Exposure to Aflatoxins Above Permissible Limits in Maize (Zea Mays) Meant for Consumption and Their Health Risk Estimations in the Volta Region of Ghana

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Exposure to aflatoxins above permissible limits in maize (*Zea mays*) meant for consumption and their Health risk estimations in the Volta Region of Ghana

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Abstract

Aflatoxin contamination in foods is a vital health challenge for low and middle-income countries in subtropical regions. Maize (*Zea mays* L.), a staple food most widely grown in Africa including Ghana, and extensively consumed as much as three times per day is a source of aflatoxin contamination owing to its susceptibility to fungal attack. This study aimed at evaluating aflatoxin levels above international (European Food Safety Authority, EFSA) and local (Ghana Standards Authority, GSA) standards and health risks associated with maize sampled from the Volta Region (Hohoe, Ho, Battor Dugame, and Keta) of Ghana. Total aflatoxins (AF_{Total}) and the constituent aflatoxins (AFB_1, AFB_2, AFG_1, and AFG_2) were measured with High-Performance Liquid Chromatography (HPLC). Risk assessments were also conducted using models prescribed by the Joint FAO/WHO Expert Committee on Additives (JECFA). The degree of occurrence of aflatoxins was observed to be in decreasing order of $AFG_2 < AFG_1 < AFB_2 < AFB_1$ and were within the ranges of 0.78±0.04−234.73±3.8 µg/kg, 0.47±0.03−21.6±0.33 µg/kg, 1.01±0.05−13.75±1.2 µg/kg and 0.66±0.06−5.51±0.26 µg/kg respectively. Out of the 100 samples analyzed for total aflatoxins (AF_{Total}), 68% exceeded the limits of EFSA and were of range 4.98±0.6−444.01±8.9 µg/kg whereas 58% and ranged between 12.12±1.4− 444.01±8.9 µg/kg exceeded for GSA limits. Risk assessments of total aflatoxins (AF_{Total}) for infants, children, and adolescents, and adults ranged between 0.0083-0.3427 µg/Kg.bw/day, 0.116-4.8192, 0-0.0396 ng Aflatoxins $kg^{-1}bwday^{-1}$ and $3.2868x10^{-4}$ -0.0136 for Estimated Daily Intake (EDI), Margin of Exposure (MOE), Average Potency, and Population Risks respectively. It was inferred that the consumption of maize posed adverse health effects on all age categories studied because all calculated MOE values were greater than 0.04 $ngkg^{-1}bwday^{-1}$.

Keywords: Aflatoxins, toxigenic fungi, maize, mycotoxins, cereals, Ghana, HPLC

Introduction

Among the major problems in recent times of escalating urbanization and human population, are food safety and security. These are mainly determined by three key facets namely; sufficient food availability, access to safe food, and application of the food in terms of quality, nutritional and cultural purposes for a healthy life (Unnevehr 2015). The failure of any of these aspects leads to food insecurity and malnutrition that further influences human health, in addition to the socio-economic aspect of society. Furthermore, food and feed contamination by mycotoxins are one of the key factors responsible for creating food insecurity (Agriopoulou et al. 2020).

Mycotoxin is the term used to accurately describe natural poison of fungal origin. These poisons are used as defense mechanisms in the ecosystem. Among the thousands of species of fungi, only about 100 are acknowledged to produce mycotoxins and belong to the genera *Aspergillus, Penicillium,* and *Fusarium*. Out of the 300–400 mycotoxins known, the most important are aflatoxins, ochratoxins, deoxynivalenol (DON or vomitoxin), zearalenone, fumonisin, T-2 toxin, and T-2 like toxins (trichothecenes)(Cinar and Onbaşı 2019, Agriopoulou et al. 2020). Aflatoxins are biochemical by-products of difurano coumarin synthesized by a polyketide pathway by some strains of *Aspergillus* notably *flavus* and *parasiticus*. *A. flavus* is a well-known communal contaminant in agriculture as well as food safety. Although the species *A. bombycis, A. ochraceus, A. nomius,* and *A. pseudotamari* are also aflatoxin-producing species, they rarely do produce aflatoxins (Klich et al. 2000). Variations in isolates of each aflatoxigenic species have resulted in great qualitative and quantitative differences in their aflatoxin-producing capabilities. They have gained so much popularity due to their ability to cause adverse health effects in both humans and animals and are categorized as a class 1 carcinogenic toxin.

Maize is a staple food for an estimated 50% of the population of sub-Saharan Africa (FAOSTAT 2006) and is indispensable in Ghana because it is widely consumed and cultivated across all the agro-ecological zones. It accounts for more than one-quarter of calories consumed, about double that of the second crop, cassava (GSS 2018). About three-quarters of maize consumption is from own production, suggesting maize has limited appeal as a cash crop (Gage et al. 2012). Ghana in

its quest to increase output and improve food security has developed New varieties with enhanced attributes. Some improved maize varieties available in Ghana include *Abeleehe, Aburotia, Dobidi, Dorke, Kawanzie, Kwadaso local, Obatanpa, Okomasa, Mamab*a, *Abontem*, and *Aburohema* (Manga, 2010; Tweneboah Koduah, 2013). Nonetheless, maize is highly susceptible to fungal contamination, thus its contamination with mycotoxins especially, aflatoxins infiltrates the human and animal populace (Agbetiameh et al. 2018, Kortei et al. 2021a) owing to its extensive usage in the preparation of countless delectable dishes such as "kenkey" (boiled fermented corn dough), "banku/akple", "waha", "goya" (tubani), "Tuo zaafi", "aboloo"..etc also some beverages like "nmedan", "asana", and "pito" (Kortei et al. 2020b) (Ghana Tourism Authority, 2016) just to mention a few are also obtained from maize. Interestingly, maize is one of the most favorable substrates for aflatoxin amplification and even crops in the field can get high levels of infection (Wagacha and Muthomi 2008, Williams et al. 2014). Conversely, other cereal crops like wheat, barley, oat, and sorghum are not very prone to wide preharvest aflatoxin contamination. Conidia of *Aspergillus flavus* are the major source of primary inoculum in maize fields (Scheidegger and Payne, 2003).

Ghana recorded a between birth and the age of 20 months rapid percentage rise of malnourished children from 7 to 48% (Macro 2005, Africa Nutrition Chartbooks 2005). It is very depressing to report that about 40% of deaths of children under-five in Ghana are attributed to malnutrition (Macro 2005, Africa Nutrition Chartbooks 2005). The adverse effects of malnutrition on the populace especially children have prompted the documentation of strategic plans to reduce malnutrition, one of which is the recommendation to include protein- and energy-rich foods, such as cereals and legumes, for the feeding of infants and young children (Organization 2008).

A perusal of pertinent literature revealed some evidence of the incidents of acute aflatoxicosis in children in Ghana (Kumi et al. 2014, Blankson and Mill-Robertson 2016, Opoku et al. 2018, Omari and Anyebuno 2020, Tuffour and Steele-Dadzie, 2019) presumably via infant complementary foods. This troubling and uncomfortable situation stems from the over-reliance and seemingly inevitable use of the two most frequently fungi-contaminated foodstuffs maize and groundnuts as ingredients for the preparation of complementary foods (porridges) for infants, children, and toddlers (Achaglinkame et al. 2017, Kortei et al. 2021a).

Given the potential of maize becoming a non-traditional export commodity of Ghana considering its high productivity, intolerable aflatoxin levels could be an enormous setback. Usually, indicators of these natural toxins in most of our foods that exceed permissible limits at European boundaries (Rapid Alert System for Foods and Feed (RASFF) (AF_{Total} = 4µg/kg, AFB_1 = 2µg/kg) has ended in the refusal of many food commodities and as a result, negatively affected the local trade sector and other economic activities.

Besides setting regulatory limits for aflatoxins (AF), it is also essential to conduct health risk assessments in the population due to dietary exposure. A low-dose extrapolation approach introduced by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1997 and the margin of exposure (MOE) method proposed at the 64th JECFA meeting in 2005 (Authority 2005) were both recommended and have been widely used worldwide (Sun et al. 2011, Huong et al. 2016) to assess the risk of dietary exposure to aflatoxins.

The objectives of this study were therefore to evaluate the quantities above permissible limits and health risks posed by aflatoxins via maize (*Zea mays*) meant for consumption sold in Hohoe, Ho, Battor Dugame, and Keta in the Volta Region of Ghana.

Materials and Methods

Study Area and Site

The Volta Region of Ghana has Ho Municipal as its administrative capital. It is one of the twenty-five (25) Municipalities and Districts of the region. This Municipality is also the viable nucleus of

the region. The municipality consists of seven hundred and seventy-two (772) communities and a Land Size of 2660 sq per records of the Ghana Statistical Service (Service 2014).

The Volta Region was the study site for this research. Fig. 1 describes the geographical location of the Volta region and the sampling sites (Hohoe, Ho, Battor Dugame, and Keta districts). The sites of sampling for this study were categorized into two agro-ecological zones; deciduous forests (Hohoe and Ho) and coastal savannah (Battor Dugame and Keta) with rainfall ranging between 536.2-1985 mm and temperature ranging between 25-28.4 ºC. (Table 1).

Sample collection

Maize (*Zea mays*) samples were randomly purchased concurrently from the Ho central market in the Volta Region of Ghana (Figure 1) from April 2020 to October 2020 (Tables 3-6). Aliquots of Twenty (20) grams and twenty milliliters (20 ml) each of the samples were fetched and kept in sterile bags and sent to the laboratory where they were stored in a deep freezer at −20 °C until ready for chemical analysis (Kortei et al. 2020a).

Extraction of samples

The European Committee for Standardization (CEN) official method EN14123 (Stroka and Anklam 2002) was used to extract and quantify AFB_1, AFB_2, AFG_1, and AFG_2 from the samples. Methanol in water (200 ml) (8+2) and 5 g NaCl were used to extract 20 g or 20 ml of sample. Fat samples containing more than 50 % fat were extracted with 100 ml of hexane by the normal methanol extraction of solvent. 100 ml of hexane was added to 200 ml of the methanol. After homogenization, a separation funnel was used to separate the hexane which became the upper layer. The mixture was homogenized for 3 min at 3000 rpm (2 min) and 3500 rpm (1 min). The extracts were filtered and 10 ml of filtrate added to 60 ml of phosphate buffer saline (PBS) for solid-phase extraction using a pre-conditioned immune-affinity column specific for AFB_1, AFB_2, AFG_1, and AFG_2. The 70 ml filtrate-PBS mixture was loaded onto the pre-conditioned column

and allowed to elute by gravity at a flow rate of 1 ml min^{-1}. This was followed by a cleanup with 15 ml distilled water at a flow rate of 5 ml min^{-1}. Aflatoxins were eluted in two steps into a 5 ml volumetric flask with 0.5 ml followed by 0.75 ml of methanol (HPLC grade) and allowed to elute by gravity. Deionized water was used to make up the volume of eluates to 5 ml and eluate vortexed and 2 ml pipetted into HPLC vials for quantification.

HPLC parameters

Injection volume: 10 µl flow rate: 1 mL/min, column temperature: 35 ℃, excitation wavelength: 360 nm, emission wavelength: 440 nm, mobile phase composition: water/acetonitrile/MeOH (65:15:20 v/v/v), post-column derivatization: Kobra cells. HPLC Column Specification Spherisorb ODS1- Excel (4.6 mm x 25 cm), 5 µm particle size, 250A pore size.

Limit of detection/quantification (LOD/LOQ)

The limit of detection and quantification (LOD/LOQ) of the HPLC was estimated by making a calibration curve around the least standard used for spiking, 5 µg/kg (lowest concentration range of calibration curve). Blank did not produce any signal, so the LOD and LOQ were calculated as;

LOD = 3 x standard deviation/slope. (1)

LOQ = 3 x LOD. (2)

Measurement accuracy

Spiking of pure aflatoxin standard solution was done to ensure the measurement accuracy of the analysis. Three levels of spiking were done at the lower, mid, and upper concentration range of the calibration curve concentrations (5 ppb, 15 ppb, and 30 ppb). Spike volumes of pure standards were calculated as;

[Sample weight (g) x spike concentration (ppb)] / [Concentration of standard (ug/ml)]. (3)

Spike volumes were distributed evenly on aflatoxins free sample (blank) and the spiked sample analyzed for percentage recovery which was calculated as;

[(Concentration measured in spike – concentration measured in blank**) / (**spiked amount**)]** x 100 (4)

Measurement precision

Repeatability and intermediate precision analyses of an Internal Reference Material (IRM) were used to ensure the measurement precision of the method. For repeatability analysis, 10 parallel extractions of the IRM were done by the same analyst at the same time using the same HPLC, and the relative standard deviation among results calculated. For intermediate precision, 10 extractions of the IRM were done on different days by different analysts, and the relative standard deviation among results calculated. The relative standard deviations were calculated as;
[Standard deviation/mean] x 100.

Required Performance Criteria for Accuracy and Precision

Repeatability: Relative standard deviation among repeatable results should be less than **15%.**

Intermediate Precision: Relative standard deviation among results obtained under intermediate precision conditions should be less than **20%.**

Recovery: Percent recovery of measurement procedure should be in a range of **80-120%.**

Limit of Detection: The limit of detection should be less than **1** μg/kg for all aflatoxins.

Limit of Quantification: The limit of Quantification should be less than **3** μg/kg for all aflatoxins.

Linearity: Linearity from the regression curve should be **0.99 (B_1, B_2, G_1) and 0.98 (G_2)**.

Experimental Data

Repeatability: Relative standard deviation was;

B_1 = 5.5%; B_2 = 6.7%; G_1 = 7.4%; G_2 = 12.1% and Total aflatoxins = 5.2%.

Intermediate Precision (Reproducibility): Relative standard deviation was;

B_1 = 13.2%; B_2 = 13.4%; G_1 = 13.7%; G_2 = 12.2% and Total aflatoxins = 11.9%.

Recovery: Percent recovery of measurement procedure was;

Low concentration: B_1 = 107%; B_2 = 87.2%; G_1 = 113.4%; G_2 = 112.8% and Total aflatoxins = 108.2%

High concentration: B_1 = 102.6%; B_2 = 101.6%; G_1 = 104.2%; G_2 = 104.4% and Total aflatoxins = 103.3%

Linearity: Linearity from regression curve was;

B_1 = 0.991; B_2 = 0.997, G_1 = 0.994; G_2 = 0.995

Human risk assessment of exposure to total aflatoxins via consumption of cereals

Exposure estimation

Estimated Daily Intake (EDI) was considered by using the mean quantities of aflatoxins derived from the cereal samples, the quantity of samples consumed daily, and the average body weight. The EDI for mean aflatoxins was premeditated according to the following formula (5) and expressed in µg/kg of body weight/day (µg/kg b.w/day)(dos Santos et al. 2013, Chain et al. 2020)

$$EDI = \frac{\textit{daily intake (food)} \times \text{mean level of Aflatoxins}}{\text{average bodyweight}} \quad (5)$$

The daily intake of maize in Ghana according to MOFA-IFPRI (2018) is approximately 0.107 kg/day (39.3 kg/year).

Population Risk Characterization for Aflatoxins

Genotoxic compounds such as aflatoxins have their risk assessments fittingly computed based on the Margin of Exposures (MOEs) approach, which was estimated by dividing the Benchmark dose lower limit (BMDL) for aflatoxins - 400 $ngkg^{-1}bwday^{-1}$ by toxin exposure (Adetunji et al. 2018) (EFSA, 2020) as expressed in equation (6).

$$MOE = \frac{\text{Benchmark dose lower limit}}{\text{EDI (Exposure)}} \quad (6)$$

A public health alarm is raised in instances where MOEs are less than 100,000, which implied that aflatoxin exposures above 0.04 $ngkg^{-1}bwday^{-1}$ (derived by dividing 400 $ngkg^{-1}bwday^{-1}$ by 100,000) (Adetunji et al. 2018, Kortei et al. 2021a)

Estimated Liver Cancer Risk due to Consumption of food samples.

The ingestion of aflatoxins can be linked to the onset of liver cancer (Shephard 2008). Therefore, liver cancer risk estimation for Ghanaian adult consumers was calculated for aflatoxins (Shephard 2008, Adetunji et al. 2018). This involved estimating the population cancer risk per 100,000 which is a product of the EDI value and the average hepatocellular carcinoma (HCC) potency figure from individual potencies of Hepatitis B surface antigen (HBsAg) (HBsAg-positive and HBsAg-negative groups).

The JECFA (1999) estimated potency values for AFB_1 which corresponded to 0.3 cancers $year^{-1}$ $100{,}000^{-1}$ population/ $ngkg^{-1}bwday^{-1}$ (uncertainty range: 0.05–0.5) in HBsAg-positive individuals and 0.01 cancers $year^{-1}100{,}000^{-1}$population/ $ngkg^{-1}bwday^{-1}$ (uncertainty range: 0.002–0.03) in HBsAg-negative individuals (Shephard 2008) were adopted for this calculation. Also, the HBsAg+ prevalence rate of 10.2% for Ghana (Ofori-Asenso and Agyeman 2016) was adopted and 89.8% (100 – 10.2%) was extrapolated for HBsAg-negative groups. Hence the average potency for cancer

in Ghana was estimated as follows according to equation (7) as prescribed by (Shephard 2008) and (Adetunji et al. 2018):

Average potency = [0.03 x HBsAg –negative individuals in Ghana] + [0.01 x HBsAg- positive individuals/prevalence rate in Ghana] (7)

$$(0.3 \times 0.102) + (0.01 \times 0.898)$$

$$= 0.03958 \text{ cancers per year per 100,000 population per ng Aflatoxins } kg^{-1}bwday^{-1}.$$

Thus the population risk was estimated using the following formula in equation (8) (Adetunji et al. 2018):

Population risk = Exposure (EDI) × Average potency (8)

Statistical Analysis

The aflatoxin concentrations were calculated using regression analysis from the curves generated from the standards of the aflatoxins with Excel for Microsoft Windows (version 10). SPSS 22 (Chicago, USA) was used in the analysis of data. Descriptive analysis was performed to describe the concentration of aflatoxins in maize samples by using the mean ± standard deviation. Probabilistic risk assessment model calculations for Aflatoxins dietary exposure (Estimated Dietary Intake), MOE values, Average potency, and cancer risk were calculated. The results are summarized as median, standard deviation, variance, skewness, standard error of skewness, kurtosis, and standard error of kurtosis and the mean values (range from the 25th percentile to the 75th percentile).

Results

Most of the food samples tested, produced good linearity or coefficients of correlations (R^2 > 0.990) within the tested range. For the recovery analysis, samples previously tested to guarantee the nonappearance of studied mycotoxins were used in the validation procedure. The Limits of

Detection for AFB_1 and AFB_2 likewise AFG_1 and AFG_2 ranged between 0.13-0.15 while Limits of Quantification ranged between 0.26-0.30 respectively for both (Table 2).

The number of food samples contaminated with AFB_1, AFB_2, AFG_1, AFG_2, and AF Total (Total Aflatoxins) is presented in Tables 3-6. The level of occurrence of the aflatoxins was in decreasing order of AFG_2< AFG_1< AFB_2<AFB_1 and were in the ranges of 1.89±0.06−338.3±8.6 µg/kg, 0.62±0.03−103.6±2.5 µg/kg, 0.84±0.01−13.41±0.37 µg/kg and 1.00±0.02−5.51±0.26 µg/kg respectively. As explained by and Al-Zoreky and Saleh (2019), the lethal furan moiety of AFB_1 is the life-threatening point for determining the degree of biological activity of this group of fungal toxins. Out of a total of one hundred (100) samples investigated, concentrations of AFB_1 and AF_{Total} ranged between 1.89±0.06−338.3±8.6 µg/kg and 1.89±.0.06−444.01±8.9 µg/kg respectively for BD10 and KT5. The total aflatoxin yields of 338.3±8.6 µg/kg and 444.01±8.9 µg/kg obtained for BD10 and KT5 respectively were significantly ($p<0.05$) higher than all other samples studied in that category.

For Hohoe (HH), values of 19.43 and 314.33 µg/kg were recorded from the summary statistics as median and variance respectively while 0.65 and -0.83 were recorded as the skewness and kurtosis and implied asymmetrical and normally distributed for Total Aflatoxins (AFtotal) (the distribution is not outside the range of normality) (Table 3b).

Ho (Ho) had recorded 19.83 and 2622.20 µg/kg for median and variance respectively while the skewness and kurtosis were 2.54 and 7.5 respectively and showed that the data set of Total aflatoxins (AFtotal) obtained in this town was asymmetrical and light-tailed (Table 4b).

The median and variance recorded for Battor Dugame (BD) were 44.42 and 3739.67 µg/kg respectively. While the data set showed symmetrical and light-tailed as the skewness and kurtosis were 1.25 and 1.43 respectively for Total aflatoxins (AFtotal) (Table 5b).

For Keta (KT) recorded a median and variance of 4.72 and 9297.30 µg/kg respectively. The data set for Keta was asymmetrical and heavy-tailed (3.46 and 13.25 for skewness and kurtosis respectively Total aflatoxins (AFtotal) (Table 6b).

The European Food Safety Authority (EFSA) and Ghana Standards Authority (GSA) regulatory limits for total aflatoxins (AF_{Total}) and aflatoxins B_1 (AFB_1) (Table 7) were used in this study. Toxin quantity limits prescribed by the Ghana Standards Authority are a sub-set of the European Food Safety Authority (EFSA).

Regarding the frequency and (percentage %) of positive (Yes) total aflatoxins (AF_{Total}) contaminated maize samples above the various permissible limits, Hohoe (HH) recorded 19(76%) and ranged between 8.72±0.8−61.4±1.8 µg/kg and 17(68%) of range 12.51±0.26−61.4±1.8 µg/kg tested positive for EFSA and GSA, respectively. For AFB1, values of 19(76%) within the range of 4.31±0.26−48.5±0.99 µg/kg and 18 (72%) of range 7.18±0.25−48.5 ±0.99 µg/kg tested positive for EFSA and GSA respectively.

For Ho (Ho), total aflatoxin values of 17(68%) which ranged between 8.5±0.95−224.31±6.4 and 14(56%) of range 15.18±0.44−224.31±6.0. For AFB1, values of 18(72%) within the range of 2.89±0.4−195.5±2.0 and 15(60%) within the range of 5.46±0.11−195.5±2.0 were recorded as positive for EFSA and GSA respectively.

Battor Dugame (BD) recorded total aflatoxin values of 19 (76%) within the range of 4.98±0.6-229.0±6.0 µg/kg and 18 (72%) within the range of 19.4±0.85−229.0±6.0 µg/kg were recorded. AFB1 showed 20 (80%) within the range of 2.26±0.6−182.29±8.33 µg/kg and 18 (72%) of range 11.86±0.45−182.29±8.33 µg/kg for EFSA and GSA.

From Keta (KT), total aflatoxins values of 13 (52%) within a range of 4.72±0.28−445.01±8.9 µg/kg and 9 (36%) within a range of 12.12±1.4−444.01±8.9 µg/kg were recorded. AFB1 values of 14 (56%) with a range of 3.93±0.22−339.3±8.6 µg/kg was recorded for EFSA while, 13 (52%) within a range of 5.18±0.25−339.3±8.6 µg/kg was recorded for GSA.

Out of the 100 samples analyzed for total aflatoxins (AF_{Total}), 68% exceeded the limits of EFSA and were of range 4.98±0.6−445.01±8.9 µg/kg. While for GSA, 58% of samples exceeded and ranged between 12.12±1.4−444.01±8.9 µg/kg. For AFB_1, 71% of samples of the range

2.26±0.6−338.3±8.6 µg/kg exceeded the tolerable limit of the EFSA, whereas 64 % of range 5.18±0.25−338.3±8.6 µg/kg exceeded the limits of GSA (Table 7).

Risk Assessment

The weights for the age categories used in this study were 6, 40, and 60.7 kg respectively for infants (Abeshu et al. 2016, Glover-Amengor et al. 2016), children and adolescents (Biritwum et al. 2005), and adults (Walpole et al. 2012) (Table 9). The Estimated Daily Intakes (EDI) of the total aflatoxins in the maize samples from Hohoe (HH) were 0.1499, 0.0519, and 0.0343 µg/Kg b.w/day for infants, children and adolescents, and adults respectively. The Margin of Exposure (MOE) values recorded were 0.2668, 0.7707, and 1.1662 respectively. The average potency of the aflatoxins was 0.0396 aflatoxins $kg^{-1}bwday^{-1}$ and produced a population risk of $5.94x10^{-3}$, $3.05x10^{-3}$, and $1.35x10^{-3}$ respectively (Table 8).

Samples from Ho recorded EDI values of 0.1529, 0.0530, and 0.0349 for infants, children and adolescents, and adults respectively. MOE values of 0.2616, 0.7547, and 1.1461. A population risk of 0.0298, 2.0988x10-3, and 1.3820x10-3 respectively for these age categories were recorded.

From BD, the EDIs values recorded for infants, children and adolescents and adults were 0.3427, 0.1188, and 0.0364 µg/Kg b.w/day respectively. MOE values recorded were 0.1167, 0.3367, and 0.5109 respectively. The average potency was the same as other towns while the population risks were 0.0136, $4.704x10^{-3}$, and $3.100\ x10^{-3}$ respectively (Table 8).

Lastly, for Keta (KT), the EDIs values recorded for infants, children, and adolescents, and adults were 0.0364, 0.0126, and 0.0083 µg/Kg b.w/day respectively. MOE values recorded were 1.0989, 3.1746, and 4.8192 respectively. The average potency was the same as other towns while the population risks were $1.441x10^{-4}$, $4.9896x10^{-4}$, and $3.2868\ x10^{-4}$ respectively (Table 8).

Discussion

Ghanaian maize farmers have continued to cultivate different maize varieties with the vision of improving food security and boosting economic development at large in the sub-region. Yet, the challenge of aflatoxin contamination thwarts these advances, as most varieties grown in Ghana are defenseless to aflatoxin contamination and their accumulation emanating from *Aspergillus flavus* infection that usually begins from infested soils where conditions are favorable.

The variation in the mean aflatoxin levels as observed in this study could partly be attributed to the different agro-ecological zones of the region where the maize samples were taken. Ho and Hohoe belong to the deciduous forest zone while Keta and BD also belong to coastal savannah zones. Presumably, the rainfall and humidity patterns of these zones contributed to the conducive growth conditions which encouraged fungal growth and subsequent production of persistence aflatoxins in the maize samples. The range of values recorded in this study was consistent with values reported by Kpodo et al. (1996) in earlier surveys reported aflatoxin levels in the range of 20–355 ng/g maize samples from silos and warehouses in Ejura while fermented corn dough collected from major processing sites also contained aflatoxin levels of 0.7–313 ng/g. From Tolon-Kumbungu district in the northern region of Ghana, Aklaku et al. (2020) reported aflatoxin values 60 ppb.

James et al. (2007) also found high average aflatoxin levels in maize samples collected from North Kwahu (153 ng/g), Ejura Sekyere-Dumasi (121 ng/g), and Nkoranza (134 ng/g).

Agbetiameh et al. (2018) reported values of range 1-341 ppb in maize from different ecological zones in Ghana. Likewise, from the Brong-Ahafo region of Ghana, (Benson-Obour et al. 2018) recorded aflatoxins of levels up to 113.56 ppb from three different maize varieties; *Obaatanpa*, *Abontem*, and *Aburohemaa.*

The incidence of mycotoxins is principally attributed to the inaccessibility or insufficient execution of mycotoxin guidelines which aim at packaged processed foods envisioned for local markets and international markets as well as imported foodstuffs in Ghana. Recent works done on the occurrence of aflatoxins in maize by some researchers (Agbetiameh et al. 2020, Aklaku et al. 2020, Kortei et al. 2021a) in some parts of Ghana, points to the persistence of aflatoxins.

From Kenya, Nduti et al. (2017) reported values of range 7.92±1.57− 22.54±4.94 ppb from maize flour samples obtained from three regions. Likewise, studies by Atter et al. (2015) and Kpodo et al. (1996) revealed greater quantities of aflatoxin levels up to 2000 μg/kg measured in maize products, and maize kernels sampled from various markets and maize processing sites across Ghana. AF levels up to 15000 ppm and 320,000 ppm in corn have also been measured in India and the USA respectively (Abbott 2002).

Perrone et al. (2014) reported heavily contaminated maize samples from the districts of Brong-Ahafo (83-290 mg/kg), Kpalsogu (4-1400 mg/ kg) and Kintampo (1200 mg/kg) in Ghana, and Lafia (1200 mg/kg) and Mokwa (5-480 mg/kg), in Nigeria. From Ejura- Sekyeredumase municipality again, Akowuah et al. (2015) reported surpassing values of 4831.42 ng/g in maize samples.

Lewis et al. (2005) reported greater values of aflatoxin quantities of > 1000 ppb from maize samples as they investigated aflatoxin contamination of commercial maize products during an outbreak of acute aflatoxicosis in Eastern and Central Kenya.

Cotty and Jaime-Garcia (2007) observed that aflatoxin contamination often occurred during a preliminary period during crop development and a second period during crop senescence. In warm, humid, and even hot deserts and drought atmospheres, the contagion is increased and optimally produced in adverse periods.

Again, Poor harvesting practices, improper storage, and less than optimal conditions during transportation, marketing, and processing can also contribute to fungal growth and increase the risk of mycotoxin (aflatoxins) production. These climatic conditions as well as the food production chains are characteristic in most parts of Africa not excepting Ghana.

Thus, the human health impact will be greatest if there are no monitoring and control mechanisms for aflatoxin contamination of food. For both food safety and economic reasons, there is the need to develop effective ways to mitigate the high and unacceptable levels of aflatoxins in food as it is becoming a serious public health and economic concern throughout the world. In other parts of

Sub-Saharan Africa, aflatoxins have caused severe lesions of the liver in malnourished children and adults, often with fatal outcomes (Wagacha and Muthomi 2008). Richard et al. (2020) as well as Wagacha and Muthomi (2008) emphasized that due to the seasonal appearance of childhood diseases such as kwashiorkor, Reye's syndrome, and neonatal jaundice, neonatal neurotoxicity in tropical countries, which coincides with periodical high concentrations of aflatoxins in food, it is believed that aflatoxins are involved in the etiology of these diseases. Aflatoxins are extremely difficult to handle since they can continue to persist in food even after the inactivation of the fungi despite all the rigorous processing methods because of their ability to resist chemical and thermal changes (Mahato et al., 2019).

Recently, Etwire and Martey (2020) asserted that aflatoxin invasion is dependent on pre-production and post-production influences, rather than farmer characteristics as previously thought. Again, the heaping location, source of seed, and region of residence are the considerable fundamentals of aflatoxin infestation in northern Ghana. Notwithstanding, interventions such as dietary diversification are highly recommended since high levels of mycotoxin exposure are directly linked to the non-diversification of diets (Anitha et al. 2020). The right to use a wider range of foods and substitute those at high risk of contamination will reduce the probability of exposure by lessening the intake of these commonly contaminated foods such as groundnuts (Kortei et al. 2021b). Increased dietary diversity is one intervention for which robust evidence of enhancement of health exists (Anitha et al. 2020), however the most difficult to accomplish due to constraints such as food insecurity, environmental factors, cultural traditions, and economic factors affecting Africa. Another intervention is to promote the use of certified seed and encourage good post-harvest practices.

Risk assessment

Risk estimations as explained by Kuiper-Goodman (1990) are modeled to predict the adverse health implications of mycotoxin exposure and guide food regulators to set thresholds for these toxins in foodstuffs. MOE results obtained in this study implied a high risk for infants, children, and adolescents (total aflatoxins).

Risk assessment results obtained in this study were comparable to published findings of Blankson and Mill-Robertson (2016) as they reported Total aflatoxins EDI values of range 0.005−1.054 μgkg-¹bwd-¹ and 0.004−0.838 μgkg-¹bwd-¹ for infants and young children respectively in infant cereal-based formula and were risky for children to consume in Ghana.

In a related study, Kortei et al. (2021a) also reported total aflatoxin EDI values of 109.7, 58.8, 33.08, and 33.08 μgkg-¹bwd-¹ for infants, children, adolescents, and adults respectively with corresponding MOE values of 3.64, 6.80, 12.09, and 6.75 and pointed to possible adverse health effects via consumption of maize sold in Ghana.

Kabak (2021) reported 95th percentile dietary exposure values of range 0.022-0.439 ng kg-1 b.w. per day with corresponding MOE values (995–860 at mean and 336 at 95th percentile exposure) and cancer potency estimates, based on the current exposure levels indicated a potential health concern for Turkish adults.

In Guangzhou China, Zhang et al. (2020) reported EDI values of range 0.02-0.04 respectively for the age ranges of 3-6, 7-17, 18-59, and above 60 yrs for maize and products. Additionally, all their computed MOE values were below the safe threshold of 10,000 and so risk analysis results showed that most of the lower bound MOE values ranged from 10 to 100, indicating a concern for risk management. Age-group analysis suggested that we should pay close attention to the 3~6 years of age group, whose MOE value was the lowest. Their results reflected that preschool children might have the highest risk of being exposed to AF. Their findings agreed with results from a similar study from Taiwan in 2018 that found that babies and toddlers were at the highest risk of AF exposure. The results of both studies agreed with our findings.

It is worthy to note that although our equipment used in this study recorded "Below Detection Limits (BDL)" for some samples, it does not necessarily imply a complete absence of aflatoxins but were just below detectable thresholds. Again, no amount of aflatoxin above zero levels is regarded as safe. "Reduction to As Low As Reasonably Achievable" is the endorsement of JECFA concerning the safe level of aflatoxins in foods following the significant genotoxic carcinogenic possibility of this toxin (Matumba et al. 2015). Recent, toxicological assessments estimate that every 1 ng/kg body weight/day increase in aflatoxin ingestion results in an increased risk of 0.01

to 0.03 cases of liver cancer per 10,000 individuals, (depending on the prevalence of hepatitis B infection) (Wu et al. 2013). From that standpoint, any decrease in aflatoxin intake will decrease the risk of death due to liver cancer.

Conclusion

Based on the results obtained in our study as compared with standards set by the European Food Safety Authority (EFSA) (2 and 4 µg/kg) and the Ghana Standards Authority (GSA) (5 and 10-15 µg/kg), it can be construed that out of the 100 samples analyzed for total aflatoxins (AF_{Total}), 68% exceeded the limits of EFSA and were of range 4.98±0.6−444.01±8.9 µg/kg whereas 58% and ranged between 12.12±1.4− 444.01±8.9 µg/kg exceeded for GSA limits. In the case of AFB_1, 71% of samples exceeded EFSA limits and were within the range of 2.26±0.6−338.3±8.6 while 64% exceeded GSA limits and were in the range of 5.18±0.25−338.3±8.6 µg/kg.

Human health risk assessment for aflatoxins exposure via maize sold and consumed in the Volta Region by infants, children, and adolescents, and adults showed a significant adverse health risk in all the age categories of humans since all calculated values for Margin of Exposure (MOE) were greater than 0.04 $ngkg^{-1}bwday^{-1}$ (derived by dividing 400 $ngkg^{-1}bwday^{-1}$ by 100,000). This study scratches the surface of a dire situation that calls for attention by all stakeholders involved in mitigating the harmful effects associated with these exposures.

We therefore strongly advocate some the strict compliance to good agricultural practice (GAP), good manufacturing practice (GMP) as well as good hygiene practice (GHP) which are critical ingredients to alleviate the formation of aflatoxins in the field as well as during storage of foodstuffs. By impeding the aflatoxins formation in foods, there is the protection of both public health and prevention of economic losses. Monitoring foods prone to fungal infection and the presence of mycotoxins regularly is cautious to assess the public level of awareness.

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Authors' contributions

NKK, TA, and JD performed the experiments and wrote the manuscript. TA and JD were responsible for aflatoxin analysis. NKK, TA, and JD helped conceive the experiments and prepare the manuscript. NKK and TA conceived the original study and NKK, and TA led the sampling and study in Ghana. All authors read and approved the final manuscript.

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Availability of data and Materials

Data shall be made available by the corresponding author upon request

Ethics approval

The data generated for this article did not involve both animal and human subjects which would have warranted the need for ethical clearance. All data obtained and used in the study were on plant material (maize)

Consent for publication

The data herein presented in this article does not contain any individual's personal data

Competing interest

The authors declare that they have no competing interest.

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Figures

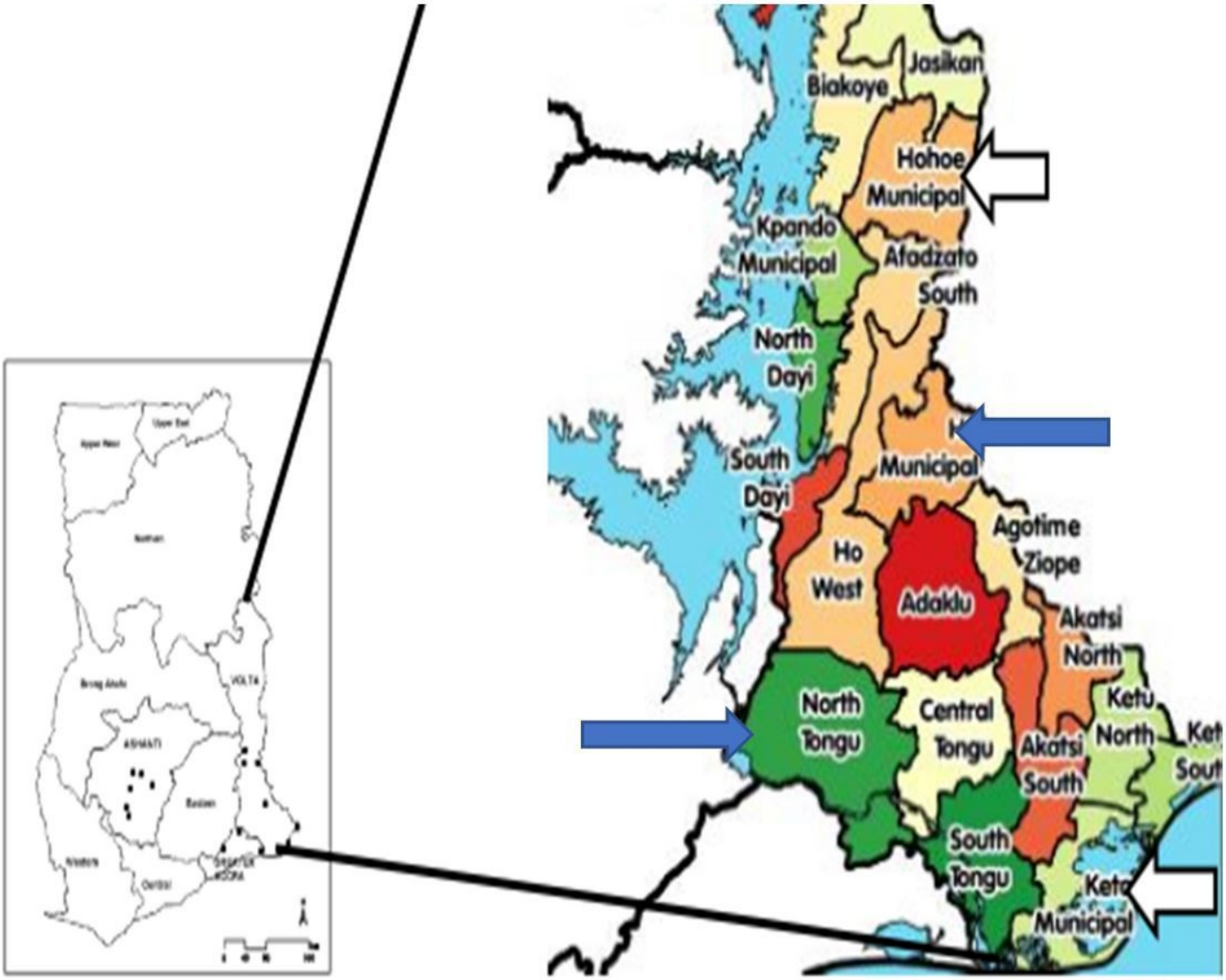

Figure 1

Map of Ghana and the Volta Region with arrows showing sampling sites. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.