



## Exposure to aflatoxins and ochratoxin A from the consumption of selected staples and fresh cow milk in the wet and dry seasons in Ghana

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### ABSTRACT

Across sub-Saharan Africa, the heavy reliance on mycotoxin-susceptible staple foods means that populations in the region are particularly vulnerable to chronic mycotoxin exposure. This study assessed the exposure risk to ochratoxin A (OTA) and aflatoxins (AFs) from 18 samples of selected staple foods (maize, millet, groundnut) and 56 fresh cow milk samples collected from across Ghana. The foods were sampled simultaneously to maximise comparability, and at two timepoints in March/April (during the dry season) and July/August (during the rainy season) to assess the effects of duration of storage and seasonal conditions on the mycotoxin levels as measured by high-performance liquid chromatography. The margin of exposure (MOE) approach was used to assess the exposure risk from consumption of the sampled foods. Each of the sampled staples was contaminated with OTA (0.19–3.11 ng/g) and at least one AF (0.75–13.05 ng/g B<sub>1</sub>, ND-12.12 ng/g B<sub>2</sub>, 0.1–9.95 ng/g G<sub>1</sub>, ND-16.78 ng/g G<sub>2</sub>). Up to 67% had contamination above European Food Safety Authority (EFSA) maximum limits, and 50% were above Ghana Standards Authority (GSA) limits. The fresh cow milk samples were contaminated with AFM<sub>1</sub> in the range of 0.05–1.49 ng/g, with 95% above EFSA limits and 36% above GSA limits. Aflatoxin contamination in the staples was high, particularly in July/August when the wet conditions may have adversely impacted the handling and storage of farm produce. Variation in AFM<sub>1</sub> between the two sampling periods mirrored total aflatoxin in the staples, suggesting that even if dairy cattle were grazing in open pasture and not being rationed on stored feed, then there was a high environmental presence of aflatoxigenic fungi. The MOE estimates were ≤533.09, far below the safe cut-off of 10,000 for suspected carcinogenic compounds. The high mycotoxin levels indicate a priority risk to child nutrition which relies heavily on cereal mixes based on one or all the three sampled staples. The data from this study underscore the urgent need for interventions to better appreciate and address mycotoxin exposure for enhanced food security and public health in Ghana and across sub-Saharan Africa.

### 1. Background

Maize, millet, and groundnut are major staples in Ghana and several other countries in sub-Saharan Africa (SSA). Particularly for maize and groundnut which are important for food security and economic

development across the sub-region, the literature continues to report increasing contamination with mycotoxins (Edgar Mugizi et al., 2021; Gbashi et al., 2020). This trend raises serious food safety and public health concerns, because of the adverse effects of mycotoxin exposure even at low doses (Omotayo et al., 2019). Mycotoxin contamination

**Abbreviations:** AEZ, Agroecological Zone; AF, Aflatoxin; CS, Coastal Savannah; GSA, Ghana Standards Authority; GS, Guinea Savannah; SDF, Semideciduous Forest; OTA, Ochratoxin A.

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renders foods unfit for human and other animal consumption, leading to market rejection and revenue loss from trade, and a reduced local supply of safe food (Adeyeye et al., 2022; Gbashi et al., 2020; Imade et al., 2021; Udomkun et al., 2017). Dietary intake of mycotoxins is a well-documented risk factor for several adverse health effects, including organ damage, autoimmune diseases, cancer, and neurological dysfunction in humans and other animals (Chu et al., 2017; Sirma et al., 2019). Aflatoxins (AFs<sup>1</sup>) and ochratoxin A (OTA) are two of the most frequent mycotoxin contaminants of Africa’s food crops (Blankson et al., 2019). They have also been shown to occur in animal-derived products such as dairy (Sirma et al., 2019; Zhang et al., 2019), eggs (Emmanuel K et al., 2020), and meat (Pleadin et al., 2021), and have been shown to have neurotoxic, carcinogenic, hepatotoxic, nephrotoxic, immunotoxic, and teratogenic effects (Benkerroum, 2020; Kumar, 2020; Kumar et al.,

2022). Although the mycotoxin contamination of foods in SSA is well-acknowledged, the human exposure risks from dietary sources, and the relationships between mycotoxin levels in foods and mycotoxin levels in humans, and are still under-described. In this study, maize, millet, and groundnut were sampled to assess the levels of contamination and margins of human exposure from consumption of the contaminated foods. For the first time in Ghana, the sampling of food to assess mycotoxin contamination was designed (1) to capture the combined effects of duration of storage and seasonal conditions by sampling at two time-points, (2) to simultaneously assess food samples collected from the most densely populated cities spread across the length of country, and (3) to assess multiple staple food material simultaneously to maximise the relevance of comparisons and exposure assessments. Fresh cow milk was also sampled to determine AF contamination levels

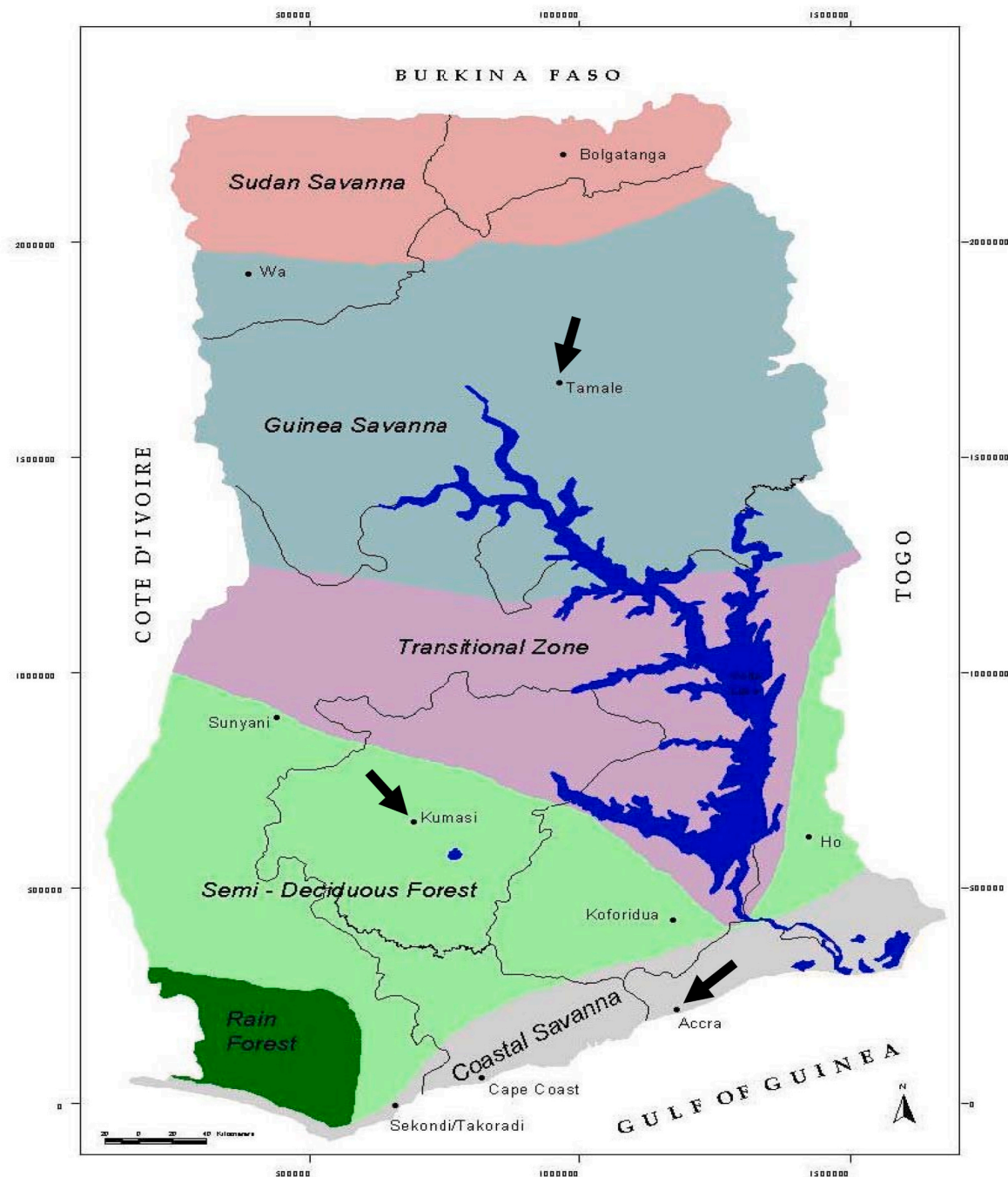


Fig. 1. Ghana’s agroecological zones with the sampling areas marked by arrows. Source of map image: (Owusu-Ansah & Smardon, 2015).

and assess human exposure.

## 2. Materials and methods

### 2.1. Chemicals and reagents

HPLC-grade acetonitrile, methanol, and absolute ethanol were purchased from Park Scientific Limited (Northampton, UK). Formic acid, sodium chloride, cyclooctadecanonaene (C18), chloroform, 2-propanol, anhydrous magnesium sulphate and ethylenediaminetetraacetic acid (EDTA) were purchased from BDH Chemicals Limited (Poole, England). Chem-Lab (NV, Belgium) supplied trifluoroacetic acid (TFA), and glacial acetic acid was from Merck KGaA (Darmstadt, Germany). Primary secondary amine (PSA) was from Thermo-Fisher Scientific (Waltham, MA, USA). Mixed AFB<sub>1</sub>/B<sub>2</sub>/G<sub>1</sub>/G<sub>2</sub>, AFM<sub>1</sub>, and OTA standards were purchased from Romer Labs, Inc (Newark, USA).

### 2.2. Preparation of mycotoxin standards

Stock solutions of mixed AF (200 ng/g) and OTA (100 ng/g) standard were each prepared in 5 mL methanol and stored at  $-20^{\circ}\text{C}$ . Working solutions of 20 ng/g AF and 10 ng/g OTA were prepared in methanol and used for analyses.

### 2.3. Sample collection and preparation

Sampling was done in March/April 2022 (dry period) and July/August 2022 (wet period) according to published methods (The European Commission, 2014). Maize, millet, and groundnut were purposively sampled from three major markets in each of Tamale, Kumasi, and Accra; the biggest cities in Ghana by population, and with the busiest market activities serving as the hubs for smaller markets across three of the country's agroecological zones (AEZs): Guinea Savannah, Semi-Deciduous Forest, and Coastal Savannah (Fig. 1, Table S1). The AEZs were chosen because they are the most densely populated, and because they capture the range of rainfall and farming seasonal variations in Ghana. The sampling was done at timepoints which coincided with the end of the dry season and the peak of the rainy season in Ghana, to provide insight into the influence of seasonal variation on the mycotoxin levels. In each location, 0.9–1.5 kg of each staple was purchased from three vendors in three different markets into sterile Ziplock bags, placed on ice, and transported to the laboratory for mycotoxin extraction within 48 h. Approximately 1 L of fresh cow milk was purchased from fourteen randomly selected vendors in two of the busiest markets in Accra (Nima and Madina) in each of the sampling windows.

A total of 110 samples from both periods, consisting of 18 samples each of groundnut, maize, and millet, and 56 samples of fresh milk were processed for testing. For each staple, equal weights of samples collected from the same market and during the same collection period were pooled and mixed thoroughly to provide three lots. From each lot per market, a 1 kg quantity was fetched and finely milled in a high-speed blender (Silver Crest Commercial Blender 3000W). The three milled samples per staple representative of the AEZ were packed separately in sterile Ziplock bags and stored at  $-20^{\circ}\text{C}$  pending further processing for testing. Each milled pack was sampled and tested in duplicate, and all six results were averaged for each staple per AEZ. The milk samples from each market and each sampling time were pooled, and three 100 mL portions were aliquoted from each pool for testing.

### 2.4. Detection and quantification of mycotoxins

OTA and AFB<sub>1</sub>, -B<sub>2</sub>, -G<sub>1</sub>, -G<sub>2</sub>, -M<sub>1</sub> were extracted and quantified by high-performance liquid chromatography with fluorescence detection (HPLC-FLD) (ISO 14501:2021, 2021; Pantano et al., 2021).

#### 2.4.1. Mycotoxin extraction

The quick, easy, cheap, effective, rugged, and safe (QuEChERS) dispersive solid-phase extraction (dSPE) method (Pantano et al., 2021) with slight modifications, was used to extract mycotoxins in the milled samples. Briefly, 5 g of each milled sample was mixed with 10 mL double distilled water and 10 mL acetonitrile/2% formic acid solution at 80/20 v/v. The mixture was vortexed briefly and placed on a reciprocating shaker for 15 min at 5000 rpm. The mixture was incubated at  $-20^{\circ}\text{C}$  for 15 min, 5 g QuEChERS dSPE salts (4 g MgSO<sub>4</sub>; 1 g NaCl) were added, and the mixture was vortexed for 5 min. The mixture was centrifuged at 5000 rpm for 5 min, and 1 mL of supernatant was transferred into a 2 mL Eppendorf tube containing QuEChERS dSPE clean-up salt mix (150 mg MgSO<sub>4</sub>; 50 mg PSA; 50 mg C18). The mixture was vortexed for 5 min and centrifuged at 5000 rpm for 5 min. Approximately 500  $\mu\text{L}$  of supernatant was evaporated to dryness at  $40^{\circ}\text{C}$  and reconstituted in methanol or derivatization solution (water/TFA/glacial acetic acid 70/20/10 v/v/v) (Hofhine et al., 2016) for mycotoxin testing.

AFM<sub>1</sub> in fresh milk was extracted as described in (ISO 14501:2021, 2021). For each sample, 100 mL was warmed to  $35\text{--}37^{\circ}\text{C}$  and filtered through Whatman no. 4 filter paper. The filtrate (50 mL) was run down an AFM<sub>1</sub> IAC immunoaffinity column (AFLAPREP® M WIDE, R-Biopharm Rhône Ltd) at a rate of 2 mL/min, followed by 20 mL of phosphate-buffered saline (pH 7.4) at a rate of 5 mL/min. To eliminate any remaining liquid, air was passed from a syringe through the column. To elute the toxin, 1.25 mL of methanol: acetonitrile (40:60 v/v) was loaded on the column and eluted at a rate of one drop per second into an amber glass vial. The column was rinsed with 1.25 mL of distilled water which was eluted to make a total volume of 2.5 mL in each collection vial. This eluent was used for all analyses.

#### 2.4.2. Mycotoxin analysis

AFs B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>, M<sub>1</sub>, and OTA in the sample extracts were analysed on a Phenomenex® C-18 Synergi (00G-4375-E0) column on a Waters e2695 HPLC Separations Module (Waters Corp., Milford, MA) with a fluorescence detector (2475 FLR Detector). Pre-column derivatization was done to enhance the detection of AFB<sub>1</sub> and AFG<sub>1</sub> (Hofhine et al., 2016; Ok et al., 2016). Briefly, the dried standards or sample extracts were reconstituted in 100  $\mu\text{L}$  of derivatization solution, vortexed, and incubated in an ultrasonic water bath (Clifton SW3H, Switzerland) at  $65^{\circ}\text{C}$  for 15 min. The solution was cooled after which 900  $\mu\text{L}$  of 50% methanol was added, vortexed, and analysed by HPLC. No derivatization was required for OTA analysis. The dried standards and sample extracts were reconstituted in 1 mL of 100% methanol for HPLC analysis. The samples from milk were analysed as eluted. The HPLC conditions are as shown in Table S2. The levels of OTA and AF in the samples were compared to the Ghana Standards Authority (Kortei et al., 2021; Omari et al., 2022) and European Food Safety Authority (The European Commission, 2023, pp. 103–157) maximum limits.

#### 2.4.3. Performance of analytical methods

The performance of the analytical methods was assessed for linearity and sensitivity (AOAC Official Methods of Analysis, 2016; Rahmani et al., 2009). The calibration points ranged from 2 to 10 ng/g for AFB<sub>1</sub>/B<sub>2</sub>/G<sub>1</sub>/G<sub>2</sub>, 0.02–0.8 ng/g for AFM<sub>1</sub>, and 0.5–10 ng/g for OTA. The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the calibration plot for each mycotoxin (International Committee on Harmonization, "Validation of Analytical Procedures: Text and Methodology, Q2(R2), 2022). For assessment of the accuracy of the analytical methods, in-house reference materials of maize, millet, groundnut and milk were spiked with three concentrations each of AFB<sub>1</sub>/B<sub>2</sub>/G<sub>1</sub>/G<sub>2</sub> (0.5, 5, and 50 ng/g), OTA (0.5, 5, and 15 ng/g), or AFM<sub>1</sub> (0.02, 0.2, and 2 ng/g). The reference samples were extracted as described for the experimental samples, the mycotoxin levels in the extracts were measured by HPLC, and the recovery for each was calculated as:

$$\text{Recovery} = \frac{\text{Concentration in spiked material} - \text{Original concentration in material}}{\text{Spiking concentration}} \times 100 \dots\dots (1)$$

To assess precision, intra-day repeatability and inter-day reproducibility values were determined and expressed as relative standard deviations, RSD<sub>r</sub> and RSD<sub>R</sub>, respectively. Briefly, two standards — 2 and 4 ng/g of AF<sub>TOTAL</sub> and OTA, or 0.04 and 0.8 ng/g AFM<sub>1</sub> — were added to the samples for each batch of HPLC analyses, and were both run before and after the test samples. The results before and after for each day were compared to assess intra-day repeatability. Inter-day reproducibility was estimated by comparing the data of the control runs between days.

2.5. Determination of exposure risk to aflatoxins and OTA

Published estimates of Ghana’s daily intake per capita of groundnuts, maize, millet, and milk (Achaglinkame et al., 2023; Agriculture in Ghana Facts and Figures 2021, n.d.; Ghana: An Overview of Ghana’s Dairy Industry | USDA Foreign Agricultural Service, n.d.) were adopted for this study (Table 1). The average bodyweight of a Ghanaian adult as adopted for this study is 60.7 kg (Kortei et al., 2021). The mycotoxin levels (AFB<sub>1</sub>, AFM<sub>1</sub>, OTA) per staple across the two sampling periods were averaged. The estimated daily intake (EDI) values were calculated based on the upper and lower limits of the averages, estimated daily intake of the staples, and the average adult body weight:

$$\text{EDI} = \frac{\text{Daily food intake} \times \text{Average mycotoxin concentration}}{\text{Average bodyweight}} \dots\dots (2)$$

The benchmark dose lower limits (BMDLs) for AFB<sub>1</sub> and OTA, respectively, are 400 ng/g bw/day and 17.86 ng/g bw/kg, and the BMDL for AFM<sub>1</sub> is 40 ng/g bw/day (EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain) et al., 2020). The risk due to mycotoxin exposure from the contaminated foods was estimated by margin of exposure (MOE) calculations based on the BMDL and EDI values (upper and lower limits):

$$\text{MOE} = \frac{\text{BMDL}}{\text{EDI}} \dots\dots (3)$$

2.6. Data analyses

Comparison of mycotoxin levels between staples from different locations or seasons were done by Welch ANOVA with Dunnett’s multiple comparisons, or by multiple unpaired t-test with Welch correction on GraphPad Prism 9.0.0 (121). The data is reported as mean ± SD to reflect the distribution of data points. Differences were considered statistically significant at P < 0.05.

**Table 1**  
General per capita consumption of maize, millet, and groundnut in Ghana.

Food	Per Capita Consumption	
	Annual (kg/year)	Daily (kg/day)
Maize	75.03	0.206
Groundnut	14.68	0.04
Millet	5.31	0.015
<sup>a</sup> Fresh Milk	<sup>b</sup> 1.71	0.0046

<sup>a</sup> Fresh milk refers to locally produced fresh cow milk and milk products.  
<sup>b</sup> Based on the estimation that 19% of the 9 kg annual per capita milk consumption in Ghana is locally sourced.  
 Sources: (Achaglinkame et al., 2023; Agriculture in Ghana Facts and Figures 2021, n.d.; USDA, 2022)

3. Results

3.1. Method performance

Recovery of mycotoxin from the spiked samples ranged between 73.31 and 97.9% for AFs and ≥86% for OTA (Table 2); all were within the acceptable recovery ranges of the European Commission (The European Commission, 2014). The calibration plots showed strong linear relationships with coefficients of determination ≥0.996 for all the mycotoxins (Table S3). The limit of detection (LOD) and limit of quantification (LOQ), respectively, were 0.10 and 0.31 (AFG<sub>1</sub>), 0.54 and 1.64 (AFG<sub>2</sub>), 0.02 and 0.05 (AFB<sub>1</sub>), 0.05 and 0.17 (AFB<sub>2</sub>), 0.02 and 0.06 (AFM<sub>1</sub>), and 0.05 and 0.15 (OTA) (Table S3).

3.2. Occurrence of aflatoxins and OTA in maize, millet, groundnut, and milk

In all, 9 samples of each staple were analysed per collection time: three pooled samples from each of the three markets in each AEZ. All the pooled samples had detectable mycotoxin contamination at both collection times (Figs. 2 and 3, Tables S4–S6). AF-B<sub>1</sub>, -G<sub>1</sub>, and OTA were detected in 100% of the staples, and AFM<sub>1</sub> was detected in 95% of the milk samples. The overall total mycotoxin levels were compared between the AEZs and between staples across sampling periods (Figs. 4 and

**Table 2**  
Precision and accuracy of the test method.

Matrix	Mycotoxin	Precision			Accuracy	
		Range (ng/g)	<sup>1</sup> RSD <sub>r</sub> (%)	<sup>2</sup> RSD <sub>R</sub> (%)	Range (ng/g)	<sup>3</sup> Rec ± RSD (%)
Maize	AFG <sub>1</sub>	2–10	5.63	5.18	0.5–50	90.76 ± 5.49
	AFG <sub>2</sub>	2–10	9.17	8.59	0.5–50	83.31 ± 6.93
	AFB <sub>1</sub>	2–10	5.69	5.01	0.5–50	90.90 ± 4.92
	AFB <sub>2</sub>	2–10	5.84	4.76	0.5–50	76.55 ± 4.08
	OTA	2–5	11.73	8.78	0.5–15	86.01 ± 12.81
Millet	AFG <sub>1</sub>	2–10	3.66	5.70	0.5–50	97.89 ± 2.80
	AFG <sub>2</sub>	2–10	4.85	6.33	0.5–50	86.60 ± 5.92
	AFB <sub>1</sub>	2–10	7.60	7.04	0.5–50	85.95 ± 2.69
	AFB <sub>2</sub>	2–10	2.81	4.15	0.5–50	86.80 ± 6.80
	OTA	2–5	11.46	9.30	0.5–15	95.13 ± 8.68
Groundnut	AFG <sub>1</sub>	2–10	4.52	3.98	0.5–50	93.39 ± 3.93
	AFG <sub>2</sub>	2–10	5.98	5.36	0.5–50	89.86 ± 6.76
	AFB <sub>1</sub>	2–10	4.77	5.25	0.5–50	92.05 ± 5.87
	AFB <sub>2</sub>	2–10	7.44	5.15	0.5–50	80.89 ± 4.90
	OTA	2–5	14.76	12.03	0.5–15	98.52 ± 10.50
Fresh milk	AFM <sub>1</sub>	0.04–0.8	4.67	2.18	0.02–2	95.67 ± 3.87

RSD, Relative Standard Deviation; AFG<sub>1</sub>, Aflatoxin G<sub>1</sub>; AFG<sub>2</sub>, Aflatoxin G<sub>2</sub>; AFB<sub>1</sub>, Aflatoxin B<sub>1</sub>; AFB<sub>2</sub>, Aflatoxin B<sub>2</sub>; OTA, Ochratoxin A. Figures shown are (Mean ± SD) of n = 6.  
<sup>1</sup>intra-day repeatability.  
<sup>2</sup>inter-day reproducibility.  
<sup>3</sup>Recovery ± Relative standard deviation.

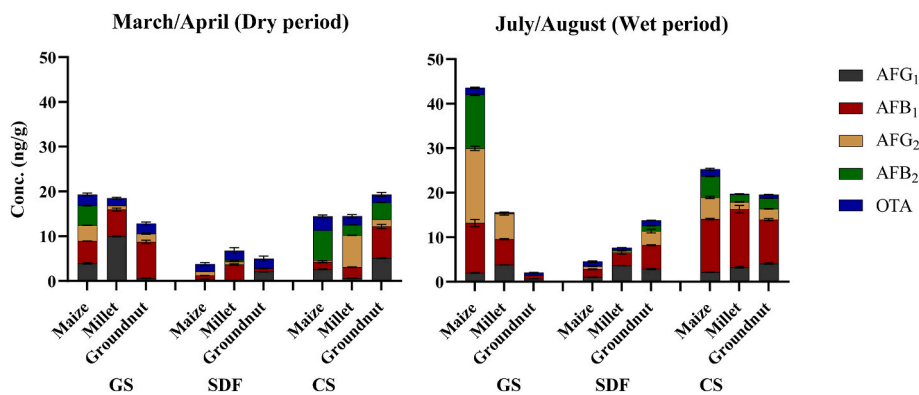


Fig. 2. Total mycotoxin concentration in staples sampled in March/April (dry period) and July/August (wet period). Aflatoxin (AF)-B1, -B2, -G1, -G2; Ochratoxin A (OTA); Guinea Savannah (GS); Semi-Deciduous Forest (SDF); Coastal Savannah (CS).

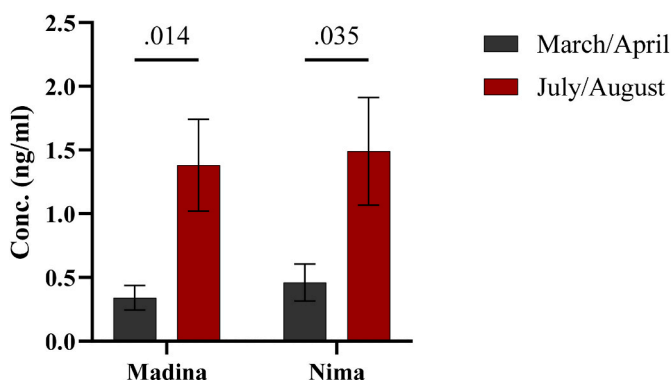


Fig. 3. Aflatoxin M<sub>1</sub> (AFM<sub>1</sub>) concentration in fresh cow milk sampled at different times from two locations in the coastal savannah agroecological zone in Ghana.

5; Tables S4 and S5). The range for mean AF<sub>TOTAL</sub> across AEZs was 10.09–11.35 ng/g in the dry sampling period (March/April), and 10.94–23.09 ng/g in the wet sampling period (July/August) (Fig. 2; Tables S4 and S5). Mean OTA ranged between 1.90 and 2.42 ng/g in the dry period and 0.33–1.37 ng/g in the wet period (Fig. 2; Tables S4 and S5). Mean AFM<sub>1</sub> in the milk samples ranged between 0.34 and 0.46 ng/g in the dry period and 1.38–1.49 ng/g in the wet period (Fig. 3; Table S6). Overall, during the dry period across all AEZs, millet had the highest mean AF<sub>TOTAL</sub> (11.35 ng/g;  $P \leq 0.007$ ), and maize had the highest OTA (2.42 ng/g;  $P \leq 0.001$ ) (Fig. 4A and B). During the wet period, maize had both the highest mean AF<sub>TOTAL</sub> (23.09 ng/g;  $P \leq 0.043$ ) and the highest OTA (1.37 ng/g;  $P \leq 0.001$ ) (Fig. 4A and B).

The mean of AF<sub>TOTAL</sub> contamination in samples collected from the semi-deciduous forest (SDF) and coastal savannah (CS) zones in the wet period was higher than in the dry period for maize ( $P < 0.001$ ), millet ( $P < 0.001$ ) and groundnut ( $P < 0.035$ ) (Fig. 5). Although AF<sub>TOTAL</sub> in maize was higher in the wet period for the GS samples ( $P < 0.0034$ ), the level in groundnut was lower ( $P < 0.001$ ) than in the dry period and the level in groundnut did not change ( $P = 0.062$ ). Conversely, OTA levels were higher in the dry period than in the wet period for samples from all the zones (Fig. 6): maize ( $P \leq 0.024$ ), millet ( $P \leq 0.015$ ), and groundnut ( $P$

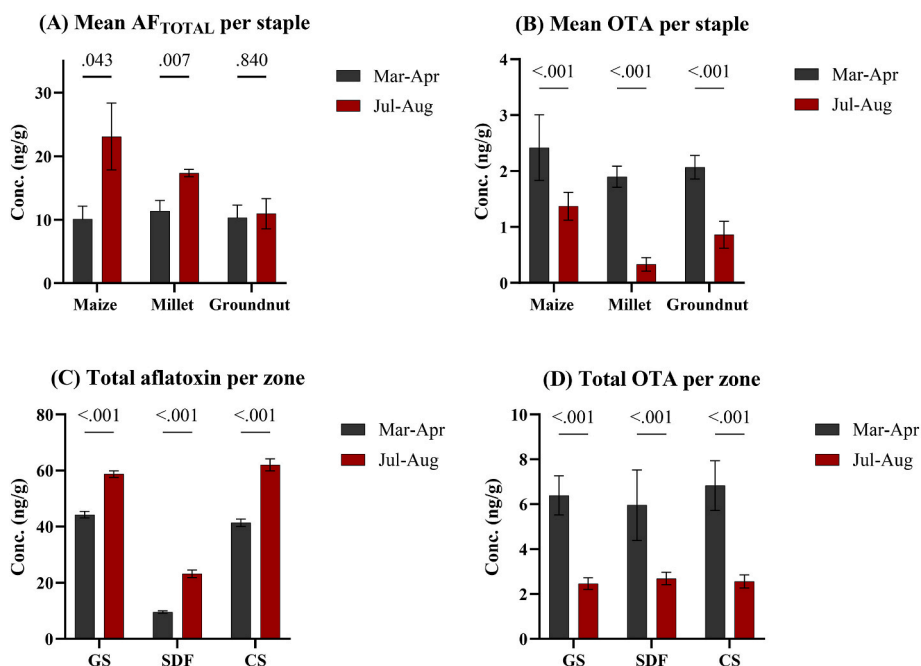
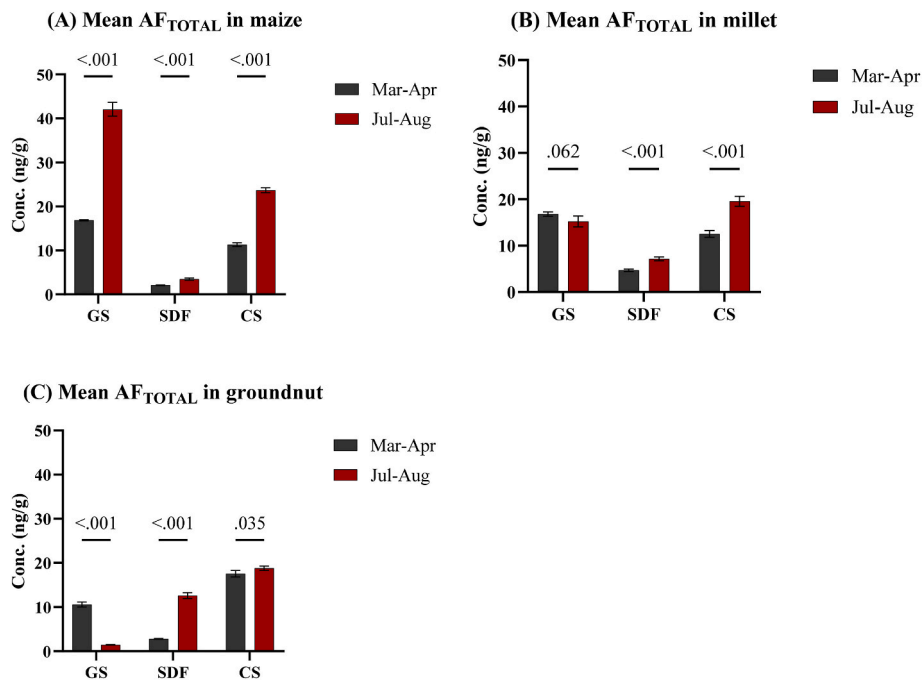


Fig. 4. Mean mycotoxin concentration per staple across agroecological zones (A, B); and total mycotoxin concentration in all staples per agroecological zone (C, D). Total Aflatoxin (AF<sub>TOTAL</sub>); Ochratoxin A (OTA); Guinea Savannah (GS); Semi-Deciduous Forest (SDF); Coastal Savannah (CS).



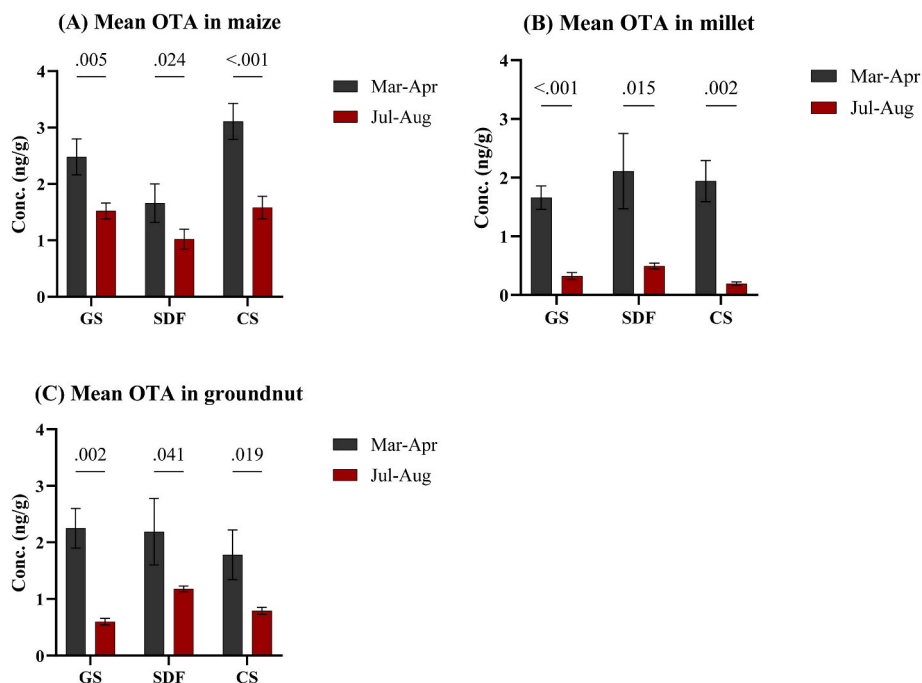
**Fig. 5.** Mean total aflatoxin (AF<sub>TOTAL</sub>) concentration per agroecological zone in maize (A), millet (B), and groundnut (C). Guinea Savannah (GS); Semi-Deciduous Forest (SDF); Coastal Savannah (CS).

< 0.041). In the two sampling locations for fresh cow milk, AFM<sub>1</sub> in the wet period was higher ( $P \leq 0.035$ ) than in the dry period (Fig. 3).

Across all AEZs and over the two sampling periods, at least 50% of the staples and milk were contaminated with aflatoxin exceeding both the GSA and EFSA maximum limits for AFB<sub>1</sub> and AF<sub>TOTAL</sub> (Table 3). The mean contamination levels in the samples above limits ranged from 2.45 to 13.05 ng/g for AFB<sub>1</sub> and 10.57–42.08 ng/g for AF<sub>TOTAL</sub>. AFM<sub>1</sub> in the fresh milk samples above regulatory limits ranged from 0.055 to 1.56 ng/g (EFSA) and 0.54–1.56 ng/g (GSA). The recorded OTA levels were all below the thresholds of both the EFSA (4 ng/g) and GSA (5 ng/g).

### 3.3. Exposure risks to aflatoxins and OTA through maize, millet, groundnut, and milk

Groundnut presented the lowest risk as an exposure route to both AFB<sub>1</sub> and OTA (Table 4), although the MOE from groundnuts was still unacceptably high (Table 5). Across the AEZs, estimated daily intake (EDI) for AFB<sub>1</sub> was 0.75–4.61 ng/kg bw/day from groundnut, 4.48–27.40 ng/kg bw/day from maize, and 3.86–5.44 ng/kg bw/day from millet (Table 4). For OTA, EDI values ranged from 0.05 to 0.25 ng/kg bw/day for groundnut, 0.88–5.01 ng/kg bw/day for maize, and



**Fig. 6.** Mean ochratoxin A (OTA) concentration per agroecological zone in maize (A), millet (B), and groundnut (C). Guinea Savannah (GS); Semi-Deciduous Forest (SDF); Coastal Savannah (CS).

**Table 3**

Proportion of samples with mycotoxin concentration exceeding the maximum limits of European Food Safety Authority or Ghana Standards Authority.

Matrix	<sup>1</sup> N	EFSA			GSA		
		Limit (ng/g)	<sup>2</sup> Percentage (%)	Range (ng/g)	Limit (ng/g)	<sup>2</sup> Percentage (%)	Range (ng/g)
<b>AFB<sub>1</sub></b>							
Maize	18	5	66.67	5.01–11.91	5	66.67	5.01–11.91
Millet	18	2	100	2.45–13.05	5	66.67	2.45–13.05
Groundnut	18	2	66.67	5.32–9.88	5	66.67	5.32–9.88
<b>AF<sub>TOTAL</sub></b>							
Maize	18	10	66.67	11.30–42.08	15	50.00	16.85–42.08
Millet	18	4	83.33	12.53–19.57	15	83.33	12.53–19.57
Groundnut	18	4	66.67	10.57–18.76	10	66.67	10.57–18.76
<b>AFM<sub>1</sub></b>							
Fresh cow milk	56	0.05	94.64	0.06–1.56	0.5	35.71	0.54–1.56

<sup>1</sup>Total number of samples tested. <sup>2</sup>Proportion exceeding tolerable limits.EFSA, European Food Safety Authority; GSA, Ghana Standards Authority; AFB<sub>1</sub>, Aflatoxin B<sub>1</sub>; AF<sub>TOTAL</sub>, Total of AFG<sub>1</sub>, AFG<sub>2</sub>, AFB<sub>1</sub>, and AFB<sub>2</sub>; OTA, Ochratoxin A. Sources: (Kortei et al., 2021; Omari et al., 2022; The European Commission, 2023, pp. 103–157).**Table 4**

Estimated daily intake of mycotoxins from the consumption of the sampled foods by an average adult in Ghana.

Sampled Food	Estimated Daily Intake (ng/kg bw/day)		
	AFB <sub>1</sub>	OTA	AFM <sub>1</sub>
Groundnut	0.75–4.61	0.05–0.25	–
Maize	4.48–27.40	0.88–5.01	–
Millet	3.86–5.44	0.17–0.77	–
Milk	–	–	0.03–0.04

AFB<sub>1</sub>, Aflatoxin B<sub>1</sub>; OTA, Ochratoxin A. Figures shown are the ranges of n = 6.**Table 5**

Margins of exposure to mycotoxins from the consumption of the sampled foods by an average adult in Ghana.

Sampled Food	Margin of exposure		
	AFB <sub>1</sub>	OTA	AFM <sub>1</sub>
Groundnut	86.84–533.09	70.76–326.26	–
Maize	14.60–89.34	3.56–20.19	–
Millet	73.55–103.55	23.22–103.99	–
Milk	–	–	1070.29–1535.81

AFB<sub>1</sub>, Aflatoxin B<sub>1</sub>; OTA, Ochratoxin A. Figures shown are the ranges of n = 6.

0.17–0.77 ng/kg bw/day for millet (Table 4). Margin of exposure (MOE) estimates for AFs were 86.84–533.09 for groundnut, 16.60–89.34 for maize and 73.55–103.55 for millet (Table 5). MOEs for OTA were 70.76–326.26 for groundnut, 3.56–20.19 for maize, and 23.22–103.99 for millet. The EDI and MOE for milk ranged between 0.03 and 0.04 ng/kg bw/day and 1070.29–1535.81, respectively.

#### 4. Discussion

Foods and food products may be co-contaminated with different filamentous fungal species and their toxins (Crudo et al., 2019; Gam-bacorta et al., 2018; Zhao et al., 2021). This study assessed the levels of aflatoxin (AF) and ochratoxin A (OTA) contamination in major staple foods (maize, millet, groundnut) and fresh cow milk in Ghana, and estimated the margins of exposure to the mycotoxins across different age categories. AFs and OTA were of interest in this study because they are two of the most important mycotoxin contaminants of foods in sub-Saharan Africa (Udomkun et al., 2017), and they have frequently been reported to co-occur in food matrices (Asghar et al., 2023; Daou et al., 2021; Iqbal et al., 2018; Joubrane et al., 2020; Kabak, 2019). This co-occurrence was recorded in all the sampled foods (Figs. 2 and 3), with 67–100% of the staple samples above EFSA and/or GSA limits for AFB<sub>1</sub>, and 36–95% of fresh milk samples above limits for AFM<sub>1</sub> (Table 3). The more potent B<sub>1</sub> and G<sub>1</sub> aflatoxins occurred in all the staples, whereas the

less potent B<sub>2</sub> and G<sub>2</sub> types occurred in 89% and 61% of samples, respectively (Fig. 2). These findings are instructive to the urgency and scale of interventions required to mitigate the public health effects of the chronic low-dose exposure of the population in Ghana and across the rest of SSA.

Interestingly, AFs and OTA are noted to show an inverse 'level of contamination' relationship — relatively high AF concentrations are frequently recorded in matrices where OTA levels are low, and vice versa (Heshmati et al., 2017; Iqbal et al., 2018; Zain, 2011). The reason for this is unclear, but the inverse relationship was seen from our data (Fig. 4). An under-appreciated inference from this is that there is the potential to harness atoxigenic strains of fungi that are traditional OTA-producers, such as *A. niger* and *A. ochraceus*, in the biocontrol of aflatoxigenic fungi, and vice versa. This potential has recently been highlighted and explored in field isolates of *A. niger* (Oduro-Mensah et al., 2023).

The levels of mycotoxin contamination varied between the various agroecological zones (AEZs) and sampling periods (Fig. 4). These variations may be attributable to interactions between the contaminating fungal species, storage duration, and seasonal changes that affected the storage conditions, all of which influence fungal colonization and mycotoxin contamination of stored foods. Because the major harvest in Ghana is between September/October of each year, it was expected that the foods that were sampled in this study had been stored from the previous year's harvest. This meant that the samples collected in the wet period (Jul/Aug) had been in storage longer than the samples collected in the dry period (Mar/Apr). Also, rainfall in Ghana is either bimodal in the south where the SDF and CS zones are located, or unimodal in the north where the GS zone is located (Zubairu, 2021). The unimodal rainfall starts in June/July and ends in November; the bimodal is from March to July for the major rainy season, and from September to October for the minor season (Ministry of Food and Agriculture (MoFA), 2022; Rainfall Pattern Has Changed in Ghana - EPA - Ghana Business News, n.d.). Therefore, the wet period samples from the SDF and CS zones had been exposed to more humid conditions that would encourage fungal growth and mycotoxin contamination, relative to the samples from the GS zone.

For all the AEZs, AF<sub>TOTAL</sub> in maize was higher ( $P < 0.001$ ) in the wet period than in the dry period (Fig. 5). Because the wet period sampling took place after the rains had started in all AEZs, our suggestion is that the wetter weather affected the storage conditions of crops in market storehouses, making it more conducive for fungal growth and mycotoxin production. Particularly in the SDF zone which has earlier and more rainfall than both the GS and CS, there was a 142% increase in AF<sub>TOTAL</sub> in maize in July/August, relative to 33% for GS and 51% for CS. The CS zone also has earlier and more rainfall than the GS zone (Yeleele et al., 2023).

Overall, AF<sub>TOTAL</sub> contamination in maize in the GS and CS zones was

higher ( $P < 0.001$ ) than in SDF (Fig. 5). In the southern AEZs which have bimodal rainfall, harvest of the second maize crop is completed in January of the following year (FAO GIEWS Country Brief on Ghana -, n.d.). Therefore, the maize crop sampled in the southern AEZs were of fresher stock than the maize from the northern GS zone. In the unimodal GS zone, the major harvest of cereals including maize is completed in October/November (FAO GIEWS Country Brief on Ghana -, n.d.), and so the sampled maize had been in storage for 2–3 months longer than the SDF samples. This may account for the higher  $AF_{TOTAL}$  in GS maize than in SDF. The CS samples also had higher contamination ( $P < 0.001$ ) than SDF. The CS zone is not a significant producer of maize but relies on the GS and SDF zones for supply. Therefore, the combination of wet conditions and longer storage than the SDF zone may explain why CS had higher aflatoxin contamination.

The factors driving the variations in mycotoxin levels in the maize samples may be applicable to the groundnut samples. Over 85% of Ghana's groundnuts are grown in northern farming communities in the GS zone (Ministry of Food and Agriculture (MoFA), 2022). Groundnuts are harvested twice in Ghana; the major harvest in April–June and the minor in August–September (Jolly et al., 2008). In addition to relying on groundnut supplies from the GS AEZ, the groundnuts grown in the SDF zone tends to be harvested during the wet months of the year. This presents serious challenges with effective drying, as opposed to the harvest in the GS zone which tends to be around April/May when it is relatively dry. This may explain why  $AF_{TOTAL}$  in the groundnut samples were particularly high during the wet period sampling in the SDF and CS zones (Fig. 5c). The  $AF_{TOTAL}$  levels in the GS zone may have been lower during the wet period stored groundnut had been sampled earlier in the dry period whereas the fresh groundnut had recently arrived on the market at the time of second sampling. Notably, this data points to rather rapid colonization by aflatoxigenic fungi and aflatoxin contamination of groundnuts. This is because the fresh stock was distributed to markets across Ghana and by the time of sampling, was already heavily contaminated in the wetter zones (Fig. 5).

Like groundnuts, millet in Ghana is produced almost exclusively in the GS zone (Ministry of Food and Agriculture (MoFA), 2022), and the harvest is between July and November (Asungre et al., 2021; Bawa, 2019). Therefore, the millet sampled in all the AEZs was likely to have been stored since November of the previous year. Between the two sampling periods,  $AF_{TOTAL}$  in millet did not change significantly in the GS zone ( $P = 0.062$ ) but increased by  $\geq 53\%$  ( $P < 0.001$ ) in the SDF and CS zones (Fig. 4b). The reason for this is unclear but may be attributable to increased fungal contamination due to the handling and transport of stored millet from the primary storehouses in the northern GS zone to the wetter south of Ghana. Millet is reputed for being relatively resistant to aflatoxin contamination (Sirma AJ et al., 2016), unlike maize and groundnut which are more susceptible. Curiously, our millet samples were contaminated above EFSA and GSA aflatoxin limits more frequently (83–100%) than maize and groundnut samples (50–67%) (Table 3).

Whereas several studies have documented the contamination of staple foods in Ghana with mycotoxins, only a few have demonstrated the potential health risks (Kortei et al., 2021). Human exposure to carcinogenic and genotoxic compounds like aflatoxins and OTA are of public health concern when the margins of exposure (MOEs) are below 10,000. The estimated MOEs in this study for aflatoxins and OTA from all the sampled foods were well below the safe threshold (Table 5). The MOEs were highest from groundnuts and lowest from maize for both  $AFB_1$  and OTA. This is an indication of dangerously high exposure of an average adult in Ghana to the mycotoxins. Even for children under 3–10 years of age (average bodyweight 26 kg), we estimate maize-associated MOE values of 22.06–135.04 ( $AFB_1$ ) and 5.39–30.58 (OTA), respectively, based on the per capita maize intake of 136 g/day by children under 5 years as reported by the AFRICAP Household Vulnerability Survey in Malawi in 2019 and 2021 (Galani, Ligowe, et al., 2022). We have adopted this estimate from Malawi because in sub-Saharan Africa,

most foods for weaning babies and toddlers are maize based (Ekpa et al., 2019; Galani, Orfila, & Gong, 2022). This usually implies that all their meals throughout the day will consist of maize products. These estimates are an indication of a major challenge to wholesome nutrition to support child development. Additionally, the estimates highlight the danger across the lifespan from exposure to mycotoxins in the sampled foods.

A key limitation in this study, however, is our pooling of samples from individuals in a subset of market vendors at the respective sampling sites. Fungal spot contaminations can lead to heterogeneously distributed mycotoxin patterns over grain samples. In our analyses, however, samples from different vendors in the same markets were pooled and considered as point estimates. Therefore, caution must be exercised with the generalisation of our findings to cover all groundnuts, maize, and millet under similar conditions in Ghana. We will need continuous monitoring over a few years with far greater sample sizes in order to obtain a clear overview of the mycotoxin contamination patterns and levels.

The high levels of  $AFM_1$  in the milk samples suggest a pervasive environmental presence of aflatoxigenic fungi. This is despite on-going efforts to reduce mycotoxin contamination of crops in the field; for example, using products which specifically target aflatoxigenic fungi and aflatoxin contamination, and have been deployed in Ghana since 2018 (Aflasafe: Safeguarding Health and Livelihoods with Biocontrol Technology in Africa - CGIAR, n.d.; Bandyopadhyay et al., 2019). However, we note that the sampling of milk was done in Ghana's capital city, Accra, which is in the CS zone, popular for cattle rearing for meat and dairy (Ferguson Gidiglo, 2014). Accra does not have much farming activity and so its environment is unlikely to enjoy the benefits, if any, of the application of such products for aflatoxin control. The dairy cattle in Ghana are generally accommodated in open kraals and released to graze, but their diets may be supplemented with composites from stored feed and fodder in the dry season. The high levels of  $AFM_1$  contamination in both the dry and wet periods, mirroring  $AF_{TOTAL}$  in the staples, suggest that even during open pasturing, the animals consume aflatoxin-contaminated material. It is important to probe this further to identify which feed substrates are the source(s) of the aflatoxins, to provide insight into the environmental presence of aflatoxigenic fungi (Xu, 2022) and the bioaccumulation of aflatoxins in farmed meat and other farm animal produce in Ghana.

#### 4.1. Conclusion

The data from this study indicate that staple foods in Ghana may be a source of dangerously high levels of population-wide exposure to mycotoxins, increasing with duration of storage and wetter weather conditions. Overall, the levels of mycotoxin contamination point to a dire outlook on healthy life years and public health costs in Ghana. The range of MOEs indicates a priority threat to public health and signals the need for dedicated work to address the persisting challenges of mycotoxin contamination and human exposure. The contamination of fresh milk that was observed is a strong indication of a high level of environmental presence of mycotoxigenic fungi. Studies are needed to reassess the trail of contamination from field to table. Urgent cooperation is needed between regulatory agencies, research institutions, farmer groups, bulk purchasers, cereal marketers, and other stakeholders to formulate strategies for mitigating the contamination of crops and the levels of human exposure to mycotoxin-contaminated produce on local markets. Continuing efforts are needed to map the distribution of mycotoxigenic fungi in Ghana and across the sub-region, and to continue the development of sustainable, effective control strategies, including biocontrol approaches.

#### CRedit authorship contribution statement

**Abdul Hamid Nuhu:** Writing – original draft, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation. **Winfred-**

**Peck Dorleku:** Writing – original draft, Validation, Supervision, Resources, Methodology, Formal analysis, Conceptualization. **Beatrice Blay:** Writing – original draft, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation. **Evans Derban:** Investigation, Data curation. **Clara Owusuwah McArthur:** Investigation, Data curation. **Simon Elikplim Alobuia:** Investigation, Data curation. **Araba Incoom:** Methodology, Investigation. **Derry Dontoh:** Methodology. **Isaac Williams Ofosu:** Writing – original draft, Validation, Supervision, Resources, Methodology, Formal analysis, Conceptualization. **Daniel Oduro-Mensah:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodcont.2024.110968>.

## Data availability

Data will be made available on request.

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