentration is higher than 51ppb reported by Jimoh and Kolapo, 2008 in Ibadan, Nigeria. Evidently, dried yam chip had the least aflatoxin contamination. Dried yam chips used to be exposed to the heat of the sunlight before being sold in the markets for drying purpose which inhibits aflatoxin (Kpodo, 1996). This could be the reason why low aflatoxin level was recorded. Valente *et al.* (2020) reported that proper heat drying can effectively limit the spread of harmful fungi that produce different mycotoxins especially aflatoxin.

Maize had most fungal infestation and aflatoxin contamination in most markets. This may be associated with chemical composition of maize been a better growth substrate for *Aspergillus spp* that are capable of aflatoxins production than the chemical components of most crops including yam chips and groundnuts**.** Hence, the higher fungal contamination with subsequent aflatoxin production in maize than the yam chips and groundnut is possible (Nsabiyumva *et al.,* 2023). The mean value for total aflatoxin concentration in maize is 885ppb which is higher than 177ppb with a similar finding reported by (Abdurrazaq *et al.,* 2022).

There was little variation in aflatoxin concentration among the markets as the highest total aflatoxin concentration was recorded in samples from the Igbona market. This might be due to higher humidity and temperature during storage as compared to the other three markets enrolled in the study. In addition, it might be due to the dusty nature of the Igbona market which promotes the growth of mold. No aflatoxin contamination was recorded in food products from the Akindeko market. This may be attributed to the relatively low dusty nature of the road within the market. From this study, household food products may likely represent a significant source of exposure to aflatoxin in Nigeria as it was the case in Ghana where health concerns have been raised (Kortei *et al.,* 2024).

CONCLUSION

The total aflatoxin level found in all the food products was higher than the range of the acceptable limit for consumption and exportation (2-20 ppb) from the TLC analysis. The data from this study suggested that the Nigerian government agency in charge of food control and regulation should set maximum tolerable limit standards for aflatoxin in other food products meant to be consumed by Nigerians as well as their entire food chain as results from this study are far above the recommended limit by SON.

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Table 1. **C**olonial morphology of the *Aspergillus* spp on AFPA

Organism	**Colonial Morphology on AFPA**
Aspergillus flavus	White mycelium, buffy spores, orange underside
Aspergillus parasiticus	White mycelium, cream spores, orange underside
Aspergillus brasiliensis	White mycelium, black spores, yellow underside

Table 2. Frequency of *Aspergillus spp* isolates present in selected foodstuffs

Foodstuffs	***A. flavus***	***A. parasiticus***	***A. brasiliensis***	**Total**
Maize (n=67)	36	12	6	54
Groundnut (n=66)	14	11	9	34
Yam chip (n=66)	5	6	6	17
Total	55 (52.4%)	29 (27.6)	21 (20.0%)	

Key: n- Total number of food products sampled

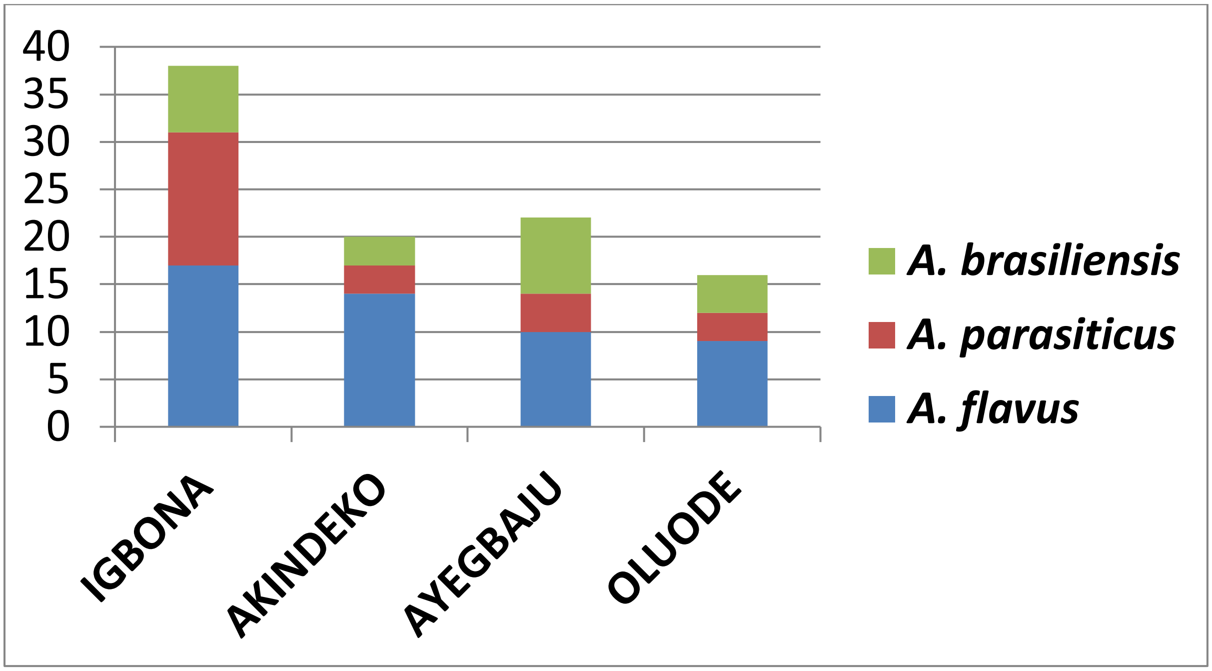

Figure 1. Rate of occurrence of *Aspergillus* spp associated with markets in Osogbo

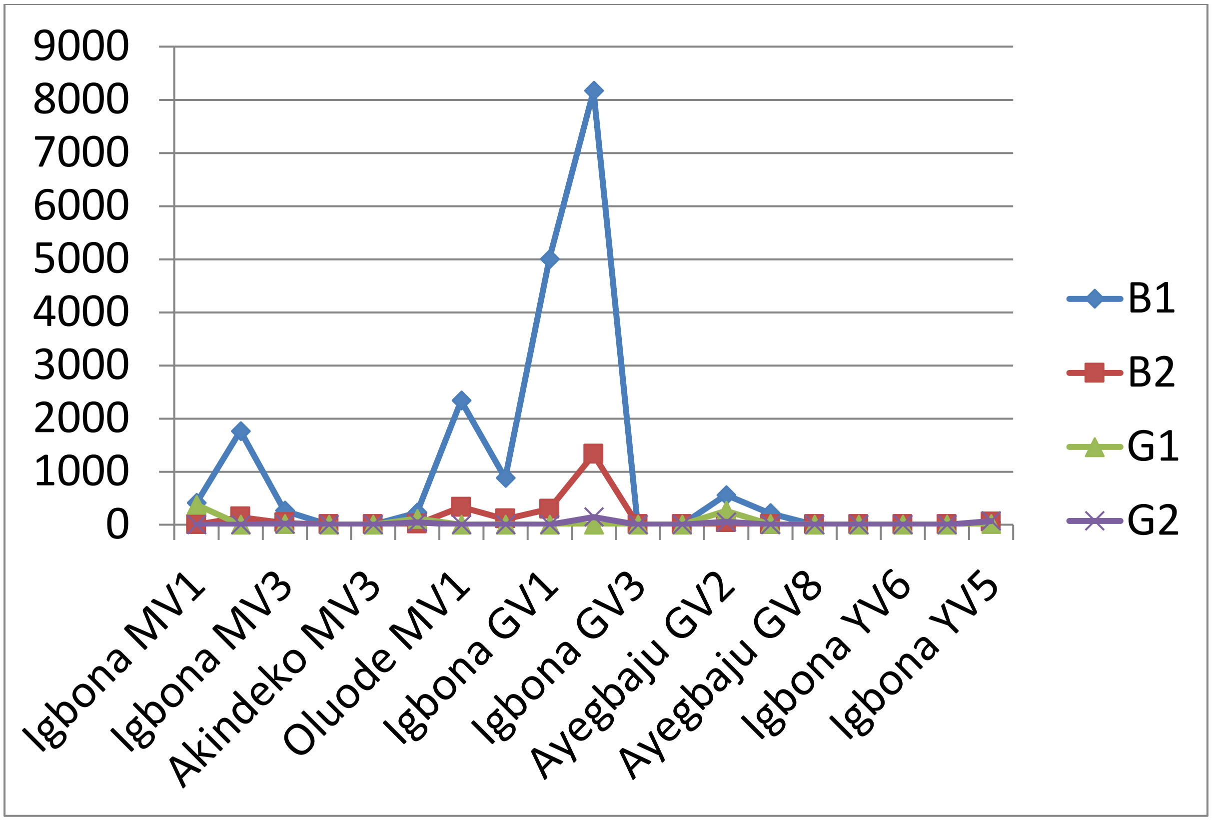

Figure 2. Concentration of aflatoxins B_1, B_2, G_1 and G_2 (Part per billion, ppb) based on the results obtained using Thin Layer chromatography with scanning densitometer