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Mycoflora, Aflatoxin Occurrence, and Consumer Risk Assessment in Marketed Groundnuts from Kinshasa (Democratic Republic of the Congo) and Minna (Nigeria)

[*1,2]Calvin Ciuma Ilunga K., [1,4]Helen Shnada Auta, [1,5]Hadiza Lami Muhammad, [3]Van Emery Tshiombe Mulamba, [1]Aminu Shaibu, [2]Freddy Bulubulu Otono, [2]Chantal Mbuyi Musadi
[1]African Centre of Excellence for Mycotoxins and Food Safety, Federal University of Technology, Minna.
[2]Department of Radiobiology, General Commission for Atomic Energy, Kinshasa Regional Centre for Nuclear Studies.
[3]Department of Radioagronomy, General Commission for Atomic Energy, Kinshasa Regional Centre for Nuclear Studies.
[4]Department of Microbiology, Federal University of Technology, Minna
[5]Department of Biochemistry, Federal University of Technology, Minna

Abstract
Aflatoxins, a group of highly toxic mycotoxins primarily produced by Aspergillus flavus and Aspergillus parasiticus, are common contaminants of groundnuts, particularly in subtropical regions. These toxins cause hepatocellular carcinoma (HCC), a type of liver cancer that ranks among the leading causes of cancer-related deaths worldwide, with a disproportionate impact on populations in developing countries, like SSA. This study aimed to assess the risk of aflatoxin exposure and estimate the HCC risk for populations in the Democratic Republic of the Congo (DRC) and Nigeria when eating groundnut. A total of 256 groundnut samples were collected from local markets in Kinshasa (DRC) and Minna (Nigeria). The fungal load, contaminating fungal species, and their occurrence were determined using standard microbiological techniques. Aflatoxin contamination levels were quantified using ultra-high-performance liquid chromatography (UHPLC). Risk characterisation was performed using the Margin of Exposure (MoE) approach, and the population-level HCC potency was also estimated using the standard protocol. The results revealed Aspergillus as the most prevalent fungal genus, followed by Penicillium, Fusarium, and Alternaria. A significant difference ($p < 0.05$) was observed in fungal loads between groundnuts from Kinshasa (14.4×10^2 CFU/g ± 1.92) and Minna (7.4×10^2 CFU/g ± 1.14). Aflatoxin contamination levels, with groundnuts from Kinshasa showing significantly higher levels (151.80×10^3 ng/kg ± 47.62) compared to those from Minna (14.20×10^3 ng/kg ± 1.55). The MoE indicated a potential health risk. The estimated HCC cases attributable to aflatoxin exposure were approximately 514,000 in the HBsAg+ Congolese population (8%) and 62,000 in the Nigerian population (13%).
***Key words**: Aflatoxins, Risk assessment, Groundnut, Fungi, Hepatocellular carcinoma.*

1.0 INTRODUCTION

Groundnuts, like many other agricultural products, are highly susceptible to contamination by mycotoxins, harmful secondary metabolites produced by fungal species such as *Aspergillus, Penicillium,* and *Fusarium* (Badmos *et al.*, 2023). Of these, aflatoxins are among the most prevalent and hazardous, contaminating a wide range of food products at multiple stages of the food supply chain, including farming, harvesting, storage, and processing (FFSA, 2009; Makun *et al.*, 2009; Ilunga *et al.*, 2025). The primary producers of aflatoxins are *Aspergillus* species, notably *A. flavus, A. parasiticus*, and *A. nomius*, which thrive under conditions of high temperature and humidity (Smith, 2020). In addition to environmental factors, poor agricultural practices, inadequate storage, improper handling, and weak regulatory enforcement further exacerbate the risk of contamination (FAO, 2018). These challenges are especially pronounced in sub-Saharan Africa (SSA), where the burden of mycotoxin contamination remains alarmingly high (Braicu *et al.*, 2019). Consequently, an estimated 500 million people in developing countries are exposed to dangerous levels of aflatoxins (Ezekiel *et al.*, 2019), prompting international regulatory bodies to set maximum allowable limits of 15 µg/kg by Codex Alimentarius (FAO/WHO JEFCA, 2022) and 4 µg/kg (Aflatoxin total), 2 µg/kg (aflatoxin B1) by the European Union for groundnuts intended for further processing (EFSA, 2020).

The health implications of aflatoxin exposure are profound, with aflatoxins recognised as major contributors to hepatocellular carcinoma (HCC), a leading cause of cancer-related deaths globally (Hamid *et al.*, 2013). This risk is particularly acute in developing regions such as SSA, where aflatoxin exposure is estimated to cause approximately 250,000 deaths annually (Li and Liu, 2010). Among the various aflatoxin types, aflatoxin B_1 (AFB_1) is the most toxic and frequently encountered, followed by B_2 (AFB_2), G_1 (AFG_1), and G_2 (AFG_2). Metabolites such as aflatoxins M_1 (AFM_1) and M_2 (AFM_2) are found in animal products such as milk when livestock consume contaminated feed (WHO, 2023). The International Agency for Research on Cancer (IARC) classifies aflatoxins as Group 1 carcinogens, with B forms being 10 to 100 times more toxic than G forms (IARC, 1993). The risk of HCC is further amplified in populations with high rates of hepatitis B virus (HBV) infection, as individuals who are HBV-positive have a 30-fold increased risk of developing HCC from aflatoxin exposure compared to HBV-negative individuals (Nugraha *et al.*, 2018). With SSA harbouring approximately 350 million HBV and 170 million hepatitis C virus (HCV) infections, the public health threat posed by aflatoxins *is* particularly severe (WHO, 2023).

Despite significant health risks, groundnuts remain a staple food and vital cash crop for millions of households across sub-Saharan Africa (SSA). Globally, groundnuts rank as the 13th most important food crop and the 4th most important oilseed crop, serving as both a vital dietary staple and a significant source of income for nearly a billion people in Africa (Manizan *et al.*, 2018). In this region, approximately 43 % of the population, particularly children, pregnant, and lactating women, suffer from chronic malnutrition, characterised by anaemia and deficiencies in vitamin A and iron (WHO, 2023). Recognising this, the Food and Agriculture Organisation of the United Nations (FAO, 1990) recommended that consuming a modest daily portion of groundnuts (~30g) could address many cases of malnutrition in developing regions such as sub-Saharan Africa (Makun *et al.*, 2009). However, this nutritional promise is overshadowed by a serious health risk. According to Atanda *et al.* (2013), aflatoxin contamination in maize and groundnuts is linked to an estimated 77,761 liver cancer cases annually in the region, resulting in over 100,000 disability-adjusted life years (DALYs) lost each year. This starkly highlights the paradox of groundnuts: while they are essential for nutrition and livelihoods, they also represent a major source of harmful aflatoxin exposure.

The prevalence of aflatoxin food contamination has been the subject of a few studies across the Democratic Republic of the Congo (DRC) (Udomkun *et al.*, 2018), and reports widespread contamination, with groundnuts and other crops (maize, cassava, rice, potatoes, banana) frequently exceeding international safety thresholds. For instance, Brudzynski *et al.* (1977) found all tested samples from local markets contaminated, and Kamika *et al.* (2013) reported that 95 % of groundnut samples from Kinshasa markets contained aflatoxin B_1, with 75% exceeding safety limits. Similarly, Matendo *et al.* (2022) found 100 % of maize samples in South Kivu contaminated with aflatoxins and fumonisins. More recently, Kasongo *et al.* (2024) reported that a significant proportion of maize flour and groundnut paste from Kinshasa surpassed EU aflatoxin limits. However, the true extent of the risk to public health remains uncertain due to a paucity of data and the absence of a comprehensive national policy on mycotoxin management.

Comparable trends are observed in Nigeria, where groundnut contamination is pervasive. Over 80 % of groundnut samples have been found to contain aflatoxins, with about a quarter exceeding the national regulatory limit of 20 µg/kg (Akullo *et al.*, 2025). High levels of aflatoxin B_1 and total aflatoxins have been reported, with margin of exposure (MoE) values indicating a significant carcinogenic risk, particularly for children (Oyedele *et al.*, 2017; Misihairabgwi *et al.*, 2019; Magomya and Mbatsav, 2023). Wenndt *et al.* (2023) estimated an HCC incidence rate of 2.6 cases per 100,000 population in Nigeria attributable to dietary aflatoxin exposure from maize and groundnut consumption. Similarly, the risk of aflatoxin exposure is not limited to groundnuts and maize. Badmos *et al.* (2023) estimated that aflatoxin exposure from sorghum contributes to approximately 5.99×10^5 HCC cases annually among HBsAg-positive individuals, based on a 13.6 % prevalence of hepatitis B virus (HBV) infection. This finding highlights the significant public health risk and dietary exposure to aflatoxins through the consumption of other food crops, raising important concerns for both health and trade in Nigeria.

Given these challenges, this study aims to evaluate dietary exposure to aflatoxins and the associated health risks for consumers of groundnuts purchased in open markets in Kinshasa (DRC) and Minna (Nigeria). Although these two countries differ ecologically, they share similar socio-economic challenges, including informal marketing systems, inadequate transportation, limited access to essential materials and equipment, insufficient knowledge of pre- and postharvest management, and weak regulatory enforcement. By comparing these settings, the study highlights the urgent need for targeted interventions to reduce aflatoxin exposure and associated cancer risks. Ultimately, this research underscores the importance of localised risk assessments in informing effective mycotoxin management policies tailored to the diverse conditions across African regions.

2.0 MATERIALS AND METHODS

2.1. Study area

This study was conducted in Kinshasa, RDC, and Minna, Niger State, Nigeria. The city of Kinshasa is both the capital and a province of the DRC. It is located between latitudes 4° and 5° and longitudes 15° and 16°32 East as illustrated by the location map in Plate 1. It covers an area of 9,965 square kilometres on the southern bank of the Congo River, opposite the capital of the Republic of Congo, Brazzaville, at an average altitude of 300 m. The climate is characterised as hot and humid tropical (type AW4), with an average annual temperature of 25°C and an average annual rainfall of 1,400 mm, accompanied by 87 % humidity. The soil is mainly sandy and poor for farming (Nyembo *et al.*, 2013), and agricultural produce is supplied from other provinces in the country by road or river (Congo River) to cover the food needs of a population estimated at over 17 million. The DRC's socio-economic context significantly exacerbates the risk of mycotoxin contamination. Informal marketing systems and inadequate transportation infrastructure lead to prolonged storage and handling under suboptimal conditions, increasing fungal growth and toxin accumulation (Mulunda *et al.*, 2013). Lack of governmental regulations and poor enforcement of food safety standards further compound the problem. Additionally, the country's history of conflict has negatively impacted health, education, and living standards, which in turn affect food security and the capacity to implement effective mycotoxin control measures. Food insecurity and malnutrition are common, especially among children, making the interplay between food safety and food adequacy a critical challenge in the country (Udomkun *et al.*, 2018)

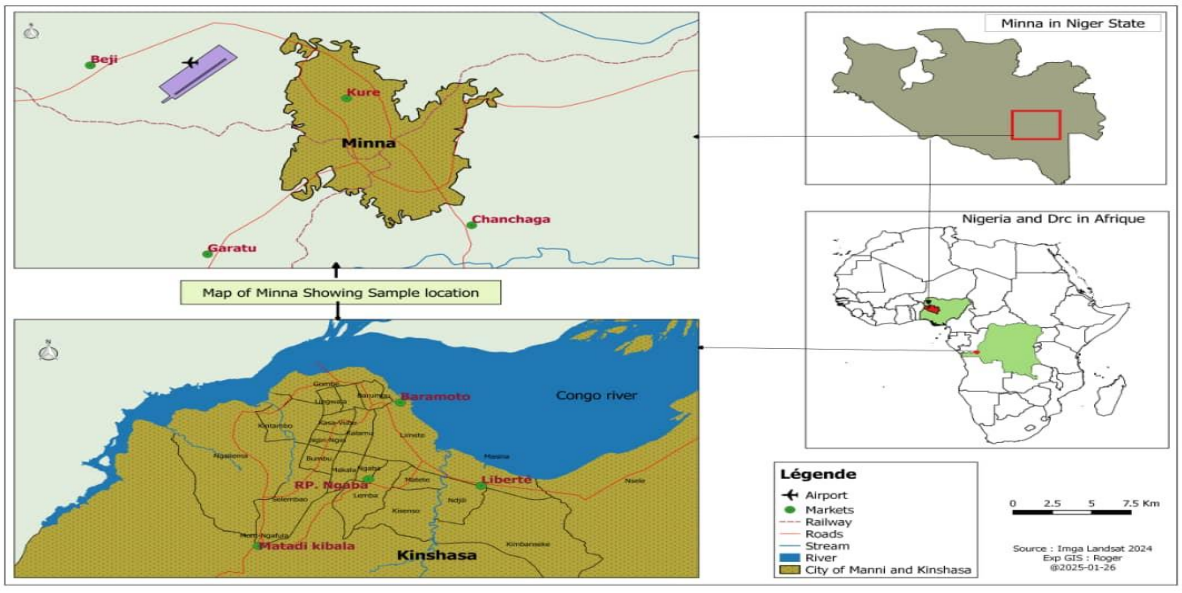

Figure 1 Location of sampling site

Minna is the capital of the Niger State. Located in North-central Nigeria (Lat. 90 27' N and Long. 60 33' E). It is the largest state in the country, covering 76,363 Km^2 and having an estimated population of 1.2 million inhabitants (Omalu *et al.*,2015). Niger State, in the middle belt of Nigeria is categorised into four microclimatic zones, namely: the wettest with annual rainfall above 1400 mm; the damp and dry with annual rainfall ranging from 1200 to 1400 mm and 1000 to 1200, respectively and the driest, with an

annual rainfall on less than 1000 mm (Merem *et al.*, 2017). The climatic and ecological conditions in Niger State combine to support flourishing agricultural practices (groundnuts, maize, sorghum, rice) which have earned the region the status of a "food basket". Niger state is categorised under a hot and humid climate for most of the year, especially between the fifth and tenth month of every year (29.5 ℃ and 73.1 %) which is favourable for fungal growth and mycotoxin production (Mukhtar, 2019). Regulatory frameworks and enforcement mechanisms for mycotoxin control remain weak or underdeveloped compared to developed countries, allowing contaminated food to enter the market and food chain. These socio-economic factors are compounded by food insecurity and poverty, which limit consumers' ability to avoid contaminated foods (Wenndt *et al.*, 2023).

2.2 Sample collection

A total of 256 groundnut samples were randomly purchased from vendors in four major markets in Kinshasa and Minna, with 32 samples collected per market, resulting in a total of 128 samples from each city. The markets selected in Kinshasa are nominally Liberte, Baramoto, Matadi Mayo, and Rond Point Ngaba, whereas Beji, Garatu, Kure, and Chanchaga markets were selected in Minna. Sample collection was conducted during the rainy season in March 2024 (Kinshasa) and June 2024 (Minna) following the European Food Safety Authority (2020): 1kg of each sample was stored in sterile Ziplock bags, properly labelled, and transported to the laboratory at the African Centre of Excellence for Mycotoxins and Food Safety (ACEMFS) in Minna, Niger State, Nigeria. In the laboratory, four groundnut samples from each market were combined to form a composite sample, resulting in eight composite samples per market and a total of 64 composite samples. One kilogram of each composite sample was aseptically transferred into sterile polyethene bags and stored at 4 °C until laboratory analysis.

2.4 Sample Preparation

Each 1 kg groundnut sample was ground using a Numeral HC-250Y automatic grinder, which has a capacity of 250 g, a grinding fineness of 300 μm, and operates at a rotation speed of 2800 rpm, with a working time of 5 minutes and 10-minute intervals, and a nominal power of 1500 W. The resulting powder was fine and well mixed to obtain a homogeneous sample. The powders were stored in sterile Ziplock bags at 4 °C for laboratory analysis (Silva *et al.*, 2019). To prevent cross-contamination, the blender was disinfected after each sample grinding by cleaning it with water and 70 % ethanol, followed by drying, before processing the next sample.

2.5 Moisture Content Determination

The moisture of groundnut samples was determined using the AOAC 925.10 (2000) gravimetric technique. An empty weighing dish was cleaned, dried, and weighed (MO). Five grams of groundnut samples were added to the dish, and the new mass was taken (M1). The vessel containing the sample was then placed in an oven at 105° C for 24 hours. After drying, the dish was removed from the oven and cooled inside a desiccator before weighing (M2) again. The moisture content of the samples was calculated using equation 1:

$$(\%M) = \frac{(M1-M2)}{M1-M0} \times 100 \qquad (1)$$

2.5 Fungal Isolation, Enumeration, and Identification

Fungi isolation was carried out using the plate dilution method as outlined in International Standard ISO 7218 (2017), with a slight modification (Badmos *et al.*, 2023). The milled sample (1g) was weighed into a sterile tube, suspended in physiological water solution (9ml of 0.9% NaCl), and shaken for 2 minutes on a vortex mixer. The suspension was serially diluted 10-fold. Aliquot (100 μL) of 10^2 diluent was inoculated on solid potatoes dextrose agar (PDA) containing 1 % Chloramphenicol in 90-mm Petri dishes and incubated at 28°C for 48-72 hours. After incubation, the fungal colonies were counted using a colony counter. The number of colonies /g of the sample was counted and expressed in colony-forming units/g as represented in equation 2.

$$cfu/_g = \frac{Number\ of\ colonies\ x\ Reciprocal\ of\ Dilution\ factor}{Volume\ plated} \qquad (2)$$

The frequency of occurrence of the isolated fungi species from the groundnut sample was calculated using Equation 3.

Frequency rate of fungal species (%) = $\frac{Number\ of\ isolates}{Total\ number\ of\ isolates} \times 100$ (3)

Fungal isolates were sub-cultured onto fresh malt extract agar (MEA), yeast extract agar (YEA). Identification was based on the examination of colonial morphology (conidial and conidiogenous cells) on agar plates, coupled with microscopy observations using lactophenol cotton blue stain. The observations were compared to relevant identification keys and atlases in the literature to confirm the identity of the fungal isolates (Pitt and Hocking, 2006).

2.6 Determination of Aflatoxin by Ultra High-Performance Liquid Chromatography (UHPLC-Rayleigh LC 100)

2.6.1 Aflatoxins Extraction and Purification Procedure

The extraction and purification of mycotoxins from food samples followed a modified version of the European Standard EN 14123 as outlined by Kortei *et al.* (2022). This process involved blending, filtering, diluting, and clean-up steps to isolate mycotoxins for analysis. A 50 g portion of each ground sample was weighed into a solvent-resistant blender jar and combined with 5 g of sodium chloride. To extract the toxins, 100 mL of ~~70%~~ 80% methanol was added, and the mixture was homogenised at high speed for 2 minutes. The blend was filtered through Whatman No. 113 filter paper, and the clear extract was collected in a conical flask. From this extract, 2 mL was diluted with 14 mL of phosphate-buffered saline (PBS) to prepare it for clean-up. A 10 mL volume of this diluted solution was passed through an immunoaffinity column designed for mycotoxins at a flow rate of 2 mL/min. This allowed the target toxins to bind to the column. The column was then washed with 20 mL of ultrapure water at about 5 mL/min to remove impurities. The bound toxins were then released using 2 mL of HPLC-grade methanol at a rate of one drop per second. The eluate was collected in amber glass vials to prevent breakdown by light and stored at 4°C until analysed using ultra-high-performance liquid chromatography with a fluorescence detector (UHPLC-FLD).

2.6.2 Standard Preparation

The certified reference standard (purchased from Sigma-Aldrich, Vienna, Austria) was a mixture of aflatoxins B_1, B_2, G_1, and G_2 at a 10 µg/ml concentration. Working standards of 10 µg/kg, 5 µg/kg, and 2.5 µg/kg were prepared by appropriate dilution with acetonitrile. These standards were then injected into the UHPLC machine under the chromatographic conditions indicated (wavelength: 360 nm, column temperature: 26.4°C and humidity 49%, flow rate: 0.8 ml/minute). Calibration was performed automatically by the HPLC and used to calculate sample values.

2.6.3 Chromatographic Separation Using UHPLC

Mycotoxins, including AFB_1, B_2, G_1, and G_2 in grains and nuts, were quantified using UHPLC-FLD, adapted from Kortei *et al.* (2022). Separation was achieved using a Spherisorb ODS1-Excel column (4.6 mm × 25 cm, 5 µm, 250 Å) with a mobile phase of methanol and water (50:50 v/v) at a flow rate of 1 mL/min. The detector was set at 360 nm excitation and 440 nm emission. The injection volume was 10 µL, and the column temperature was maintained at 40°C. Sensitivity of the method was evaluated using the limit of detection (LOD) and limit of quantification (LOQ) calculated from a standard curve using the formulas:

LOD = (3 × SD)/slope and LOQ = 3 × LOD.

To confirm method accuracy, mycotoxin-free samples were spiked at three levels: 5, 15, and 30 ppb. The volume of standard added was calculated using:

Volume (mL) = (sample weight (g) × desired ppb) / standard concentration (µg/mL).

Recovery percentage was calculated using:

% Recovery = [(measured concentration – blank concentration) / added concentration] × 100.

Precision was assessed with internal reference material (IRM) under repeatability and intermediate conditions. Ten parallel runs by the same operator and ten separate runs by another operator on different days were performed. Relative standard deviation (RSD) was calculated as: RSD = (SD/Mean) × 100.

2.7 Risk characterisation of aflatoxins

In this study, characterisation risks were assessed using the margin of exposure (MoE) approach, as proposed by Benford *et al.* (2010) and EFSA (2013), for all mycotoxins classified as genotoxic and carcinogenic, such as aflatoxins (IARC, 2013). The MoE is defined as the ratio between the no-observed-adverse-effect level (NOAEL) and the estimated or predicted human intake of a substance. Specifically, it

was calculated by dividing the Benchmark Dose Lower Limit (BMDL) by the estimated daily intake (EDI) of each aflatoxin, as outlined in equation (4). The BMDL represents the lower confidence limit (95%) of the dose associated with a small but measurable increase in risk (typically a 10% extra cancer risk) in rodent studies. Since the magnitude of the Margin of Exposure (MOE) based on human data has not yet been established, the Benchmark Dose Lower Confidence Limit for a 10% increased cancer risk (BMDL10) of 170 ng/kg body weight per day derived from animal study data modelling was selected as the Point of Departure (POD) for dose-response modeling of aflatoxins (EFSA, 2007).

$$MoE = \frac{Benchmark\ Dose\ lower\ Limit\ (BMDL)}{Estimated\ Daily\ Intake\ (EDI)} \quad (4)$$

The MoE concept suggests that an MoE value of > 10,000 should be considered 'safe', while an MoE value ≤ 10,000 could pose a potential risk to public health. Furthermore, it has been demonstrated that the lower the value, the higher the risk (Heshmati *et al.*, 2017).

The EDI (ng/kg bw/day) for each aflatoxin was calculated using the quantity of aflatoxin in the groundnut samples multiplied by the average groundnut consumption and then divided by the body weight of consumers as demonstrated by Liu and Wu (2010) in equation (5).

$$\text{EDI (ng/kg. bw/day)} = \frac{Cm \times k}{bw} \quad (5)$$

**Cm: average level of Aflatoxin present in a sample (ng/kg), k: the amount of groundnuts ingested daily (g/day), $body$: body weight.*

The body weights of groundnut consumers (60 kg for those above 18 years of age) and the amount of groundnuts consumed per person per day (52 g) were considered in this study. These data were obtained from each county's Global Environmental Monitoring System Food Cluster Database (Liu and Wu, 2010; Petersen, 2018) and the African Union Commission & Health Status SSA data (He *et al.*, 2020).

2.8 Estimation of Population Risk for Aflatoxin-Induced Hepatocellular Carcinoma

The population risk for aflatoxin-induced HCC was estimated using the approach described by the JECFA in 1998 and updated during the eighty-third meeting of the JECFA held in Rome in 2016 (WHO, 2017a). The AFs-induced HCC risk was simulated as shown in Equation (6) by multiplying the probable average cancer potency.

$$\boldsymbol{Population\ HCC\ Risk} = \frac{\boldsymbol{EDI(ngkg \times bw/day) \times HCC\ Potency \times N}}{\boldsymbol{100{,}000}} \quad (6)$$

N = Total population (HBsAg + and HBsAg-)

Given the synergistic hepatocarcinogenic effects of AFs and hepatitis B virus infection, the International Programme on Chemical Safety (IPCS)/WHO (1998) and WHO, (2017a) selected two distinct cancer potency factors for aflatoxin based on the presence of the HBV surface antigen (HBsAg), which is a biomarker of chronic HBV infection. For individuals without chronic HBV infection (HBsAg-), the cancer potency P was estimated to be 0.01 cases per year per 10^5 population per nanogram aflatoxin per kg bw per day. For individuals with chronic HBV infection (HBsAg+), the cancer potency P was estimated to be 0.30 cases per year per 10^5 nanograms of aflatoxin per /kg. body weight per day. The prevalence rate of HBsAg+ in the Congolese and Nigerian populations was 8 % and 13 % respectively (Liu and Wu, 2010). The average cancer potency P of Congolese people, as shown in equation (6), was estimated with the percentage of both carriers (% Population HBsAg+ = 0.08) and non-carriers (% Population HBsAg- = 0.92), recalling the JECFA, (WHO, 2017a) estimated carcinogenic potency of AFs for carriers (PHBsAg+ = 0.3 cancer/year/100,000 persons) and non-carriers (PHBsAg- = 0.01 cancers/year/100,000 individuals).

Then

$$Average\ HCC\ Potency = 0.3 \times P + 0.01 \times (1 - P) \quad (7)$$

Considering P, the hepatitis-B-virus surface antigen (HBsAg+) prevalence rate for the Congolese general population:

Then

$$\textbf{Average HCC Potency} = 0.3 \times \mathrm{P} + 0.01 \times (1 - \mathrm{P}) = 0.3 \times 8\% + 0.01 \times 92\% = 0.0332$$

case of cancers per year per 10^5 population per nanogram of aflatoxins per kg body weight per day.

For the Nigerian population, the average HCC Potency $= 0.3 \times P + 0.01 \times (1 - P) = 0.3 \times 13\% + 0.01 \times 87\% = 0.0477$ case of cancer per year per 10^5 population per nanogram of aflatoxins per kg body weight per day.

2.9 Statistical analysis

The results were expressed as the mean ± standard error of the mean (SEM) and analysed using one-way analysis of variance (ANOVA). Pearson's correlation coefficient was employed to assess the relationship between aflatoxin levels and fungal load in contaminated groundnut. These analyses were conducted using SPSS Version 28.0, developed by IBM Corp., Armonk, NY, USA. The uncertainty of the results was set at the acceptability threshold of 5%.

3.0 RESULTS AND DISCUSSION

3.1 Mycoflora isolates in groundnut

The analysis of groundnut samples from Kinshasa and Minna local markets (figure 2 and Figure 3) reveals a diverse spectrum of fungal contamination, with a clear dominance of *Aspergillus* species, particularly *Aspergillus niger* and *Aspergillus flavus*. These two species together account for over half of all fungal isolates, with *A. niger* representing 29.10% and *A. flavus* 26.12% of the total isolates. Other notable fungi include *Penicillium* spp. (12.69%), *Mucor fragilis* (12.69%), and several less frequent genera such as *Fusarium, Candida, Absidia, Geotrichum*, and rare occurrences of *Rhizopus arrhizus* and *Curvularia* spp.

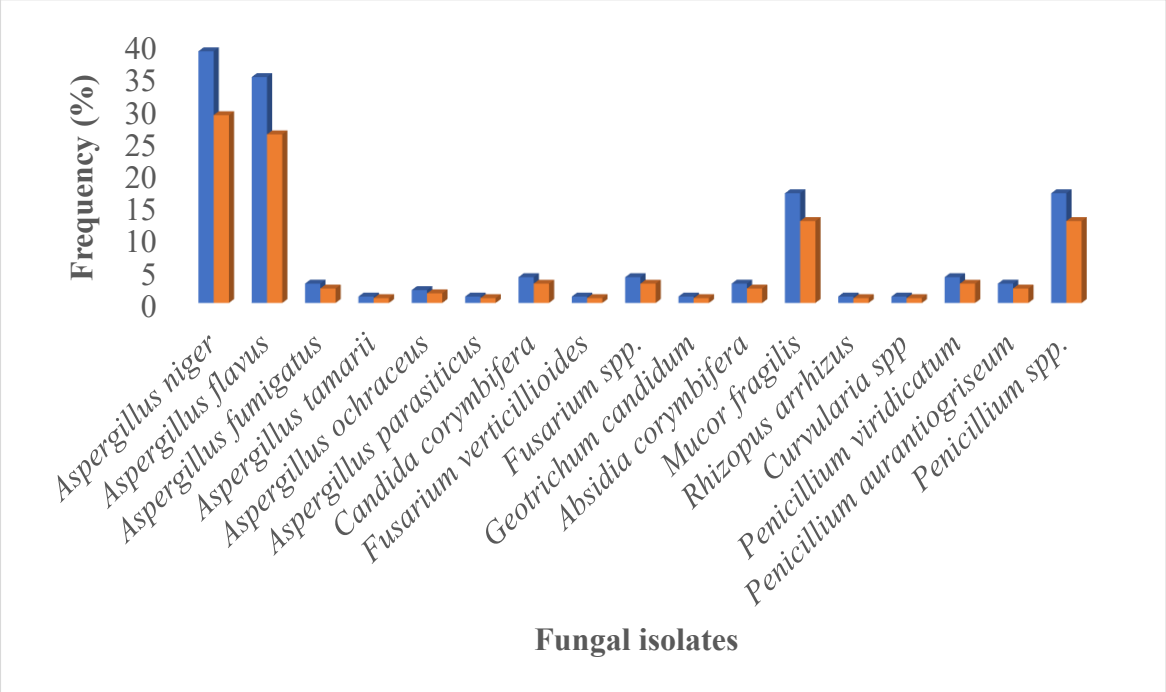

Figure 2: Fungal Isolates of Groundnut Samples from Kinshasa Local Markets

The predominance of *Aspergillus niger* and *Aspergillus flavus* in groundnut samples aligns with findings from other African countries, especially those with warm and humid climates that favor the growth of these fungi (Kamika *et al.*, 2013). This pattern is consistently observed across the Democratic Republic of the Congo (Yalala *et al.*, 2019; Mulunda *et al.*, 2013), Nigeria (Makun *et al.*, 2009; Oyedele *et al.*, 2017; Badmos *et al.*, 2023), and other SSA countries (Bediako *et al.*, 2019; Bidounga *et al.*, 2023). Collectively, these studies highlight *Aspergillus* as the most pervasive fungal contaminant of groundnuts and other agricultural produce, with *Penicillium* and *Fusarium* genera following in prevalence.

The high susceptibility of groundnuts to fungal contamination is further explained by their geocarpic nature, the pods develop and mature underground, creating a natural interface between the soil and the crop. This permanent soil-pod contact provides an ideal environment for fungi, particularly moulds, to colonise the pods (Dorner, 2008). Consequently, the abundant fungal flora found in groundnuts reflects both environmental conditions and the crop's unique growth characteristics, underscoring the challenges in managing fungal contamination in these staple foods.

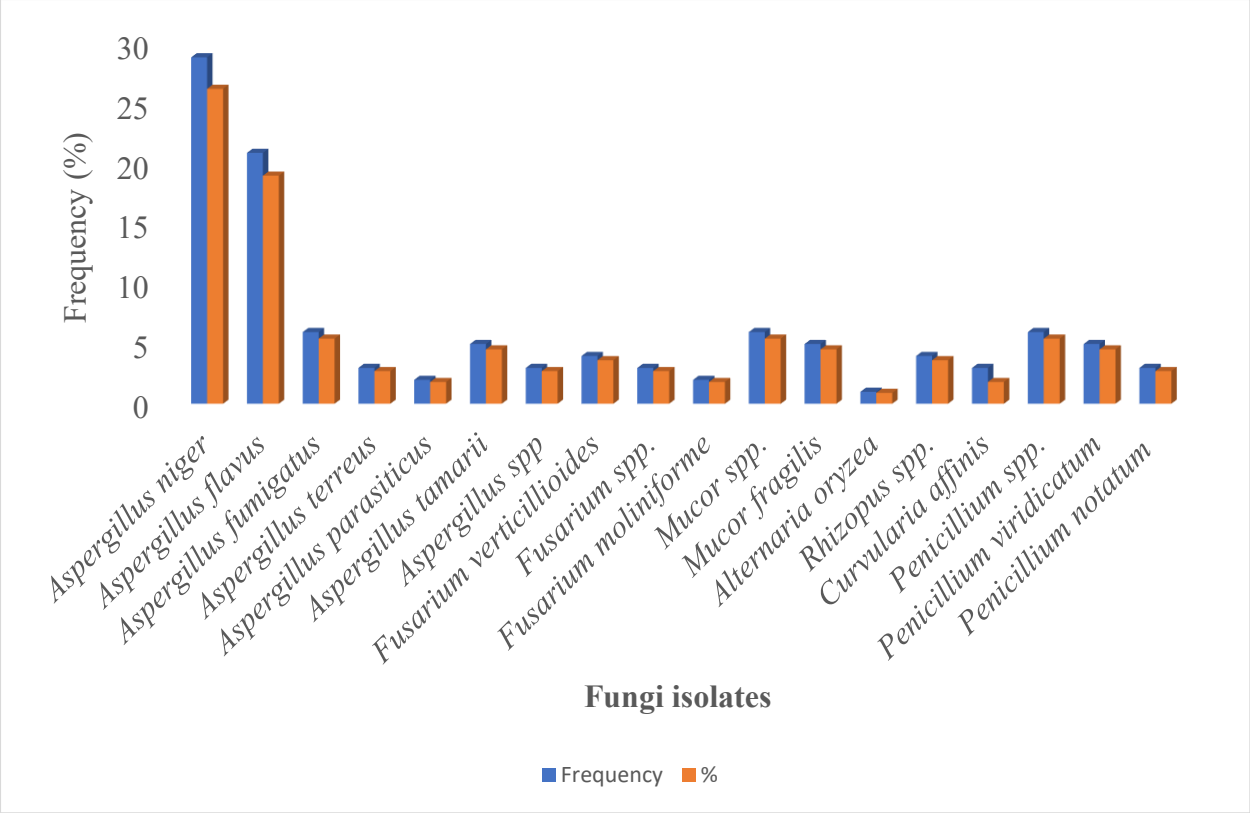

Figure 3 Fungal Isolation of Groundnut Samples from Minna Local Markets

The fungal load of the analysed groundnut samples from Minna and Kinshasa local markets exhibited variability, as demonstrated in Table 1. Variations were observed in the mean fungal load between markets and between countries. The fungal load of groundnut samples from Bara ($21.5\pm4.53x10^2$ CFU/g), Matadi Kibala (15.0 ± 3.42 $x10^2$ CFU/g), Liberte (10.9 ± 3.63 $x10^2$ CFU/g), Rond point Ngaba (10.0 ± 2.65 $x10^2$ CFU/g) markets in Kinshasa and Chanchaga ($10.1\pm2.57x10^2$ CFU/g) and Garatu ($9.0\pm2.40x10^2$ CFU/g) markets in Minna were statistically similar but higher ($P<0.05$) compared to fungal load counts of groundnuts samples from Kure ($5.9\pm1.75x10^2$ CFU/g) and Beji ($4.5\pm2.17x10^2$ CFU/g) in Minna.

While the mean fungal count between samples from the two study locations was found to be significantly different ($P<0.05$), with Kinshasa markets (14.4 ± 1.92 $x10^2$ CFU/g) recording higher mean fungal counts compared to Minna markets (7.4 ± 1.14 $x10^2$ CFU/g).

However, there is a very strong positive correlation between fungal load and moisture content in groundnut samples from both Kinshasa and Minna markets (Pearson's $r \approx 1.0$), highlighting the critical role of moisture control in minimising fungal contamination and the associated health risks during groundnut storage and marketing. This correlation aligns with the observed differences in fungal contamination between the two locations. In particular, the moisture content of samples from Kinshasa was marginally above the critical threshold (<10%) necessary to inhibit microbial growth, creating a favourable environment for fungal proliferation (Oyedele et al., 2017). The region's relatively high temperature and humidity further exacerbate this risk, promoting the growth of fungal species and increasing the susceptibility of groundnuts to contamination, thereby compromising their safety for consumption (Kalule Okello *et al.*, 2013; Zuza Júnior, 2016). In contrast, groundnut samples from Minna exhibited lower fungal loads, likely due to the combination of hotter temperatures and lower humidity levels, conditions less conducive to fungal growth (Atanda *et al.*, 2013). Together, these findings underscore the importance of maintaining moisture levels below critical limits and considering local climatic factors to effectively reduce fungal contamination in groundnut supply chains.

Table 1: Average fungal count and Humidity of groundnut samples collected from local markets in Kinshasa and Minna.

Location	Market (n=8; N=64)	Markets Mean Fungal count ($x10^2$ CFU/g) ± SEM	Country Mean Fungal count ($x10^2$ CFU/g) ± SEM	Market Mean Moisture content ± SEM	City Mean Moisture content (%H) ± SEM
Kinshasa	Matadi mayo	15.0±3.42[a]	14.4±1.92[a]	9.07±0.45[a]	9.55±1.16[a]
	Liberte	10.9±3.63[a]		9.97±1.56[a]	
	Baramoto	21.5±4.53[a]		11.05±2.38[a]	
	Rond point Ngaba	10.0±2.65[a]		8.1±0.25[a]	
Minna	Chanchaga	10.1±2.57[a]	7.4±1.14[b]	4.83.1±0.22[b]	4.03±0.37[b]
	Beji	4.5±2.17[b]		4.00±0.25[b]	
	Kure	5.9±1.75[b]		3.71±0.12[b]	
	Garatu	9.0±2.40[a]		3.59±0.64[b]	

Values are presented as mean ± standard error of the mean (SEM). Values in the same column with different superscripts are significantly different at $p < 0.05$, n: analysed sample, N: total sample

Table 2: Correlation between the fungal load isolates and the moisture content of groundnut

Statistic	Value
Pearson's r	~1.0
Relationship	Strongly correlation

Table 3 Aflatoxin Occurrence in groundnut sample

Cities	AFs	AFs Occurrence (%)	Occurrence (%) > threshold	
			UE	FAO
Kinshasa (n=32)	AFB_1	28 (87.50)	28 (87.50)	16 (50)
	AFB_2	26 (81.25)	18 (56.25)	10 (31.25)
	AFG_1	20 (62.50)	15 (46.88)	6 (18.75)
	AFG_2	15 (46.87)	13 (40.63)	3 (9.38)
Total		**22.25 (69.63)**	**18.50 (57.81)**	**8.75 (27.34)**
Minna (n=32)	AFB_1	27 (84.38)	24 (75)	7(21.19)
	AFB_2	23 (72.65)	9 (28.12)	0.00
	AFG_1	26 (81.25)	5(15.62)	0.00
	AFG_2	21 (65.62)	2 (6.3)	0.00
Total		**24 (75.78)**	**10 (31.25)**	**1.75 (5.47)**

3.2 Aflatoxin-contaminated groundnut

Table 3 summarises the occurrence of four aflatoxins (AFB_1, AFB_2, AFG_1, and AFG_2) in groundnut samples collected from two cities: Kinshasa (DRC) and Minna (Nigeria), based on 32 samples from each location. The results indicate that all four aflatoxins were detected in the samples, with varying frequencies across different markets and cities. Overall, the rate of aflatoxin contamination was higher in Minna (75.78 %) compared to Kinshasa (69.63 %). AFB_1 was the most prevalent toxin, found in 84.38% of samples from Minna and 87.50 % from Kinshasa. Notably, some markets such as Baramoto in Kinshasa and Garatu in Minna exhibited 100 % contamination of samples by AFB_1. The second most prevalent aflatoxin in Kinshasa was AFB_2, detected in 81.25 % of samples, while in Minna, AFG_1 was the second most frequent. The AFG2 were present in samples from both cities, with detection frequencies ranging from 46.87 % to 65 %.

The co-occurrence of aflatoxins in groundnuts observed in this study is consistent with previous research and is primarily attributed to colonisation by multi-mycotoxin-producing fungi or simultaneous colonisation by multiple fungal species, each capable of synthesising different aflatoxins. Notably, contamination with two aflatoxins affected approximately 80 % of samples in both Kinshasa and Minna, while simultaneous contamination by all four major aflatoxins (AFB_1, AFB_2, AFG_1, and AFG_2) was detected in 53.1 % of Minna samples and 33.3 % of Kinshasa samples. A high proportion of samples from Kinshasa exceeded the European Union (EU) limits, with 87.5% for AFB1, 56.25% for AFB2, 46.88% for AFG1, and 40.63% for AFG2. In comparison, the Codex Alimentarius limits were exceeded by 50% of samples for AFB1, 31.25% for AFB2, 18.75% for AFG1, and 9.38% for AFG2, highlighting a significant risk of multi-toxin exposure in this region. Conversely, in Minna, the rates of exceedance above EU limits were lower 75% for AFB1, 28.12% for AFB2, 15.62% for AFG1, and 6.3% for AFG2, with only 21.19% of samples surpassing the Codex limit. for AFB1 and none exceeding it for AFB2, AFG1, or AFG2.

The high frequency of samples from Kinshasa exceeding both EU and Codex limits signals a severe food safety risk, with half or more of the samples above international safety thresholds for AFB1. A comparison with international data, which reports an 8.1% exceedance rate and aflatoxin concentrations ranging from 15.56 to 973.21 µg/kg in commercial groundnuts, shows that Kinshasa exhibits some of the highest exceedance rates globally. In contrast, Minna's contamination levels are generally consistent with, or only slightly above, the global average for aflatoxin contamination in groundnuts (Alharbi *et al*., 2024). This indicates a pressing need for intervention and stricter control measures, as several fungal species isolated in our study are well-documented multi-mycotoxin producers. For example, *Aspergillus flavus* predominantly produces aflatoxins B_1 and B_2, whereas *Aspergillus parasiticus* synthesises all four major aflatoxins B_1, B_2, G_1, and G_2. Additionally, *Aspergillus tamarii* has been reported to produce aflatoxins B1 and B2, albeit less frequently (Frisvad *et al*., 2019). Although traditionally *A. flavus* was

considered to produce only B-type aflatoxins, some studies have reported strains capable of producing G-type aflatoxins, suggesting strain-dependent variability that warrants further investigation (Camiletti *et al.*, 2017). In addition, the complex ecology of *Aspergillus* species and their interactions can lead to synergistic effects, enhancing aflatoxin production under favourable environmental conditions (Misihairabgwi *et al.*, 2019). This multifaceted contamination underscores the importance of comprehensive monitoring and control strategies targeting multiple aflatoxin types and fungal species to effectively mitigate health risks associated with aflatoxin exposure.

Regarding the level of aflatoxin in groundnut, Table 4 presents the mean concentrations (µg/kg ± SEM) of four aflatoxin types AFB_1, AFB_2, AFG_1, and AFG_2, as well as total aflatoxins (AFs) measured in groundnut samples from local markets in Kinshasa (DRC) and Minna (Nigeria). The data reveal significant differences in aflatoxin contamination levels between the two cities, with important implications for food safety and public health. Specifically, groundnut samples from Kinshasa markets generally exhibited much higher mean aflatoxin concentrations than those from Minna. The highest contamination was recorded in the Baramoto market, where AFB_1 levels reached 229.4 ± 76.17 µg/kg, AFB_2 was 59.9 ± 26.11 µg/kg, AFG_1 was 57.4 ± 37.53 µg/kg, and AFG_2 was 47.1 ± 23.46 µg/kg, resulting in a mean total aflatoxin concentration of 393.8 ± 151.2 µg/kg. Similarly, samples from the Liberte market showed elevated levels of AFB_1 (98.8 ± 37.18 µg/kg) and AFB_2 (65.8 ± 27.79 µg/kg), with a total aflatoxin mean of 170.2 ± 62.23 µg/kg. Notably, even the lowest aflatoxin concentration recorded in Kinshasa (Rond Point Ngaba: 15.20 µg/kg) exceeded the highest levels found in Minna markets.

These findings underscore a serious and widespread aflatoxin contamination problem in groundnuts from both cities, with Kinshasa markets exhibiting particularly alarming levels. This pattern aligns with previous reports by Kamika *et al.* (2013) and Kasongo *et al.* (2024), which highlight the pervasive contamination of foodstuffs by aflatoxigenic fungi in the region. In Kinshasa, much of the groundnut supply originates from neighbouring areas and is transported under conditions that often lack adequate food safety measures. This transportation process can cause physical damage to the groundnuts, increasing their susceptibility to fungal infection and mycotoxin contamination. Additionally, poor storage conditions further exacerbate aflatoxin accumulation (Makun *et al.*, 2009).

Aflatoxin contamination levels in Minna were generally much lower, often remaining below the international safety threshold of 15 µg/kg for total aflatoxins. This difference is likely influenced by ecological and climatic factors; Minna's hot but relatively less humid climate is less favourable for fungal growth and aflatoxin production compared to the more humid Kinshasa. Additionally, groundnuts in Minna are predominantly produced locally near communities, reducing exposure time during transport and storage and thereby lowering contamination risk. Furthermore, Nigeria has made significant advances in aflatoxin management through the use of biocontrol agents such as Aflasafe™, which employs atoxigenic strains of *Aspergillus flavus* to competitively inhibit toxigenic fungi, achieving reductions in aflatoxin contamination of 80-99% under field conditions across the country (FAO, 2021).

More broadly, aflatoxin contamination in sub-Saharan Africa is known to be alarmingly high, frequently exceeding international regulatory limits. For instance, in Kenya, widespread contamination of maize and groundnuts has been reported, with some groundnut samples containing aflatoxin levels as high as 591.1 µg/kg, far above safe thresholds (Meijer *et al.*, 2021). These findings highlight the urgent need for improved monitoring, better post-harvest handling, and enhanced food safety regulations to mitigate aflatoxin exposure and its associated health risks in the region.

Table 4 Aflatoxin Concentration (µg/kg ± SEM) in groundnuts from Local Markets in Kinshasa and Minna.

City	Market	AFB_1	AFB_2	AFG_1	AFG_2	Afs
Minna	Beji	11.96 ± 3.28[a]	0.56 ± 0.22[b]	1.10 ± 0.49[a]	0.14 ± 0.05[b]	13.72 ± 3.67[a]
	Garatu	8.55 ± 2.94[a]	0.75 ± 0.19[b]	1.31 ± 0.48[a]	0.13 ± 0.05[b]	10.64 ± 2.97[a]
	Kure	6.47 ± 1.61[a]	4.67 ± 1.45[a]	1.62 ± 0.98[a]	2.12 ± 0.94[a]	14.89 ± 2.84[a]
	Chanchaga	10.77 ± 2.46[a]	4.79 ± 1.04[a]	1.90 ± 0.68[a]	0.10 ± 0.05[b]	17.57 ± 2.91[a]
	Total	**9.44 ± 1.31**	**2.76 ± 0.58**	**1.48 ± 0.33**	**0.62 ± 0.27**	**14.20 ± 1.55**
Kinshasa	Matadi kibala	12.40 ± 4.90[b]	7.74 ± 3.85[ab]	3.61 ± 1.32[b]	4.27 ± 1.72[b]	28.02 ± 11.17[b]
	Liberte	98.80 ± 37.18[b]	65.76 ± 27.79[a]	5.60 ± 2.90[b]	0.00	170.16 ± 62.23[ab]
	Baramoto	229.44 ± 76.17[a]	59.90 ± 26.11a[b]	57.36 ± 37.53[a]	47.05 ± 23.46[a]	393.75 ± 151.21[a]
	Rond Point	6.68 ± 3.07[b]	3.58 ± 1.33[b]	2.55 ± 1.35[b]	2.39 ± 1.18[b]	15.20 ± 6.44[b]
	Total	**86.83 ± 25.87**	**34.24 ± 10.47**	**17.28 ± 9.87**	**13.43 ± 6.60**	**151.78 ± 47.62**

Values in the same column with different superscripts are significantly different at p < 0.05 and < 0.001(b).*

Table 5: Correlation Between Aflatoxin in Groundnut and Fungal load

Kinshasa	Parameter	Fungal Count (CFU/g)	Afs	AFB_1	AFB_2	AFG_1	AFG_2
	Fungal Count	1					
	Afs	0.16*	1				
	AFB1	0.27*	.958**	1			
	AFB2	0.02	.836**	.761**	1		
	AFG1	-0.008	.852**	.698**	.611**	1	
	AFG2	0.071	.859**	.743**	.552**	.944**	1
Minna	Fungal Count	1					
	AFB1	0.103	1				
	AFB2	-0.151	0.095995	1			
	AFG1	0.186	-0.02247	-0.104	1		
	AFG2	-0.0701	-0.039	-0.043	0.301976	1	
	Afs	0.0718	.886**	.427*	0.213452	0.202513	1

**Correlation is significant at the 0.05 level (2-tailed), ** Correlation is significant at the 0.01 level*

The correlation analysis reveals a noteworthy association between fungal contamination and aflatoxin levels in groundnut samples, particularly in Kinshasa. Specifically, a statistically significant positive

correlation was observed between fungal load and total aflatoxin concentration ($r = 0.16$; $p < 0.05$), with significance confirmed at the 0.01 level (two-tailed), as shown in Table 5. This indicates that increased fungal presence is linked to elevated aflatoxin contamination in Kinshasa samples, likely reflecting both higher fungal proliferation and environmental conditions favourable for aflatoxin biosynthesis. Conversely, groundnuts from Minna exhibited a weaker correlation ($r = 0.072$; $p < 0.05$), suggesting that factors beyond fungal load, such as climatic conditions or post-harvest handling practices, may exert a greater influence on aflatoxin accumulation in this region.

Interestingly, certain aflatoxin types, such as AFG_1, showed no significant correlation with fungal load in both Kinshasa ($r = -0.008$; $p > 0.05$) and Minna ($r = -0.151$; $p > 0.05$) samples. This lack of association could be indicative of cross-contamination events, possibly arising from the reuse of contaminated storage bags or mixing of products during handling, which decouples fungal presence from toxin levels. Moreover, these findings align with previous studies suggesting that aflatoxins can persist in food products even when visible fungi are absent, as fungal metabolic activity may have ceased while toxins remain stable (Hell *et al.*, 2000; Williams *et al.*, 2004). Conversely, visible fungi growth does not always correlate with toxin presence, underscoring the complexity of aflatoxin contamination dynamics.

Overall, these results emphasise the multifaceted nature of aflatoxin contamination, where fungal load is an important but not exclusive predictor of toxin levels. Effective control strategies must therefore address not only fungal proliferation but also storage, handling, and environmental factors to mitigate aflatoxin risks in groundnuts (Kumar *et al.,* 2017; Wu *et al.*, 2014).

3.3 Risks characterisation and population HCC risk

As demonstrated in Table 6, the risk characterisation was conducted using the deterministic MoE approach. This assessment considered an average adult body weight of 60 kg and a daily groundnut consumption rate of 0.052 kg across both cities. The EDI of total aflatoxins through groundnut consumption was calculated, revealing a wide range from 9.22 to 341.25 ng/kg body weight per day across the study locations.

Notably, the highest EDI values were observed in groundnuts purchased from Kinshasa's local markets, particularly at Baramoto and Liberté markets, with values of 341.25 and 147.47 ng/kg bw/day, respectively. Correspondingly, the aflatoxin contamination levels ranged from 10,640 ng/kg in Minna to as high as 393,750 ng/kg in Kinshasa, indicating a substantial variation in contamination between the two cities.

The MoE analysis clearly indicated a potential health risk for consumers of these groundnuts. This is further supported by the estimated HCC cases attributable to aflatoxin exposure, which were significantly higher in the Congolese population, approximately 514,000 cases annually, assuming an 8 % prevalence of HBsAg+, compared to about 62,000 cases in the Nigerian population, based on a 13 % prevalence.

Taken together, these results highlight critical insights into the health risks posed by aflatoxin contamination in groundnuts from Kinshasa and Minna. Chronic exposure to multiple aflatoxins represents a serious threat, especially to vulnerable groups such as rural subsistence farmers and children. Aflatoxins, particularly aflatoxin B_1 (AFB_1), are highly carcinogenic, with a well-established link to increased HCC incidence in regions such as China (Wu *et al.*, 2014). Alarmingly, even very low EDI levels around 0.001 μg/kg bw/day have been reported to induce liver cancer (Liu and Wu, 2010; Hoteit *et al.*, 2024).

Limited risk assessment data exist for Kinshasa, but studies by Mulunda *et al.* (2013), Kamika *et al.* (2013), and Kasongo *et al.* (2024) highlight significant exposure to aflatoxins and other mycotoxins in food. In Nigeria, high levels of aflatoxin B_1 and total aflatoxins have been reported, with Margin of Exposure (MoE) values indicating notable carcinogenic risk, especially for children (Oyedele *et al.*, 2017; Magomya and Mbatsav, 2023). Wenndt et al. (2023) estimated an HCC incidence of 2.6 cases per 100,000 population linked to aflatoxin exposure from maize and groundnuts. Additionally, Badmos *et al.* (2023) reported that aflatoxin exposure from sorghum contributes to approximately 599,000 HCC cases annually among HBsAg-positive individuals, reflecting the broader risk beyond just maize and groundnuts.

Beyond liver cancer, aflatoxin exposure is associated with a range of adverse health outcomes, including increased rates of stillbirths and neonatal mortality, immunosuppression that heightens vulnerability to infectious diseases such as pneumonia, stunted child growth, and the exacerbation of conditions like HIV/AIDS (McMillan *et al.*, 2018). These findings underscore the urgent need for effective aflatoxin mitigation strategies and public health interventions in these regions to reduce exposure and protect at-risk populations.

Table 6: Estimation of population HCC risk due to aflatoxin-contaminated groundnuts

City	Markets	AFs Conc. (ng/kg)	Amount of groundnut (kg)	Body weight (kg)	EDI (ng/kg.bw/d)	$BMDL_{10}$ (ng/kg.bw/d)	MoE	HCC potency/ 10^5 × EDI	Pop HCC risk $\times 10^7$
Kinshasa	Liberte	170160	0.05	60	147.47	170	1.15	0.0565	5.80
	Matadi K	28020	0.05	60	24.28	170	7	0.0093	0,95
	Baramoto	393750	0.05	60	341.25	170	0.5	0.1307	13.40
	Round Point	15200	0.05	60	13.17	170	12.9	0.0050	0,52
	Total	**151780**	**0.05**	**60**	**131.54**	**170**	**1.29**	**0.0504**	**5.14**
Minna	Kure	14890	0.05	60	12.9	170	13.17	0.0071	0.73
	Chanchaka	17570	0.05	60	15.23	170	11.16	0.0084	0,86
	Garatu	10640	0.05	60	9.22	170	18.44	0.0051	0.20
	Beji	13720	0.05	60	11.89	170	14.3	0.0065	0.70
	Total	**14205**	**0.05**	**60**	**12.311**	**170**	**13.81**	**0.0068**	**0.62**

**MoE≤10,000 indicates a non-tolerable exposure level. Congolese population: 102.3 million; Nigerian population:232.68 million (Group Worldwide Bank Data, 2025)*

CONCLUSION

This study highlights the significant public health risks associated with aflatoxin contamination in groundnuts sold in Kinshasa (DR Congo) and Minna (Nigeria). Groundnut samples from both cities were frequently contaminated with aflatoxins, with contamination levels in Kinshasa markets far exceeding international safety limits and those found in Minna. *Aspergillus* species were the predominant fungi, further confirming the risk of mycotoxin contamination in these environments.

Risk assessment using the MoE approach indicated a substantial carcinogenic threat to consumers, particularly in Kinshasa, where the estimated annual incidence of hepatocellular carcinoma (HCC) attributable to aflatoxin exposure was approximately 514,000 cases among HBsAg-positive individuals. In Minna, the estimated annual HCC cases were lower (about 62,000), but still represent a significant health concern given the high prevalence of hepatitis B and ongoing exposure to contaminated foods.

These findings underscore the urgent need for comprehensive mycotoxin management strategies, including improved agricultural practices, better storage and handling, effective regulatory enforcement, and public health interventions such as hepatitis B vaccination. Addressing these challenges is critical for reducing aflatoxin exposure, safeguarding food safety, and protecting vulnerable populations in sub-Saharan Africa.

Conflict of interest

No conflict of interest in this study

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