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RESEARCH

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Exposure assessment and risk characterization of aflatoxins intake through consumption of maize (*Zea mays*) in different age populations 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 infection. Aflatoxin levels were checked against international (European Commission, EC) 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 were determined. Total aflatoxins (totalAFs) and the constituent aflatoxins (AFB_1, AFB_2, AFG_1, and AFG_2) were measured with High-Performance Liquid Chromatography (HPLC) with a Fluorescence Detector (FLD). Intake and Risk assessments were also conducted using deterministic 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 $0.78 \pm 0.04 - 234.73 \pm 3.8$ μg/kg, $0.47 \pm 0.03 - 21.6 \pm 0.33$ μg/kg, $1.01 \pm 0.05 - 13.75 \pm 1.2$ μg/kg and $0.66 \pm 0.06 - 5.51 \pm 0.26$ μg/kg respectively. Out of the 100 samples analyzed for total aflatoxins (totalAFs), 68 (68%) exceeded the limits of EC and were of range $4.98 \pm 0.6 - 445.01 \pm 8.9$ μg/kg whereas 58 (58%) and ranged between $12.12 \pm 1.4 - 445.01 \pm 8.9$ μg/kg exceeded GSA limits. Intake and risk assessments of total aflatoxins (totalAFs) for infants, toddlers, children, adolescents, and adults in the Volta Region were; 0.037–1.14 μg/kg bw/day, 0.35–10.81, and 1.47 -45.14 cases/10,000 person/yr respectively for Estimated Daily Intake (EDI), Margin of Exposure (MOE), and Cancer Risks. It was inferred that the consumption of maize posed potential adverse health effects on all age categories studied because all calculated MOE values were less than 10,000.

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 food in terms of quality, nutritional, and cultural purposes for a healthy life (Unnevehr 2015).

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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 toxins of fungal origin. These toxins are used as defense mechanisms in the ecosystem (Kunzler, 2018). Among the thousands of species of fungi, many are acknowledged to produce mycotoxins, and these genera; *Aspergillus, Penicillium,* and *Fusarium* are the most common. Out of the 400 and over mycotoxins known, the most important are aflatoxins, ochratoxins, zearalenone, fumonisin, and trichothecenes (Deoxynivalenol (DON or vomitoxin), T-2 toxin, T-2 like toxins) (Cinar and Onbaşı 2019; Agriopoulou et al. 2020). Aflatoxins are biochemical by-products of difurano coumarin synthesized by a polyketide pathway by strains of *Aspergillus,* notably *flavus* and *parasiticus*.(Borgomano 2015) *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. pseudotamarii* are also aflatoxin-producing species, they rarely do produce aflatoxins (Klich et al. 2000). Variations in the isolates of each aflatoxigenic species have resulted in great qualitative and quantitative differences in their aflatoxin-producing capabilities. Their ability to cause adverse health effects in both humans and animals has become a public health issue. They have gained so much prominence among mycotoxins in research and have been aptly classified as class 1 carcinogenic toxins by the International Agency for Research on Cancer (IARC, 2002; Ostry et al. 2017).

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 cultivated and consumed across all 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 that 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 of which were intended to discourage mold growth on the grains. 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, these new varieties of maize are still susceptible to fungal and subsequent mycotoxin contamination. Aflatoxins tend to contaminate foods and feed of human and animal populace (Agbetiameh et al. 2018; Kortei et al. 2021a) owing to its extensive usage in the preparation of countless delectable local dishes such as "kenkey" (boiled fermented corn dough), "banku/akple", "waha", "goya" (tubani), "Tuo zaafi", "aboloo"..etc. also some local beverages like "nmedan", "asaana", and "solom" (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 production as crops in the field can get high levels of fungal infection (Wagacha and Muthomi 2008; Williams et al. 2014). Conidia of *Aspergillus flavus* are the major source of primary inoculum in maize fields (Scheidegger and Payne, 2003). Conversely, Wagacha and Muthomi (2008) observed that other cereal crops like wheat, barley, oats, and sorghum are not so prone to preharvest aflatoxin contamination.

Previous work by some researchers (Kumi et al., 2014; Blankson and Mill-Robertson 2016; Opoku et al., 2018; Omari and Anyebuno 2020; Tuffour and Steele-Dadzie, 2019) in Ghana, revealed some evidence of the incidence of acute aflatoxicosis in children presumably via infant complementary foods. This troubling and uncomfortable situation stems from the overreliance and seemingly inevitable use of the two most frequently fungus-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).

Ghana recorded between birth and the age of 20 months a rapid percentage rise of malnourished children from 7 to 48% (Macro 2005. Approximately 40% of deaths of children under five in Ghana are attributed to malnutrition (Macro 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 (WHO, 2008).

Given the potential of maize becoming a nontraditional 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 (European Commission), totalAFs = 4 μg/kg, AFB_1 = 2 μg/kg) have 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, it is also essential to conduct health risk assessments in the population due to dietary exposure. A quantitative approach to the assessment of a possible number of hepatocarcinoma cancer (HCC) cases (Cancer risk estimates) was introduced by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1997 and a qualitative margin of exposure (MOE) method proposed at the 64th JECFA meeting in 2005 (EC, 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 occurrence of aflatoxins and the health risks posed by them through maize consumed 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 record of the Ghana Statistical Service (GSS, 2014).

The Volta Region was the study site for this research. Figure 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 °C. (Table 1).

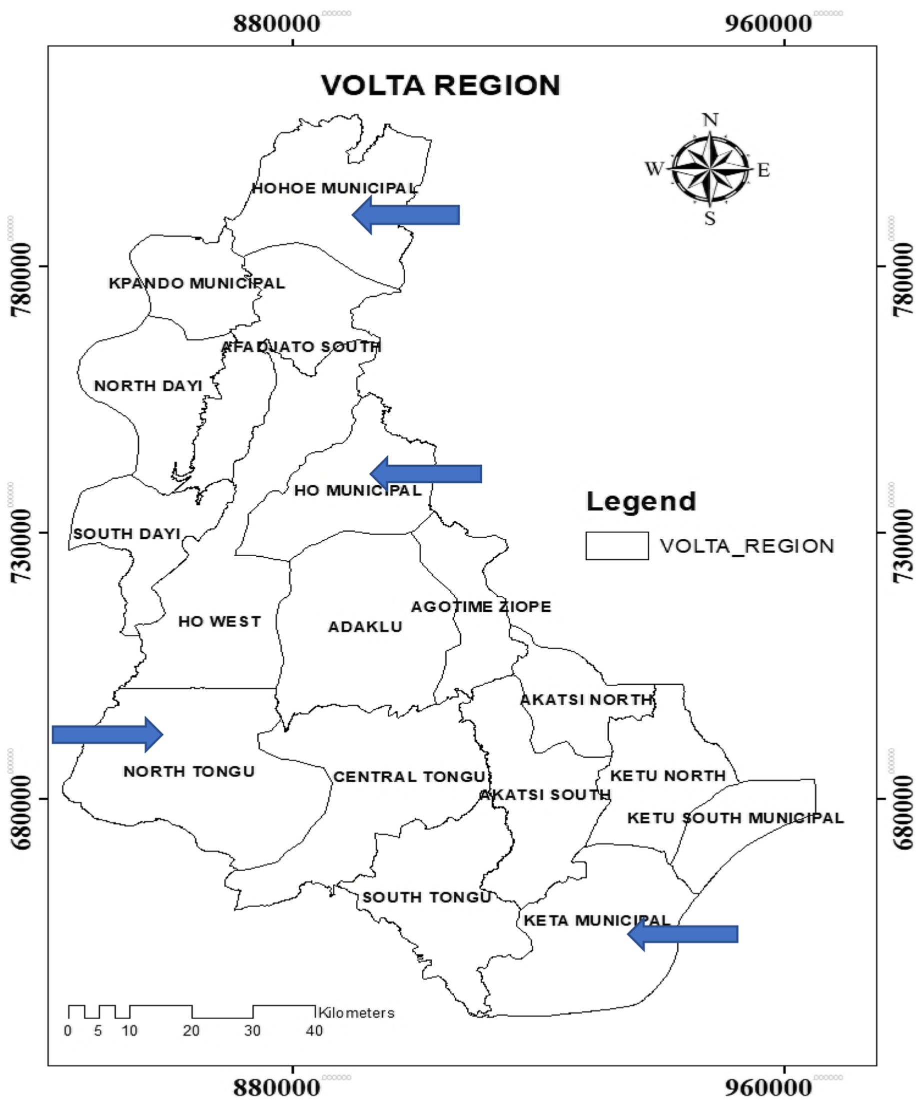

Fig. 1 Map of the Volta Region of Ghana with arrows showing sampling sites

Table 1 Geographical locations and some attributes of the origin of samples

Town in Volta Region	No. of samples	Agro-ecological zones	Rainfall (mm)	Temperature (°C)	Coordinates
Hohoe	25/100	Deciduous Forest	1985	25.5	5.8143°N, 0.0747° E
Ho	25/100	Deciduous Forest	766	25	5.5608°N, 1.0586° W
Battor Dugame	25/100	Coastal Savannah	536.2	27.9	5° 47 N, 0. 45° E
Keta	25/100	Coastal Savannah	897	28.4	6.2374°N, 0.4502° W

Sampling

Raw maize (*Zea mays*) grain samples were randomly purchased concurrently from markets in Hohoe, Ho, Battor, Dugame, and Keta, all in the Volta Region of Ghana (Fig. 1) from April 2020 to October 2020. Aliquots of twenty (20) grams 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).

Sample analysis

Extraction of aflatoxins

The European Committee for Standardization (CEN) official method EN14123 (Stroka and Anklam 2002) was used to extract and quantify AFS (AFB_1, AFB_2, AFG_1, and AFG_2) in the samples. Methanol in water (200 mL) (8 + 2) and 5 g NaCl were used to extract 20 g of the sample. Fat samples containing more than 50% fat were extracted with 100 mL of hexane by the normal methanol extraction solvent. One hundred millilitres (100 mL) of hexane was added to 200 mL of 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 the filtrate was added to 60 mL of phosphate buffer saline (PBS) for solid-phase extraction using a preconditioned immune affinity column specific for AFB_1, AFB_2, AFG_1, and AFG_2. The 70 mL filtrate-PBS mixture was loaded onto the preconditioned 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.

$$[\text{Sample weight (g) x spike concentration } (\mu g/\text{kg})] \ / \ [\text{Concentration of standard (ug/mL)}]. \quad (3)$$

Deionized water was used to make up the volume of eluate to 5 mL and eluate vortexed and 2 mL pipetted into HPLC vials for quantification.

HPLC operating conditions

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 × 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 standard used for spiking, 5 µg/kg (the lowest concentration range of the calibration curve). Blank did not produce any signal, so the LOD and LOQ were calculated as;

$$\text{LOD} = 3 \text{ x standard deviation/slope.} \quad (1)$$

$$\text{LOQ} = 3 \text{ x LOD.} \quad (2)$$

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 the studied mycotoxins were used in the validation procedure.

The left-censored data (data below LOD and LOQ) were processed by applying EFSA's substitution method (EFSA, 2010).

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, middle, and upper concentration range of the calibration curve concentrations (5 µg/kg, 15 µg/kg, and 30 µg/kg). Spike volumes of pure standards were calculated as;

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

$$[(\text{Concentration measured in spike—concentration measured in blank}) \ / \ (\text{spiked amount})] \text{ x } 100 \quad (4)$$

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 between the results was calculated. For intermediate precision, 10 extractions of the IRM were done on different days by different analysts, and the relative standard deviation between the results was calculated. The relative standard deviations were calculated as;

[Standard deviation/mean] x 100.

Intake and risk assessments of exposure to total aflatoxins through consumption of maize

Dietary estimation of Daily intake

Deterministic risk assessment model calculation for aflatoxin dietary exposure; Estimated Daily Intake (EDI) was considered by using the mean quantity of aflatoxins derived from the maize samples, the quantity of samples consumed daily, and the mean body weight. The EDI for mean aflatoxin was calculated according to the following formula (5) and expressed in µg/kg of body weight/day (µg/kg bw/day)(dos Santos et al. 2013, Chain et al. 2020).Total EDI was also calculated according to formula (5).

$$\mathrm{EDI} = \frac{Daily\ \mathrm{intake\ (food)}\ x\ \mathrm{Mean\ level\ of\ Aflatoxins}}{\mathrm{Mean\ body\ weight}} \quad (5)$$

The daily intake of maize in Ghana according to MOFA-IFPRI (2020) is approximately 0.107 kg/day (39.3 kg/year). A Daily intake of 0.107 kg/day was used for children and adults. An assumption of half of the Daily intake of maize for infants (0.5 × 0.107 kg/day).

Margin of exposure characterization for aflatoxins

Genotoxic compounds such as aflatoxins have their risk assessment fittingly computed based on the Margin of Exposure (MOEs) approach, which was estimated by dividing the Benchmark dose lower limit (BMDL) for aflatoxins—400 ng/kg bw/day by toxin exposure (EFSA, 2020) as expressed in Eq. (7).

$$\mathrm{MOE} = \frac{\mathrm{Benchmark\ dose\ lower\ limit}}{\mathrm{EDI}} \quad (7)$$

A public health alarm is raised in instances where MOEs are less than 10,000 (EFSA, 2020).

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 10,000, 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 $year^{-1}$ $10{,}000^{-1}$ population/ ng/kg bw/day (uncertainty range: 0.05–0.5) in HBsAg-positive individuals and 0.01 cancers/year/10,000 population ng/kg bw/day (uncertainty range: 0.002–0.03) in HBsAg-negative individuals (Shephard 2008) were adopted for this calculation. Moreover, 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 Eq. (8):

$$\begin{aligned}\mathrm{Average\ potency} &= [0.03\ \mathrm{x\ HBsAg} - \mathrm{negative\ individuals\ in\ Ghana}] + [0.01\ \mathrm{x\ HBsAg} - \mathrm{positive\ individuals/prevalence\ rate\ in\ Ghana}]\\ &= (0.3 \times 0.102) + (0.01 \times 0.898)\\ &= 0.03958\end{aligned} \quad (8)$$

The different age categories according to EFSA (2009) and their corresponding estimated average weights in Ghana used in this study were done as follows; Infants-2.9 (2.5–3.2) kg (Lartey et al. , 2000; Abubakari et al. , 2015), Toddler -9.8 (7–12.6) kg (Glover-Amengor et al. , 2016; Abeshu et al. 2016), Children -26 (24–28) kg (WHO, 2006; Biritwum et al. 2005), Adolescents- 46.25 (38.5–54) kg (Afrifa-Anane et al., 2015), Adults- 60.7 kg (Walpole et al. 2012).

Thus, the population risk was estimated using the following formula in Eq. (9) (Adetunji et al. 2018):

$$\mathrm{Cancer\ Risk} = \mathrm{Exposure\ (EDI)} \times \mathrm{Average\ potency} \quad (9)$$

Statistical analysis

The aflatoxin concentrations were calculated using regression analysis from the curves generated from the

Table 2 Limits of Detection (LOD) and Quantification (LOQ) of aflatoxins AFB_1, AFB_2, AFG_1, AFG_2, and Total aflatoxins (µg/kg) measured by HPLC

Aflatoxin	Limits	Amount (µg/kg)
AFB1	LOD	0.20
	LOQ	0.60
AFB2	LOD	0.17
	LOQ	0.51
AFG1	LOD	0.26
	LOQ	0.78
AFG2	LOD	0.36
	LOQ	1.08

LOD Limit of Detection

LOQ Limit of Quantification

standards of 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. Estimated Dietary Intake, MOE values, Average potency, and cancer risk were calculated. The results are summarized as mean, median, and range.

Results

Method validation

The Limits of Detection for AFB_1 and AFB_2 likewise AFG_1 and AFG2, ranged between 0.13–0.15, while Limits of Quantification ranged between 0.26–0.30 respectively for both (Table 2).

Occurrence of aflatoxins

The number of food samples contaminated with AFB_1, AFB_2, AFG_1, AFG_2, and totalAFs (Total Aflatoxins) is presented in Tables 3, 4 and 5. 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. Out of a total of one hundred (100) samples investigated, concentrations of AFB_1 and totalAFs ranged between 1.89 ± 0.06 − 338.3 ± 8.6 µg/kg and 1.89 ± 0.0.06 − 445.01 ± 8.9 µg/kg respectively. The total aflatoxin yields of 338.3 ± 8.6 µg/kg and 445.01 ± 8.9 µg/kg were ined for, respectively.

Table 3 describes the summary of statistics of the total aflatoxin in the Volta Region. For Hohoe (HH), values of 21.15, 19.43, and 61.40 µg/kg were recorded from the summary statistics as mean, median, and range respectively. Ho (Ho) recorded 35.72, 19.83, and 224.31 µg/

Table 3 Proportions of samples that exceeded totalAF limits of the European Commission (EC) and Ghana Standard Authority (GSA)

	Samples	Total samples	Mean	Median	Range	Exceeding EC regulation		Exceeding GSA regulation	
						Yes (%)	Range	Yes (%)	Range
TotalAF	Hohoe	25	21.15	19.43	61.40	19 (76%)	8.72 ± 0.8–61.4 ± 1.8	17 (68%)	12.51 ± 0.26–61.4 ± 1.8
	Ho	25	35.73	19.83	224.31	17 (68%)	8.5 ± 0.95–224.31 ± 6.4	14 (56%)	15.18 ± 0.44–224.31 ± 6.4
	Battor Dugame	25	61.81	44.42	229.00	19 (76%)	4.98 ± 0.6–229.0 ± 6.0	18 (72%)	19.4 ± 0.85–229.0 ± 6.0
	Keta	25	42.44	4.72	445.01	13 (52%)	4.72 ± 0.28–445.01 ± 8.9	9 (36%)	12.12 ± 1.4–445.01 ± 8.9
	Total	100				68	4.98 ± 0.6–445.01 ± 8.9	58	12.12 ± 1.4–445.01 ± 8.9

Ghana Standards Authority (GSA) limit for $AF_{Total} = 10$ µg/kg

European Commission (EC) limit for $AF_{Total} = 4$ µg/kg

Table 4 Proportions of samples that exceeded AFB1 limits of the European Commission (EC) and Ghana Standard Authority (GSA)

	Samples	Total samples	Mean	Median	Range	Exceeding EC regulation		Exceeding GSA regulation	
						Yes (%)	Range	Yes (%)	Range
AFB1	Hohoe	25	15.75	12.92	48.5	19 (76%)	4.31 ± 0.26–48.5 ± 0.99	18 (72%)	7.18 ± 0.25–48.5 ± 0.99
	Ho	25	27.63	14.97	195.5	18 (72%)	2.89 ± 0.4–195.5 ± 2.0	15 (60%)	5.46 ± 0.11–195.5 ± 2.0
	Battor Dugame	25	43.60	28.11	182.29	20 (80%)	2.26 ± 0.6–182.29 ± 8.33	18 (72%)	11.86 ± 0.45–182.29 ± 8.33
	Keta	25	33.50	3.93	339.30	14 (56%)	3.93 ± 0.22–339.3 ± 8.6	13 (52%)	5.18 ± 0.25- 339.3 ± 8.6
	Total	100				71	2.26 ± 0.6–339.3 ± 8.6	64	5.18 ± 0.25- 339.3 ± 8.6

European Commission (EC) limit for AFB1 = 2 µg/kg

Ghana Standards Authority (GSA) limit for AFB1 = 5 µg/kg

Table 5 Evaluation of cancer risk for Total Aflatoxins via consumption of maize

District/ Locality	Age Category	Estimated Daily Intake (EDI) (µg/Kg bw/day)	MOE	Cancer Risk (cases/10,000 persons/year)
Hohoe	Infants (0-11mths)	0.39	1.03	15.45
	Toddlers (12-35mths)	0.23	1.74	9.11
	Children (36mths-10yrs)	0.09	4.44	3.56
	Adolescents (11-17yrs)	0.05	8.00	1.98
	Adults (18-64yrs)	0.037	10.81	1.47
Ho	Infants (0-11mths)	0.66	0.61	26.14
	Toddlers (12-35mths)	0.20	2.00	7.92
	Children (36mths-10yrs)	0.15	2.67	5.94
	Adolescents (11-17yrs)	0.083	4.82	3.29
	Adults (18-64yrs)	0.063	6.35	2.49
Battor Dugame	Infants (0–11 mths)	1.14	0.35	45.14
	Toddlers (12-35mths)	0.34	1.18	13.46
	Children (36mths-10yrs)	0.25	1.60	9.90
	Adolescents (11-17yrs)	0.14	2.86	5.54
	Adults (18-64yrs)	0.11	3.64	4.36
Keta	Infants (0–11 mths)	0.78	0.51	30.89
	Toddlers (12-35mths)	0.23	1.74	9.11
	Children (36mths-10yrs)	0.17	2.35	6.73
	Adolescents (11-17yrs)	0.098	4.08	3.88
	Adults (18-64yrs)	0.075	5.33	2.97

Margin of Exposure-MOE

Mean aflatoxins- Hohoe = 21.15 µg/kg, Ho = 35.72 µg/kg, BD = 61.81 µg/kg, KT = 42.44 µg/kg

Average Potency of aflatoxins = 0.0396

Daily intake of maize for infants was halved (0.5 × 0.107 kg/day)

Daily intake of 0.107 kg/day was used for children and adults

(1 µg = 1000 ng)

Total EDIs (Volta Region) = 0.797 + 1.156 + 1.98 + 1.353 = 5.286

kg as mean, median, and range, respectively, The mean, median, and range recorded for Battor Dugame (BD) were 61.81, 44.42, and 229.00 µg/kg, respectively. Keta (KT) recorded a mean, median, and range of 42.44, 4.72, and 445.01 µg/kg, respectively. The summary of statistics for AFB1 has also been summarized in Table 4.

The European Commission (EC) and Ghana Standards Authority (GSA) regulatory limits for total aflatoxins (totalAFs) and aflatoxins B_1 (AFB_1) (Tables 3 and 4) were used in this study. Toxin permissible limits prescribed by the Ghana Standards Authority are a subset of the European Commission (EC).

Regarding the frequency and (percentage %) of positive (Yes) total aflatoxins (totalAFs) contaminated maize samples above the various permissible limits, Hohoe (HH) recorded 17(68%) of range 12.51 ± 0.26 − 61.4 ± 1.8 µg/kg and 19(76%) which ranged between 8.72 ± 0.8 − 61.4 ± 1.8 µg/kg and tested positive for GSA and EC, respectively (Table 3). For AFB1, values of 18 (72%) of range 7.18 ± 0.25 − 48.5 ± 0.99 µg/kg and 19(76%) within the range of 4.31 ± 0.26 − 48.5 ± 0.99 µg/kg tested positive for GSA and EC and respectively (Table 4).

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

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

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

Out of the 100 samples analyzed for total aflatoxins (totalAFs), 58% of samples exceeded and ranged between 12.12 ± 1.4 − 445.01 ± 8.9 μg/kg (Table 3) for GSA while 68% exceeded the limits of EC and were of range 4.98 ± 0.6 − 445.01 ± 8.9 μg/kg. For AFB_1, 64% of range 5.18 ± 0.25 − 338.3 ± 8.6 μg/kg exceeded the limits of GSA (Table 4) whereas 71% of samples of the range 2.26 ± 0.6 − 338.3 ± 8.6 μg/kg exceeded the tolerable limit of the EC.

Consumer risk assessment

The Estimated Daily Intakes (EDI) of the total aflatoxins in the maize samples from Hohoe (HH) were 0.39, 0.23, 0.09, 0.05 and 0.037 μg/kg bw/day for infants, toddlers, children, adolescents, and adults respectively. The Margin of Exposure (MOE) values recorded were 1.03, 1.74, 4.44, 8.00, and 10.81, respectively. The average potency of the aflatoxins was 0.0396 aflatoxins $kg^{-1}bwday^{-1}$ and this value was same for all towns investigated yielded cancer risks of 15.45, 9.11, 3.56, 1.98, and 1.47 cases/ 10,000 persons/ year respectively (Table 5).

Samples from Ho yielded EDI values of 0.66, 0.20, 0.15, 0.083, and 0.063 μg/kg bw/day for infants, toddlers, children, adolescents, and adults respectively. MOE values of 0.61, 2.00, 2.67, 4.82, and 6.35. Cancer risks of 26.14, 7.92, 5.94, 3.29, and 2.49 cases/ 10,000 persons/ year respectively, for these age categories were recorded.

From Battor Dugame, the EDI values recorded for infants, toddlers, children, adolescents, and adults were 1.14, 0.34, 0.25, 0.14, and 0.11 μg/kg bw/day respectively. MOE values recorded were 0.35, 1.18, 1.60, 2.86, and 3.64, respectively while the cancer risks were 45.14, 13.46, 9.90, 5.54, and 4.36, cases/10,000 persons/ year respectively (Table 5).

Lastly, for Keta (KT), the EDI values recorded for infants, toddlers, children, adolescents, and adults were 0.78, 0.23, 0.17, 0.098, and 0.075 μg/kg bw/day respectively. MOE values recorded were 0.51, 1.74, 2.35, 4.08, and 5.33, respectively. Also, the cancer risks were 30.89, 9.11, 6.73, 3.88, and 2.97, cases/10,000 persons/ year respectively (Table 5).

Discussion

Occurrence of aflatoxins

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 subregion. 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 Battor Dugame also belong to the coastal savannah zone. Presumably, the rainfall and humidity patterns of these zones contributed to the conducive growth conditions which encouraged fungal growth and subsequent production of persistent aflatoxins in the maize samples. The range of values recorded in this study was consistent with the values reported by Kpodo et al. (1996) in earlier surveys reporting 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 ng/g. 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 1–341 ng/g. in maize from different ecological zones in Ghana. Likewise, from the Brong-Ahafo region of Ghana, Benson-Obour et al. (2018) recorded aflatoxins at levels up to 113.56 ng/g. 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, pointing to the prevalence of aflatoxins. In Kenya, Nduti et al. (2017) reported values of range 7.92 ± 1.57 − 22.54 ± 4.94 ng/g 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. AFs levels up to 15,000 μg/kg and 320,000 μg/kg 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 μg/kg), Kpalsogu (4–1400 μg/ kg) and Kintampo (1200 μg/kg) in Ghana, and Lafia (1200 μg/kg) and

Mokwa (5–480 μg/kg), in Nigeria. In 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 ng/g. in 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 desert 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 (aflatoxin) production. These climatic conditions as well as 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 a 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, high exposures of aflatoxins as recorded in this study, have caused severe lesions of the liver in malnourished children and adults, often with fatal outcomes (Wagacha and Muthomi 2008). Richard et al. (2020), Abdallah et al. (2021), 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 might be 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 rigorous processing methods because of their ability to resist chemical and thermal changes (Mahato et al. 2019).

Recently, Etwire and Martey (2020) asserted that fungal invasion to produce aflatoxins depends on preproduction and postproduction 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 seeds and encourage good postharvest practices.

Consumer 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). In Ghana, not much milk is produced and so milk is only found in the nomadic minority areas. Due to this, infants are introduced to maize porridge at very early stages instead of milk. About 40% of deaths of children under five in Ghana are attributed to malnutrition (Opoku et al. 2018).

Risk assessment results obtained in this study were comparable to the published findings of Blankson and Mill-Robertson (2016) as they reported Total aflatoxins EDI values of range 0.005 − 1.054 μg/kg bw/d and 0.004 − 0.838 μg/kg bw/day 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 μg/kg bw/day 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 0.022–0.439 ng/kg bw/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 the 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 the risk analysis results showed that most of the lower bound MOE values ranged from 10 to 100, indicating a need 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 AFs. 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 AFs exposure. The results of both studies agreed with our findings.

In this study, EDI was slightly greater compared with other EDI values reported globally. This implies a significant impact of aflatoxins on the nutritional status of humans and animals. Gong et al. (2008) observed that aflatoxin exposures were to some extent linked to nutrient deficiency in humans following a suggestion that aflatoxin exposure expedites intestinal damage resulting in a decline in nutrient absorption. A noteworthy positive link between aflatoxin prevalence and zinc and vitamin A deficiencies was also established by Watson et al. (2015). Furthermore, a study in Ghana, reported that subjects with high exposure to the toxin were more likely to suffer deficiencies of vitamins A and E (Obuseh et al. 2010). A strong association between anaemia and aflatoxin has been reported in Ghana (Shuaib et al. 2010) showing that aflatoxin exposure may contribute partly to the high iron deficiency prevalent in children in developing countries including Ghana.

The consumption of aflatoxins at high levels in a single dose or repeatedly for a brief period induces acute intoxication, henceforward labeled aflatoxicosis, in humans and animals with typical symptoms, including jaundice, lethargy, nausea, edema, hemorrhagic necrosis of liver tissues, bile duct hyperplasia, and eventually death (10–60%) after severe liver damage (Peraica et al. 1999). While there is no accord on the specific dose of aflatoxins that triggers acute toxicity in humans, it is well recognized that such a dose is highly adjustable depending on many factors, including the age, gender, health, and nutritional status, presence or absence of underlying factors such as chronic viral hepatitis, alcoholism, smoking, cirrhosis, exposure to hepatotoxic microcystins); and it is lowest in youngsters, as validated by the highest death rates of this age-group in aflatoxicosis outbreaks (Opoku et al. 2018).

A recent scoping review by Soriano et al. (2020) has shown the presence of these aflatoxins appeared in greater proportion in kwashiorkor in children and in different organs and biological samples including brain (Oyelami et al., 1995a), heart (Oyelami et al., 1995b), kidney (Oyelami et al., 1998), liver (Oyelami et al., 1995b), lung (Oyelami et al., 1997), serum (Onyemelukwe et al., 2012), stool (De Vries et al., 1990) and urine (Hatem et al., 2005; Onyemelukwe et al., 2012; Tchan et al., 2010) whereas in the marasmic-kwashiorkor they were detected in liver (Hendrickse, 1985), serum (Onyemelukwe et al., 2012), and urine (De Vries et al., 1990; Onyemelukwe et al., 2012).

It is worth noting 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 level is regarded as safe. "As Low as Reasonably Achievable- ALARA" 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). 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.

Practically, some strategies such as the practice of dietary diversity, efficient and prompt drying of wet cereal grains for safe moisture levels, no late sowing dates and avoidance of high cropping density, proper selection of maize hybrids, prevention of use of soft kernel hybrids, minimizing periods between harvesting and drying etc. are among some methods suggested by some researchers (Adegoke and Letuma, 2013; Kortei et al. 2021c; Sipos et al. 2021) to help reduce aflatoxin contact in humans as well as animals.

Conclusion

Based on the results obtained in our study as compared with the standards set by the Ghana Standards Authority (GSA) and the European Commission (EC), it can be construed that out of the 100 samples analyzed for total aflatoxins (totalAFs), 58% exceeded GSA limits whereas 68% exceeded the limits of EC. In the case of AFB_1, 64% exceeded GSA limits while 71% of the samples exceeded EC limits. Cancer risk characterization for aflatoxins exposure via maize sold and consumed in the Volta Region by infants, toddlers, children, adolescents, and adults posed a potentially significant adverse cancer risk in all age categories and this indicates a need for risk management since all calculated values for margin of Exposure (MOE) were less than 10,000. This study scratches the surface of a dire situation that calls for attention from all stakeholders involved in mitigating the harmful effects associated with these exposures.

We therefore strongly advocate strict compliance with good agricultural practice (GAP), good manufacturing practice (GMP), as well as good hygiene practices (GHP), which are critical ingredients to alleviate the formation of aflatoxins in the field as well as during storage of foodstuffs. By impeding 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.

Limitations of this study

Although there exists some other data on the per capita maize consumption in Ghana, the assumptive data provided in this research article provides a reliable information to a large extent. Also, the analytical method used in the detection of aflatoxins in this study may not be of high accuracy and efficiency in this era of high technology. These were some of the limitations of this study.

Abbreviations

AFB1: Aflatoxin B1; AFB2: Aflatoxin B2; AFG1: Aflatoxin G1; AFG2: Aflatoxin G2; AFs: Aflatoxins; Total AFs: Total aflatoxin; HH: Hohoe; Ho: Ho; BD: Battor Dugame; KT: Keta; EFSA: European Food Safety Authority; EC: European Commission; GSA: Ghana Standards Authority; MOFA-IFPRI: Ministry of Food and Agriculture-International Food Policy Research Institute; HPLC: High Performance Liquid Chromatography; FLD: Fluorescence Detector; EDI: Estimated Daily Intake; MOE: Margin of Exposure; JECFA: Joint FAO/WHO Committee of Experts on Food Additives; DON: Deoxynivalenol; T1: Toxin 1; T2: Toxin 2; IRM: Internal Reference Material; HCC: Hepatocarcinoma Cancer; IARC: International Agency for Cancer Research; WHO: World Health Organization; BMDL: Bench Mark Dose Limit; HBsAg-positive: Hepatitis B surface antigen positive; HBsAg-negative: Hepatitis B surface antigen negative; Ppb: Parts per billion; BDL: Below Detection Limits; Rpm: Revolutions per minute; CEN: European Committee for Standardization; GHP: Good Hygiene Practice; GAP: Good Agricultural Practice; GMP: Good Manufacturing Practice; GSS: Ghana Statistical Service; LOD: Limit of Detection; LOQ: Limit of Quantification.

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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, DA and JD helped conceive the experiments and prepare the manuscript. NKK and TA conceived the original study and NKK, DA 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.

Declarations

Ethics approval and consent to participate

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 interests

The authors declare that they have no competing interests.

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