


Aflatoxin contamination in Tanzania: quantifying the problem in maize and groundnuts from rural households

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Abstract

Aflatoxins are toxic and carcinogenic secondary metabolites, produced by *Aspergillus flavus* and *Aspergillus parasiticus*, which contaminate food and feed and threaten human and animal health. To assess the prevalence of aflatoxins in Tanzania, 180 groundnut and 200 maize samples were collected from 9 and 10 districts, respectively. Aflatoxin contamination was quantified using high performance liquid chromatography. Aflatoxins were detected in samples collected from all districts and prevalence ranged from 92 to 100% for groundnuts and 10 to 80% for maize. The mean aflatoxin level for groundnuts was 6.37 µg/kg and the highly contaminated sample had 40.31 µg/kg. For maize, the mean aflatoxin level was 12.47 µg/kg and the highly contaminated sample had 162.40 µg/kg. The estimated average probable daily intake (APDI) of aflatoxin B₁ (AFB₁) from groundnuts consumption was 1.88 ng/kg body weight/day, while for maize, it ranged between 151.98-272.89 ng/kg body weight/day. The APDI for both groundnut and maize exceeded the provisional maximum tolerable daily intake (PMTDI) of AFB₁ for adults (1 ng/kg body weight/day), bringing about health concerns for populations in Tanzania. Another alarming finding was that 75% of the farmers who provided samples for analysis were not aware of aflatoxins or the negative health impacts from consuming contaminated products. Results reported in this paper show that aflatoxin contaminated staple crops are widely distributed in Tanzania and that the risk of human exposure is high due to diet preferences. Awareness campaigns are required to inform and protect farmers and consumers.

Keywords: mycotoxin, *Aspergillus flavus*, aflatoxin exposure

1. Introduction

Aflatoxins are poisons produced by fungi belonging to the genus, *Aspergillus*. The two most important aflatoxin producers, *Aspergillus flavus* and *Aspergillus parasiticus*, are ubiquitous in soil (Visconti and Perrone, 2008). Maize and groundnut are most susceptible to contamination with aflatoxins, but other crops such as dried cassava,

coffee, chili, tree nuts and legumes can be contaminated (Essono *et al.*, 2007; Manjula *et al.*, 2009). Aflatoxin contamination and prevalence depend on many factors including variability in climatic conditions, type of commodity, agricultural practices and processing and storage conditions (Richard, 2007). According to the United Nations Food and Agriculture Organization (FAO), about 25% of world food crops are affected by aflatoxins, and the

highest risks are in tropical countries situated between 43° North and South of the Equator (FAO, 2004). Aflatoxin is also associated with some chronic health risks, such as cancer, immune suppression, and child stunting (Gong *et al.*, 2016; Leroy, 2013).

Aflatoxin is highly regulated through international trade standards and contamination of agricultural products impacts negatively on trade. Wu (2004) showed that losses in groundnut export from rejected products due to aflatoxin contamination may exceed 450 million US dollars under the current European Union (EU) regulatory standard of 4 µg/kg. However, a risk assessment of aflatoxin in food in Africa conducted by Shephard (2008) indicated that even low levels of contamination, which might fall within regulatory limits, can have serious health implications due to high per capita consumption of aflatoxin-prone crops such as maize. Thus, meeting the regulatory limits set for maximum tolerable limits in products destined for human consumption does not in itself guarantee food safety, especially where these products are consumed in relatively large quantities.

Maximum tolerable limits of aflatoxins in food destined for human consumption vary depending on the country (FAO, 2004). The current maximum tolerable limits for aflatoxins in maize and groundnut in the EU, the East African Community (EAC) and the USA are 4, 10 and 20 µg/kg, respectively (EC, 2010; USFDA, 2015; EAC, 2011). Intake of high doses of aflatoxins can lead to acute conditions such as aflatoxicosis outbreaks and death. Consumption of food with high levels of aflatoxin can be fatal, as experienced in Kenya in 2004, where 125 people lost their lives after consuming food made from home-grown maize that contained high levels of aflatoxins (Lewis *et al.*, 2005). Tanzania experienced its first cases of acute aflatoxicosis in 2016, when 68 people were hospitalised after consuming contaminated maize and 20 lost their lives (Kamala *et al.*, 2018).

Aflatoxicosis and chronic health risks due to aflatoxin are from dietary exposure. The World Health Organization (WHO) (2018) reports that most human exposure to aflatoxin comes from nuts and grains. Aflatoxin contamination levels can vary widely, from products that meet the maximum tolerable levels set by authorities to products with levels that can pose a risk of acute aflatoxicosis. However, the consumption pattern of a given crop significantly influences aflatoxin-related problems (Gong *et al.*, 2016), thus, low concentration of aflatoxin in a product does not by itself guarantee its safety if the consumption rate per day is high. Maize and groundnut are among the most important food crops in sub-Saharan Africa with high consumption rates per day and are major ingredients in weaning food given to children (Gong *et al.*, 2016). Moreover, children are reported to be more prone

to aflatoxicosis than adults because of their less developed immune systems and high intake of foods and water per kg body weight (bw) (Lombard, 2014; WHO, 2018). In Tanzania, maize consumption rate is estimated at 429.4 g/person/day (Abt Associates, 2012), while the Tanzanian Food and Nutrition Center (TFNC) has recommended an average consumption rate of 771 g/person/day (TFNC, 2014). For groundnut, consumption rate is 15 g/person/day (Abt Associates, 2012). Using consumption rate, dietary exposure is calculated to measure parameters such as probable daily intake (PDI), average probable daily intake (APDI) and maximum probable daily intake (MPDI), and these are expressed in ng/kg bw/day (Herrman and Yunes, 1999). Estimates of mean aflatoxin dietary exposures in developed countries are generally less than 1 ng/kg bw/day whereas in some of the countries in sub-Saharan African exceeds 100 ng/kg bw/day (WHO, 2018).

Aflatoxin contamination of agricultural products starts while the crop is in the field (pre-harvest) and continues through processing, transportation, and storage when environmental conditions are favourable for fungal infection and colonisation (Milani, 2013). Factors favouring aflatoxin contamination include drought during crop growing period, crop contact with bare soil (during harvesting), high temperature and humidity (harvesting and/or storing wet/humid crops) (Schroeder, 1969). As aflatoxins are colourless, odourless and cannot be seen, the only way to detect them is through laboratory testing. However, aflatoxin assaying kits suitable for field testing are either unavailable or expensive for smallholder farmers. This complicates efforts to remove contaminated products, especially for developing countries like Tanzania, where more than 70% of maize and groundnut production is by smallholder farmers and for home consumption.

Relatively little information on the prevalence of mycotoxins across the maize and groundnut value chains in Tanzania is available (Abt Associates, 2012). A few studies focusing on a few villages and using a limited number of samples have revealed high levels of aflatoxin contamination of maize and groundnuts (Kimanya *et al.*, 2014), and the presence of aflatoxins in milk and serum (Magoha *et al.*, 2014a; Shirima *et al.*, 2013). Assessing mycotoxin contamination of maize and groundnuts across a wide area of Tanzania encompassing several agro-ecological zones is the first and essential step in addressing the problem. This study was conducted to quantify aflatoxin contamination in maize and groundnuts across the major producing regions in Tanzania. This information was necessary for selection of areas to target intervention strategies for managing aflatoxin and for targeting aflatoxin awareness raising activities. Previous reports revealed that awareness of aflatoxin and its health impacts is low in Tanzania (Kimanya *et al.*, 2014; Magembe *et al.*, 2016; Nathaniels, 2014; TFDA, 2012).

2. Materials and methods

Selection of sites to collect samples

Information on history and levels of maize and groundnut production was obtained from a stakeholders' inception workshop that was held between 18-19 April 2012 at IITA campus in Dar es Salaam, Tanzania. Priority study areas were based on where there had been reports of aflatoxin and/or food poisoning and where maize and groundnut were predominantly cultivated. This information was used to select 18 districts across Tanzania that were targeted for sample collection (Supplementary Figure S1), and to assess levels of aflatoxin awareness among stakeholders along the maize and groundnut value chains using a questionnaire.

Sample collection

With International Institute of Tropical Agriculture (IITA) leading the activities, Ministry of Agriculture (MA) in Tanzania, Tanzania Food and Drugs Authority (TFDA), Sokoine University of Agriculture (SUA) and Tropical Pesticide Research Institute (TPRI) worked together as a team to collect samples in the country. Ten villages which were at least 10 km apart were randomly selected for each district. For groundnuts, 0.5 kg of grain was collected from each participating farmer, while a 1 kg sample was collected for maize. Depending on availability, one or two samples were collected per village for each crop. Samples were collected from farmer fields, storage structures and markets. In Tanzania, farmers normally store their maize or groundnut in 90 kg sacks or containers in granaries. Samples were collected from the top, middle and bottom of the storage container and mixed together to provide a single sample of 1 and 0.5 kg for maize and groundnut respectively for laboratory analysis. A total of 200 maize and 180 groundnut samples were collected. For each collected sample, moisture content was measured, and if the moisture content was >13%, the samples were further dried in an oven at 90 °C until desired moisture content. Before analysis, samples were stored at -20 °C to avoid fungal growth. Socioeconomic data that included information on whether the responding households were aware of mycotoxins, level of education and economic status was collected using a questionnaire. GPS coordinates were recorded at each sampling site.

Extraction and purification of aflatoxin from samples

The entire 1 kg maize grain sample was ground to pass a 10-micron sieve using BUNN™ grinder (Bunn-O-Matic Corporation Springfield, IL, USA). For groundnut, the entire 0.5 kg sample was milled using Waring laboratory blender (Waring Lab, Torrington, CT, USA). The flour for each sample was thoroughly mixed and sub-sampled using a sequential quartering technique to obtain 20 g sample for analysis. The rest of the sample was placed in

a Ziplock plastic bag and stored at -20 °C. Aflatoxins were extracted by placing 20 g sample in a 100 ml glass bottle containing 50 ml of acetonitrile and water (3:1, v/v); placed on a horizontal shaker and shaken at 140 rpm for 50 min. The solution was passed through two layers of Whatman filter paper No. 1 (Whatman, Maidstone, UK); 4 ml of the filtrate were adjusted to pH 7 using 0.1 M NaOH solution, diluted with 36 ml of phosphate buffer saline solution (pH 7.4 NaCl 0.15M). The diluted extract was passed through AflaStar immunoaffinity column (Romer Labs, Getzersdorf, Austria) fitted to a solid-phase extraction manifold and allowed to flow through without application of vacuum. The columns were washed with 25 ml of sterile distilled water, and aflatoxins were eluted under vacuum using 3 ml of methanol, applied as 0.5-ml aliquots which were held in the bed for 3 min.

Aflatoxin analysis

Aflatoxins were quantified using high-performance liquid chromatography (HPLC) (Stroka *et al.*, 2000). Briefly, an aliquot of 20 µl of elute was injected into the reverse-phase HPLC and analysed using a fluorescence detection system without derivatization of the aflatoxins (Samapundo *et al.*, 2007). A Waters HPLC system (Waters, Milford, MA, USA) consisting of a Waters 600 pump and controller was used and the system was connected to an auto-injector (Shimadzu RF-10AXL fluorescence detector and Shimadzu C-R 3A chromatopac integrator; Shimadzu, Tokyo, Japan). Chromatographic separations were performed on a Bondapak ODS column (250×4.6 mm, 5 µm pore size; Waters). A methanol/water mixture (1:1, v/v) was used as mobile phase. The flow rate of the mobile phase was set at 0.7 ml/min.

Fluorescence of the aflatoxins was recorded at wavelengths of 360 nm (excitation) and 440 nm (emission). The limit of detection of the analytical method was 0.6 µg/kg for AFB₁ and aflatoxin G₁ (AFG₁), and 0.07 µg/kg for aflatoxin B₂ (AFB₂) and aflatoxin G₂ (AFG₂). To assess the repeatability of the method, aflatoxin free maize and groundnut samples were spiked with AFB₁ and AFG₁ pure toxins, each at three concentrations 0.76 µg/kg, 3.81 µg/kg, and 6.85 µg/kg. The recovery rate was 89% for AFB₁ and AFG₁. Similarly, blank maize and groundnut samples were also spiked with pure AFB₂ and AFG₂ toxins, each at a concentration of 0.56 µg/kg, 1.31 µg/kg, and 1.675 µg/kg and the average recovery rate was 87%. The aflatoxin concentrations obtained from HPLC were corrected for recovery rate.

Dietary exposure

Three parameters (PDI, APDI) and maximum probable daily intake (MPDI) were used to assess dietary exposure level to aflatoxins. PDI and APDI estimates were calculated as described by Herrman and Yunes (1999) as follows:

1. PDI (ng/kg bw/day) = maize intake (g/person/day) × levels of aflatoxins in the samples (µg/kg)/bw (kg);
2. APDI (ng/kg bw/day) = maize intake (g/person/day) × average aflatoxin concentrations in the samples (µg/kg)/bw (kg).

The estimates of the maximum probable daily intake (MPDI) of aflatoxin were calculated using the formula:

3. MPDI (ng/kg bw/day) = (L×D)/bw (kg).

where L is the 90th percentile concentration of aflatoxin in the samples; and D the daily consumption of maize based foods (g/person/day). For all estimates, the assumed typical body weight of an adult was 60 kg. Different consumption scenarios were used to estimate exposure to aflatoxins. Based on a study by Abt Associates (2012), consumption levels were assumed to be different for different regions and these were used to estimate exposure for each region. An average consumption rate (based on FAO) and a third scenario based on recommendations from Tanzania Food and Nutrition Center (TFNC, 1997) were also used.

Statistical analysis

Statistical analysis was done using SAS (SAS Institute Inc., Cary, NC, USA) version 9.4 for Windows for hypothesis testing using T-test and calculation of average, median and 90th percentile values. Total aflatoxin was derived as a summation of AFB₁, AFB₂, AFG₁ and AFG₂. One-Sample t-test after log₁₀ (x + 1) transformation was used to determine the variation of aflatoxin prevalence across districts. The data for the levels of contamination of aflatoxin were analysed using ANOVA. The means were separated by Student-Newman-Keuls Test (SNK) to assess differences in aflatoxin prevalence and quantity between districts. Chi-Square analysis was used to compare the proportion of samples with aflatoxin levels above maximum tolerable limits set for EAC (10 µg/kg) and EU (4 µg/kg). Unless otherwise stated, all statistical tests were judged significant at $P=0.05$.

3. Results

Levels of aflatoxin awareness

The level of aflatoxin awareness among stakeholders interviewed ranged from 5 to 95% with an average of 75% of people interviewed not aware of aflatoxins (Figure 1). Most farmers interviewed were unaware of aflatoxins, the health impacts from consumption of aflatoxin contaminated food, and strategies to minimise contamination. Some districts, such as Babati (90%), Masasi (95%) and Nanyumbu (95%) had exceptionally high percentage of farmers who were aware of aflatoxins (Figure 1). In contrast, farmers

interviewed in eight of the 18 districts studied were not aware of aflatoxin, and in another five districts, less than 10% were aware of aflatoxins (Figure 1). More groundnut farmers (30.1±13.8%) were aware of aflatoxins compared to maize farmers (14.5±9.5%) (Figure 1).

Prevalence of aflatoxins

Groundnut

From a total of 180 groundnut samples collected, aflatoxins were detected in 173 (96.1%) (Table 1). Aflatoxin prevalence in districts ranged between 90% and 100%, and all samples from four districts (Babati, Chamwino, Kahama and Nzega) contained detectable aflatoxin levels (Table 1). Of the aflatoxin positive samples, 55 (31%) contained aflatoxin levels above the maximum tolerable limit set by the EU (4 µg/kg), while 31 samples (17%) contained aflatoxin levels exceeding the EAC and Tanzanian maximum tolerable limit of 10 µg/kg for total aflatoxins (Table 1). No significant differences were observed in the number of groundnut samples testing positive for aflatoxin ($P>0.05$) between districts (Table 1). AFB₁ was detected in 134 (74.4%) of the 180 samples (Table 2).

Maize

In comparison to groundnut, fewer maize samples had detectable aflatoxin levels. Of the 200 samples collected, 99 (49.5%) had detectable aflatoxins and prevalence ranged from 10 to 80% (Table 3). Significant differences ($P\leq 0.001$) were observed in the number of aflatoxin positive samples in districts; Babati, Ifakara, Kilosa, Kiteto and Sumbawanga having more than 50% of the samples testing positive for aflatoxins (Table 3). Districts with samples containing total aflatoxin levels exceeding the European Union regulatory limit of 4 µg/kg and EAC regulatory limit of 10 µg/kg (respectively) were Babati (20% for both limits), Ifakara (35% and 30%), Kilosa (55% for both limits) and Kiteto (5% for both limits). Overall, 12% of the maize samples exceeded the EU regulatory limit of 4 µg/kg, while 11% exceeded the EAC and Tanzanian regulatory limit of 10 µg/kg for total aflatoxin (Table 3). AFB₁ was detected in 45 (22.5%) samples but was not detected in samples from two districts: Makambako and Mbinga (Table 4).

Aflatoxin levels

Groundnut

Average aflatoxin levels in groundnut samples ranged from 2.50 to 10.93 µg/kg, with an overall mean of 6.37 µg/kg (Table 1). Median aflatoxin levels ranged from 1.24 µg/kg in Nzega district to 6.39 µg/kg in Iringa Rural district. No significant differences were observed in levels of total aflatoxin ($F=1.66$; $P=0.111$) contamination between

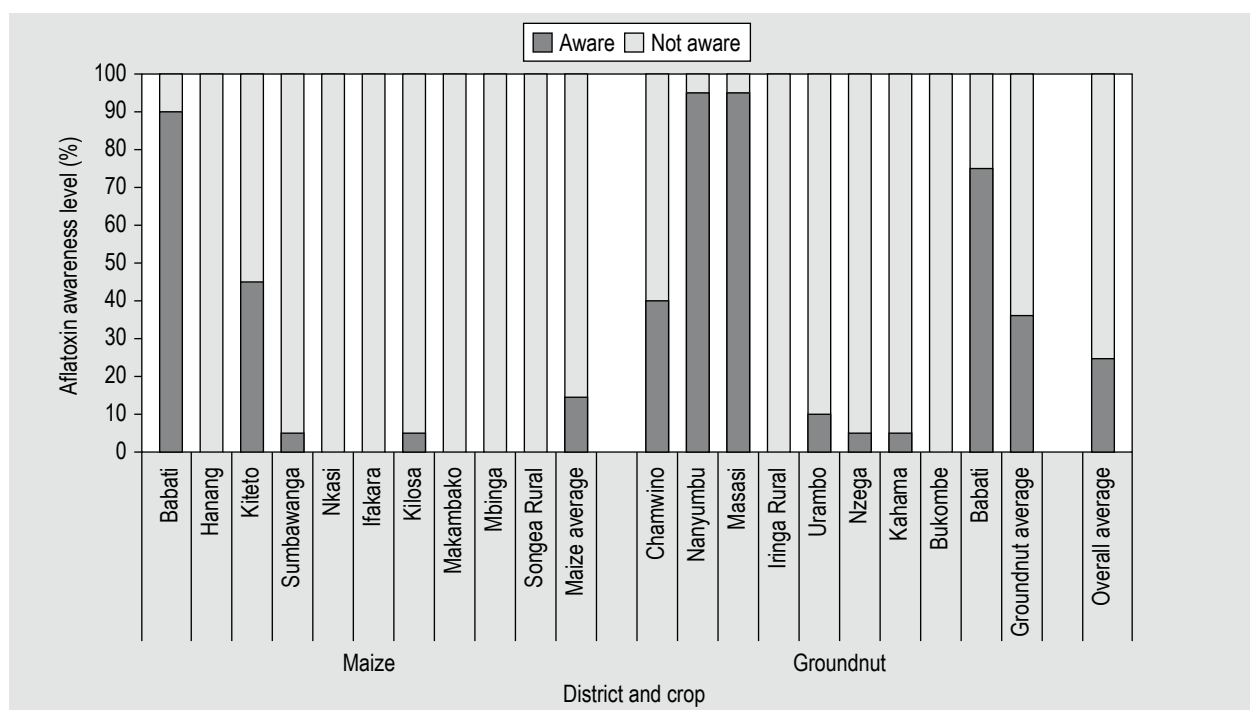


Figure 1. Proportion of aflatoxin-aware farmers who provided maize and groundnut samples in 18 districts of Tanzania in 2012 assessed based on questionnaire.

Table 1. Prevalence and levels of total aflatoxin in groundnut collected from nine districts in Tanzania in 2012 and proportion of groundnut samples with total aflatoxin levels exceeding maximum tolerable limits in the European Union (EU) and East African Community (EAC).

District	Number (n) of samples	Positive samples N (%)	Concentration range of positive samples ($\mu\text{g}/\text{kg}$)	Median ($\mu\text{g}/\text{kg}$)	Mean ($\mu\text{g}/\text{kg}$) \pm standard error ¹	Number of samples exceeding EU regulatory limit of 4 $\mu\text{g}/\text{kg}$ (%)	Number of samples exceeding EAC regulatory limit of 10 $\mu\text{g}/\text{kg}$ (%)
Babati	20	20 (100)	1.42-40.31	4.01	7.69 \pm 2.25 ^a	10 (50)	3 (15)
Bukombe	20	19 (95)	0.90-30.07	2.75	7.63 \pm 2.29 ^a	6 (30)	4 (20)
Chamwino	20	20 (100)	0.08-23.60	1.42	3.25 \pm 1.17 ^a	4 (20)	1 (5)
Iringa Rural	20	18 (90)	0.51-36.09	6.39	10.93 \pm 2.91 ^a	10 (50)	6 (30)
Kahama	20	20 (100)	1.10-33.59	2.89	6.10 \pm 1.95 ^a	5 (25)	3 (15)
Masasi	20	18 (90)	0.08-37.54	2.91	8.15 \pm 2.41 ^a	7 (35)	5 (25)
Nanyumbu	20	19 (95)	0.06-32.44	2.35	7.03 \pm 2.39 ^a	5 (25)	4 (20)
Nzenga	20	20 (100)	0.04-18.42	1.24	4.60 \pm 1.38 ^a	6 (30)	4 (20)
Urambo	20	19 (95)	0.34-14.74	1.46	2.50 \pm 0.73 ^a	2 (10)	1 (5)
All samples	180	173 (96.1)	0.04-40.31	2.57	6.37 \pm 0.68	55 (30.6)	31 (17)

¹ Means with same letter(s) are not significantly different at the 0.05 level.

districts. The highest contaminated sample contained 40.31 $\mu\text{g}/\text{kg}$ aflatoxins and this sample was from Babati (Table 1). Average AFB₁ levels ranged from 1.01 to 9.02 $\mu\text{g}/\text{kg}$, with a mean of 5.02 $\mu\text{g}/\text{kg}$ and a median of 0.58 $\mu\text{g}/\text{kg}$ (Table 2). The highest contaminated sample contained 38.32 $\mu\text{g}/\text{kg}$ AFB₁ and this sample was from Babati. No significant differences ($F=1.16$; $P=0.327$) were observed for levels of AFB₁ between districts (Table 2).

Maize

Average aflatoxin levels between districts ranged from 0.96 to 44.11 $\mu\text{g}/\text{kg}$ with an overall mean of 12.47 $\mu\text{g}/\text{kg}$ (Table 3). Significant differences ($F=4.98$; $P<0.001$) in levels of aflatoxin contamination were observed between districts, with Kilosa (average 44.11 $\mu\text{g}/\text{kg}$) and Ifakara (average 25.89 $\mu\text{g}/\text{kg}$) having high levels of contamination compared to

Table 2. Prevalence, consumption, concentration, probable daily intake (PDI), average probable daily intake (APDI) and maximum probable daily intake (MPDI) of aflatoxin B₁ (AFB₁) in groundnut consumed in different districts of Tanzania.¹

District	Number (n) of samples	Positive samples N (%)	Mean AFB ₁ (µg/kg) ± standard error ²	Concentration range of positive samples (µg/kg)	90 th percentile (µg/kg)	Consumption ³ (g/person/day)	PDI range ng/kg bw/day ⁴	APDI ng/kg bw/day ⁴	MPDI ng/kg bw/day (90 th percentile) ⁴
Babati	20	14 (70)	6.43±3.02 ^a	0.37-38.30	21.6	3.2	0.019-2.044	0.343	1.150
Bukombe	20	14 (70)	6.21±2.35 ^a	0.39-22.80	18.7	29.7	0.193-11.261	3.074	9.276
Chamwino	20	16 (80)	2.70±1.40 ^a	0.27-22.80	6.6	36.4	0.164-13.832	1.637	4.028
Iringa Rural	20	16 (80)	9.02±2.72 ^a	0.28-32.90	25.3	14.1	0.066-7.732	2.120	5.936
Kahama	20	16 (80)	4.41±2.11 ^a	0.37-30.80	16.6	29.8	0.183-15.221	2.184	8.197
Masasi	20	15 (75)	4.84±2.22 ^a	0.37-31.00	15.2	15.1	0.093-7.794	1.218	3.823
Nanyumbu	20	16 (80)	5.55±2.43 ^a	0.37-30.10	22.6	15.1	0.093-7.585	1.397	5.655
Nzega	20	11 (55)	5.52±1.90 ^a	0.26-17.10	13.7	29.7	0.129-8.460	2.730	6.796
Urambo	20	16 (80)	1.01±0.54 ^a	0.26-9.10	1.1	29.7	0.129-4.480	0.498	0.530
All samples	180	134 (74.4)	5.02±0.73	0.26-38.30	18.7	22.5	0.098-14.370	1.884	7.028

¹ AFB₁ is a genotoxic carcinogen, as such, a tolerable daily intake has not been set. Rather, the ALARA principle (As Low As Reasonably Achievable) is recommended.

² Means with same letter(s) are not significantly different at 0.05 level.

³ Groundnut consumption adopted from the report by Abt (2014).

⁴ Body weight (bw) is 60 kg.

Table 3. Prevalence of total aflatoxin in maize collected from ten districts of Tanzania in 2012 and proportion of maize samples with aflatoxin levels exceeding maximum tolerable limits in the European Union (EU) and East African Community (EAC).

District	Number (n) of samples	Positive samples N (%)	Median (µg/kg)	Mean of positive samples ± standard error (µg/kg) ¹	Percent of samples exceeding EU regulatory limit of 4 µg/kg N (%)	Percent of samples exceeding EAC regulatory limit of 10 µg/kg: n (%)
Babati	20	14 (70)	1.68	7.15±2.68 ^a	4 (20)	4 (20)
Hanang	20	5 (25)	0.99	0.96±0.29 ^a	0 (0)	0 (0)
Ifakara	20	14 (70)	4.39	25.87±9.20 ^b	7 (35)	6 (30)
Kilosa	20	16 (80)	31.26	44.11±11.96 ^b	11 (55)	11 (55)
Kiteto	20	13 (65)	0.07	1.67±1.44 ^a	1 (5)	1 (5)
Makambako	20	9 (45)	0.97	0.97±0.13 ^a	0 (0)	0 (0)
Mbinga	20	2 (10)	1.27	1.27±0.24 ^a	0 (0)	0 (0)
Nkasi	20	7 (35)	1.20	1.40±0.32 ^a	0 (0)	0 (0)
Songea	20	6 (30)	0.97	1.21±0.24 ^a	0 (0)	0 (0)
Sumbawanga	20	13 (65)	0.52	0.92±0.29 ^a	0 (0)	0 (0)
All samples	200	99 (49.5)	1.14	12.48±2.82	23 (11.5)	22 (11)

¹ Means with same letter(s) in a column are not significantly different at 0.05 level.

other districts (Table 3). Six of the districts contained low levels of aflatoxin, below the EU regulatory limit of 4 µg/kg. The maximum level of aflatoxin was detected in Kilosa (162.40 µg/kg) followed by Ifakara (92.47 µg/kg) (Table 5). AFB₁ levels in maize samples ranged between 0.46 and 159.54 µg/kg, with a mean of 21.24 µg/kg and a median of 2.60 µg/kg (Table 4). High levels of AFB₁ were observed in

Kilosa (mean 44.08 µg/kg; range 1.09-159.54 µg/kg), Ifakara (mean = 27.38 µg/kg; range 0.6-91.15 µg/kg) and Babati (mean = 12.86 µg/kg; range 0.60-23.48 µg/kg) compared to the other districts. AFB₁ levels in the highly contaminated sample was 159.54 µg/kg, which was 30-fold more than the level (5 µg/kg) recommended by EAC and TBS for maize destined for human consumption.

Table 4. Prevalence, concentration, probable daily intake (PDI) and average probable daily intake (APDI) of aflatoxin B₁ (AFB₁) in maize consumed in different districts of Tanzania.

District	Number (n) of samples	Positive samples n (%)	Mean AFB ₁ (µg/kg) ± standard error ¹	AFB ₁ concentration range (µg/kg)	90 th percentile concentration (µg/kg)	PDI range (ng/kg bw/day) ^{2,3}		APDI (ng/kg bw/day) ^{2,3}		MPDI (ng/kg bw/day 90 th percentile) ^{2,3}	
						Abt	TFNC	Abt	TFNC	Abt	TFNC
Babati	20	6 (30)	12.86±3.95 ^{ab}	0.60-23.48	23.48	4.29-168.07	7.71-301.77	92.05	165.28	168.07	301.77
Hanang	20	1 (5)	0.63±0.00 ^b	–	–	4.50	8.08	4.50	8.08	4.50	8.08
Ifakara	20	12 (60)	27.38±9.74 ^a	0.60-91.15	77.35	4.29-652.36	7.71-1,171.33	195.93	351.81	553.60	994.00
Kilosa	20	12 (60)	44.08±13.70 ^a	1.09-159.54	96.70	7.80-1,141.80	14.00-2,050.10	315.48	566.46	692.05	1,242.60
Kiteto	20	3 (15)	4.44±3.85 ^b	0.48-12.13	12.13	3.41-86.82	6.13-155.88	31.74	56.99	86.82	155.89
Makambako	20	0 (0)	–	–	–	–	–	–	–	–	–
Mbinga	20	0 (0)	–	–	–	–	–	–	–	–	–
Nkasi	20	4 (20)	0.68±0.04 ^b	0.61-0.77	0.71	4.37-5.51	7.84-9.89	4.87	8.74	5.51	9.89
Songea	20	1 (5)	0.99±0.00 ^b	0.99	0.99	7.14	12.81	7.14	12.81	7.14	12.81
Sumbawanga	20	6 (30)	0.55±0.03 ^b	0.456-0.63	0.63	3.26-4.53	5.86-8.13	3.94	7.07	4.53	8.13
Average	200	45 (22.5)	21.24±5.07	0.46-159.54	77.35	3.26-1,141.78	5.86-2,050.1	151.98	272.89	553.60	994.00

¹ Means with same letter(s) (across) are not significantly different at the 0.05 level.
² Consumption rates: Abt = 429.4 g/person/day; consumption rate recommended by TFNC = 771 g/person/day.
³ Body weight (bw) is 60 kg.

Table 5. Concentration, probable daily intake (PDI), average probable daily intake (APDI) and maximum probable daily intake (MPDI) of total aflatoxin in maize from different districts of Tanzania.

District	Concentration range of positive samples (µg/kg)	Mean concentration of positive samples (µg/kg)	90 th percentile concentration of positive samples (µg/kg)	PDI range (ng/kg bw/day) ¹		APDI (ng/kg bw/day) ¹		MPDI (ng/kg bw/day 90 th percentile) ¹	
				Abt	TFNC	Abt	TFNC	Abt	TFNC
Babati	0.07-27.50	7.15	24.65	0.50-196.79	0.90-353.38	51.17	91.88	176.39	316.74
Hanang	0.08-1.78	0.96	1.78	0.57-12.74	1.03-22.87	6.87	12.34	12.72	22.83
Ifakara	0.07-92.47	25.89	80.61	0.50-661.72	0.90-1,118.24	185.27	332.69	576.82	1035.79
Kilosa	0.10-162.40	44.11	105.92	0.72-1,162.13	1.29-2,086.84	315.65	566.81	757.97	1361.08
Kiteto	0.06-18.88	1.67	0.87	0.43-135.11	0.77-242.61	11.95	21.46	6.21	11.15
Makambako	0.08-1.34	0.97	1.34	0.57-9.59	1.03-17.22	6.94	12.46	9.60	17.24
Mbinga	1.03-1.51	1.27	1.51	7.37-10.81	13.24-19.40	9.09	16.32	10.77	19.34
Nkasi	0.07-2.42	1.40	2.42	0.50-17.32	0.90-31.10	10.02	17.99	17.32	31.11
Songea	0.09-2.42	1.21	2.42	0.64-17.32	1.16-31.10	8.66	15.55	17.33	31.12
Sumbawanga	0.06-2.70	0.92	2.62	0.43-19.32	0.77-34.70	6.58	11.82	18.76	33.69
Average	0.06-162.40	12.48	54.99	0.43-1,162.24	0.77-2,086.84	89.28	160.30	393.55	706.62

¹ Consumption rates: Abt = 429.4 g/person/day; TFNC = 771 g/person/day. Body weight (bw) is 60 kg.

Exposure to aflatoxins

The probable daily intake of aflatoxins assuming that the maize collected from farmers was destined for consumption ranged between 0.43 and 1,162.24 ng/kg bw/day in all districts when using an average maize consumption rate of 429.4 g/day/person according to method described by Abt Associates (2012). Using the maize consumption rate recommended by the Tanzanian Food and Nutrition Center (TFNC) of 771 g/person/day, exposure rates were much higher, ranging between 0.77 and 2,086.84 ng/kg bw/day (Table 5). The average probable daily intake ranged between 6.58 and 315.65 ng/kg bw / day using the Abt average consumption rate of 429.4 g/person/day; and from 11.82 to 566.81 ng/kg bw/day using the TFNC recommended consumption rate of 771 g/person/day (Table 5). The maximum probable daily intake ranged from 6.21 to 757.97 ng/kg and from 11.15 to 1,361.08 ng/kg bw/day 90th percentile using the Abt and TFNC recommended consumption rate of maize respectively (Table 5). Exposure (PDI) to AFB₁ ranged from 3.26 to 1,141.78 ng/kg bw/day, using consumption rates reported by Abt of 429.4 g/person/day, and from 5.86 to 2,050.10 ng/kg bw/day, using consumption rate of 771 g/person/day recommended by TFNC (Table 4). Average exposure rates ranged from 3.94 to 315.48 ng/kg bw/day and from 7.07 to 566.46 ng/kg bw/day using the consumption rates reported by Abts and TFNC, respectively (Table 4).

Groundnut consumptions were different depending on the region under study; using the Abt Associates (2012) rates, for instance, exposure to total aflatoxin ranged from 0.02 to 15.12 ng/kg bw/day, with an average of 2.39 ng/kg bw/day, and a 90th percentile of 8.62 ng/kg bw/day (Table 6). The probable daily intake of AFB₁ ranged between 0.10 and 14.37 ng/kg bw/day, with a mean of 1.88 ng/kg bw/day and a 90th percentile of 7.03 ng/kg bw/day (Table 2).

4. Discussion

Maize is the main dietary staple food for most Tanzanians and together with groundnuts, they are used as main ingredients for complementary foods. Unfortunately, these crops are susceptible to diverse opportunistic fungi and therefore, vulnerable to mycotoxin contamination (Suileman and Rosentrater, 2015). In the current study, all maize and groundnut samples from surveyed districts were contaminated with aflatoxin. The prevalence of aflatoxins was higher in groundnuts, where 96% of the samples had detectable aflatoxin levels, compared to 50% for maize. Similar results have previously been reported for Tanzania by different researchers (Kamala *et al.*, 2015; Kimanya *et al.*, 2014; Magembe *et al.*, 2016; Manjula *et al.*, 2009). A study conducted by Kimanya *et al.* (2008) found 18% of maize samples contaminated with aflatoxins, with levels of up to 158 µg/kg, and 12% of the samples had AFB₁ levels

above the Tanzania maximum tolerable limit of 5 µg/kg (TBS, 2004). In another study, Magoha *et al.* (2014b) found that 57% of 67 maize flour samples contained detectable aflatoxins, that ranged from 0.33-69.47 µg/kg. The high prevalence of aflatoxins in groundnut compared to maize has previously been reported (Magembe *et al.*, 2016; Seetha *et al.*, 2017). Thus, our results agree with those reported earlier for Tanzania, where aflatoxins were detected in all districts sampled, with other districts having higher incidences than others (TFDA, 2012). The current study included more districts in a single study (larger sample size) than in earlier reports. Compared to maize, groundnut was highly contaminated by aflatoxins, probably because the pods develop in the soil where *A. flavus* resides.

In Tanzania, the maximum tolerable limits for AFB₁ and total aflatoxins are 5 and 10 µg/kg, respectively (TBS, 2004). Contamination levels above these maximum tolerable limits were observed in some groundnut and maize samples. Our study provides additional information supporting earlier reports that Tanzanian maize and groundnuts are contaminated with unacceptable levels of aflatoxins (Kamala *et al.*, 2015; Kimanya *et al.*, 2008). High incidences of and levels of aflatoxin in maize and groundnut have also been reported in other countries in Africa (Lewis *et al.*, 2005; Misihairabgwi *et al.*, 2017). Two districts in central Tanzania, Kilosa and Ifakara, stood out as having high levels of aflatoxin in maize, confirming previous reports of higher aflatoxin levels in this region (Kamala *et al.*, 2015; Magembe *et al.*, 2016). This zone is prone to high incidences of drought, conditions that are conducive for *A. flavus* infection and aflatoxin contamination (Timiza, 2011). A study by Seetha *et al.* (2017) reported high levels of toxigenic *A. flavus* strains in central Tanzania, including Kilosa, and a ten-year (2007-2016) review of mycotoxin contamination of foods in Southern Africa by Misihairabgwi *et al.* (2017) shows a similar trend for the region.

It has been shown that health risks associated with mycotoxins can be reduced through awareness campaigns (Magembe *et al.*, 2016; Seetha *et al.*, 2017). However, results from this study revealed that more than 75% of the stakeholders were not aware of aflatoxins and associated health impacts from consumption of aflatoxin-contaminated products. Similar observations have previously been made in Tanzania and it was shown that awareness of aflatoxin and health impacts varied between districts (Kimanya *et al.*, 2014; Magembe *et al.*, 2016; TFDA, 2012). Aflatoxin awareness levels were high in a few districts largely because of presence of projects dealing with aflatoxin or where traders had rejected commodities for export due to aflatoxins. This variation is probably a reflection of the different aflatoxin campaign efforts that have been going on across the country (Seetha *et al.*, 2017). Lack of awareness of aflatoxins among stakeholders (farmers, traders, and consumers) and policy makers

Table 6. Prevalence, consumption, concentration, probable daily intake (PDI), average probable daily intake (APDI) and maximum probable daily intake (MPDI) of total aflatoxins in groundnut consumed in different districts of Tanzania.

District	Concentration range of positive samples ($\mu\text{g}/\text{kg}$)	Mean concentration ($\mu\text{g}/\text{kg}$)	90 th percentile concentration ($\mu\text{g}/\text{kg}$)	Consumption (g/person/day)	PDI range (ng/kg bw/day) ¹	APDI (ng/kg bw/day) ¹	MPDI (ng/kg bw/day) ¹
Babati	1.42-40.31	7.69 \pm 2.25	22.845	3.20	0.0757-2.1499	0.40997	1.21840
Bukombe	0.90-30.07	7.63 \pm 2.29	26.010	29.70	0.4455-14.8847	3.77477	12.87495
Chamwino	0.08-23.6	3.25 \pm 1.17	7.285	36.40	0.0485-14.3173	1.96924	4.41957
Iringa Rural	0.51-36.09	10.931 \pm 2.91	30.780	14.10	0.1199-8.4812	2.56868	7.23330
Kahama	1.10-33.59	6.100 \pm 1.95	20.260	29.76	0.5456-16.6606	3.02560	10.04896
Masasi	0.08-37.54	8.154 \pm 2.41	23.080	15.10	0.0201-9.4476	2.05206	5.80847
Nanyumbu	0.06-32.44	7.027 \pm 2.39	29.030	15.10	0.0151-8.1641	1.76855	7.30588
Nzega	0.04-18.42	4.627 \pm 1.38	15.845	29.70	0.0198-9.1179	2.29037	7.84328
Urambo	0.34-14.74	2.499 \pm 0.73	4.840	29.70	0.1683-7.2963	1.23698	2.39580
Average	0.04-40.31	6.373 \pm 0.68	22.990	22.50	0.015-15.1163	2.39005	8.62125

¹ Groundnut consumption adopted from the report by Abt Associates (2014). Body weight (bw) is 60 kg.

exacerbate the problem of mycotoxins in Tanzania. Regulation is unlikely to work as has been observed in other countries across Africa (Matumba *et al.*, 2014), where most of the population eat what they produce. As a result, it is difficult to enforce laws. However, there is a growing peri urban and urban population that depends on food produced in rural areas, and as such, rising aflatoxin awareness may increase the demand for aflatoxin-safe food, which in turn might facilitate adoption of aflatoxin mitigating strategies and enforcement of regulations in rural areas. There is an urgent need to raise awareness among producers and consumers on the detrimental effects of aflatoxin exposure and available strategies to minimise contamination. These strategies include good agricultural practices (such as using improved seed, use of Aflasafe (biological control), use of fertilisers, good harvesting and handling procedures, storing properly dried grain and avoiding contact of grain with soil), use of improved storage structures, such as hermetic bags, and diversifying the diet to minimise consumption of crops highly prone to contamination with aflatoxin (Hell *et al.*, 2005). Such campaigns should be streamlined according to gender and income of target populations.

AFB₁ is considered the most potent form of aflatoxin (IARC, 2002). An economic assessment conducted by Abt Associates in collaboration with Tanzania Food and Drugs Authority (TFDA) observed significantly high prevalence of AFB₁ in multiple regions around Tanzania (TFDA, 2012). In this study, AFB₁ was detected in most samples collected and levels were higher in groundnut samples from Southern parts of the country and in maize collected from Northern and Eastern parts of the country. Two districts, Kilosa and Ifakara in central Tanzania had

the highest levels of AFB₁. Central and eastern Tanzania are generally drier and hotter than other regions in Tanzania. Infection and proliferation of *A. flavus* are favoured by high temperatures and humidity and drought conditions during crop growth (Holbrook *et al.*, 2000; Richard, 2007). The weather conditions found in Kilosa and Ifakala are conducive for infection and proliferation of *A. flavus* and aflatoxin contamination.

Considering the maximum tolerable limits set by the EU of 4 $\mu\text{g}/\text{kg}$, 31% groundnut samples and 11.5% of maize samples were not suitable for human consumption. The risk of exposure to aflatoxin is high, especially for people subsisting on groundnuts and maize. Exposure to aflatoxin is a function of the level of contamination, the amount consumed and the frequency of consumption. In Tanzania, people consume an average of 429 g of maize per day (Ranún *et al.*, 2014), and consumption rates as high as 771 g/day/person have been recommended (TFNC, 1997). Therefore, even the low levels of aflatoxins observed in this study are a potential health hazard, given the high rates of maize consumption in Tanzania. More than 80% of the population in Tanzania depends on maize as a food and cash crop (Moshi, 1997). Furthermore, groundnuts and maize are major components of weaning foods in Tanzania (Magoha *et al.*, 2014b). High levels of aflatoxin have been reported in blood and urine of children up to 12 months from Morogoro district of Tanzania (Shirima *et al.*, 2013). The levels of aflatoxin increased with age, and as children started taking weaning foods. The risk of aflatoxin exposure in children receiving complementary weaning foods is very high in Tanzania (Shirima *et al.*, 2013).

Apart from being associated with some chronic health risks, such as cancer and immune suppression, aflatoxin is also associated with child stunting (Gong *et al.*, 2016; Leroy, 2013). In sub-Saharan Africa and South Asia, prevalent stunting in children under five years old has been reported (Black *et al.*, 2013) with insignificant reduction overtime through nutrition-specific interventions, such as micronutrient supplementation and fortification, as well as complementary feeding (Lutter *et al.*, 2011). This situation, however, has been linked to aflatoxin contamination of weaning foods supplied to the children (Gong *et al.*, 2016). The central region of Tanzania has one of the highest stunting levels in the country, with Dodoma district reporting over 40% stunting in children under the age of 5 years (TFNC, 2014). A recent study conducted in Iringa, Tabora, and the Kilimanjaro regions of Tanzania, showed that 67% of children had serum aflatoxin biomarkers with a mean aflatoxin-albumin adduct concentration of 4.7 pg/mg of albumin (Shirima *et al.*, 2013). Therefore, results from this study reveal a chronic problem of exposure to aflatoxins, coming from both groundnuts and maize consumption. This study revealed high levels of aflatoxins in Kilosa and Ifakara districts, and these two districts have been traditionally associated with high levels of child stunting (Shirima *et al.*, 2013).

Within the East Africa region, Tanzania has the highest maize and groundnuts consumption, at 429 g/person/day and 15 g/person/day, respectively (Abt Associates, 2012; Ranún *et al.*, 2014). Average national consumption is estimated to be over three million metric tons per year (FAOSTAT, 2014). Maize is a major staple produced mostly by smallholder farmers (71%) for own consumption (Suleiman and Rosentrater, 2015). Thus, even low levels of contamination is a potential health risk, especially for people consuming large amounts of maize, as in Tanzania. Estimated average probable daily intake of AFB₁ from groundnut consumption was 1.88 ng/kg bw/day with a maximum of 14.37 ng/kg bw/day. For maize, the average intake ranged from 151.98 to 272.89 ng/kg bw/day. These levels exceeded the provisional maximum tolerable daily intake (PMTDI) of 1 ng AFB₁/kg bw/day for adults for adults without hepatitis B, and 0.4 ng AFB₁/kg BW/day for adults with hepatitis B quoted by Kuiper-Goodman (1998), revealing that the Tanzanian population is at high risk of AFB₁ exposure. Given that both maize and groundnut are major components of complementary and weaning foods in Tanzania, the exposure levels are worrisome, as they exceeded the health concern level of 0.017 ng/kg BW/day recommended by the European Food Safety Agency (EFSA) for food to be consumed by infants (Barlow *et al.*, 2006; EFSA, 2007). High risk of exposure from consumption of maize has been reported for Tanzania (Kamala *et al.*, 2018; Kimanya *et al.*, 2014; Magembe *et al.* 2016; Magoha *et al.*, 2014b). A study by Kimanya *et al.* (2014) reported that in Rombo District, North Eastern Tanzania, the risk of

exposure for children consuming maize flour ranged from 1 to 786 ng/kg bw/day. Therefore, measures need to be put in place to educate the population on the health impacts of aflatoxins, as well as provide solutions to prevent aflatoxin contamination of maize and groundnuts (Nathaniels, 2014).

5. Conclusions and recommendations

This study revealed widespread contamination of maize and groundnuts with aflatoxin in Tanzania. Smallholder farmers, who consume what they grow, are at the highest risk of aflatoxin exposure, especially for maize because of the higher levels of contamination and secondly because the average person eats more maize per day than other foods. Aflatoxin awareness is generally low; extra efforts are required to raise aflatoxin awareness among stakeholders along the maize and groundnut value chains. More studies are required to better map the distribution and prevalence of aflatoxins, focusing on other regions that were not part of this study and in multiple seasons. This will allow mapping variations in aflatoxin contamination and levels relative to prevailing environmental conditions. In addition, sampling in multiple years will provide the required information to develop more accurate aflatoxin risk maps and better target intervention strategies. Strategies to minimise impact of aflatoxins on human health should involve educating farmers, producers, processors and consumers about aflatoxins, health impacts and strategies for minimising contamination.

Supplementary material

Supplementary material can be found online at <https://doi.org/10.3920/WMJ2020.2646>.

Figure S1. A map of Tanzania showing the geographical locations from where maize and groundnut samples were collected in 2012 to conduct a mycotoxin prevalence study.

Conflict of interest

The authors declare that there are no conflicts of interest.

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