



Prevalence of aflatoxin, ochratoxin and deoxynivalenol in cereal grains in northern Uganda: Implication for food safety and health

Richard Echodu^{a,b,*}, Geoffrey Maxwell Malinga^{a,c}, Joyce Moriku Kaducu^d, Emilio Ovuga^e, Geert Haesaert^f

^a Department of Biology, Faculty of Science, Gulu University, P.O. Box 166, Gulu, Uganda

^b Gulu University Bioscience Research Laboratories, P.O. Box 166, Gulu, Uganda

^c Department of Environmental and Biological Sciences, Faculty of Science and Forestry, University of Eastern Finland, P.O. Box 111, 80101 Joensuu, Finland

^d Department of Pediatrics, Faculty of Medicine, Gulu University, P.O. Box 166, Gulu, Uganda

^e Department of Mental Health, Faculty of Medicine, Gulu University, P.O. Box 166, Gulu, Uganda

^f Department of Applied Sciences, Faculty of Bioscience Engineering, Ghent University, Belgium

ARTICLE INFO

Keywords:

Aflatoxin
Ochratoxin
Deoxynivalenol
Food grains
Food safety
Uganda

ABSTRACT

Mycotoxin contamination of cereals is a significant health risk for humans and animals, particularly in developing countries. To gain insight into food safety related to agricultural practices, we assessed levels of mycotoxin contamination in 105 samples of food grains raised and stored for consumption by rural households in the post-conflict districts of Kitgum and Lamwo in Northern Uganda. Aflatoxin, ochratoxin and deoxynivalenol (DON) contamination was assessed by quantitative enzyme-linked immunosorbent assay. Total aflatoxin in the foods analyzed varied from nd (not detected) to 68.2 µg/Kg. Ochratoxin ranged from 0.1 to 16.4 µg/Kg. DON ranged from nd to 2606 µg/Kg. The mean concentration of total aflatoxins was significantly higher ($P = 0.002$) in sorghum than in millet, maize and sesame seeds. Frequency of co-occurrence of two mycotoxins ranged from 8.3 to 100%, with the highest being aflatoxin and ochratoxin in sorghum. Co-occurrence of all three mycotoxins ranged from 8.3 to 35.3%, with the highest again being in sorghum. Mean levels of aflatoxins concentration in sorghum samples were 11.8 µg/Kg, exceeding the Ugandan national regulatory limits of 10 µg/Kg. Furthermore, 46.5% of the sorghum consumed in both districts exceeded this limit, and 86.1% of sorghum samples exceeded the European Union (E.U.) maximum tolerable limit of 4 µg/Kg. The Estimated Daily Intake (EDI) and Hazard Indices (HI) values were in the range of 1.2×10^{-5} –91.521 and 1.3×10^{-7} to 0.0059, respectively. In conclusion, our results provide evidence of high levels of mycotoxin contamination and co-occurrence in food grains in Northern Uganda with aflatoxins and ochratoxins at high levels in all the cereal types analyzed. Consumption of cereals cultivated in this region poses no health risk of mycotoxins exposure since HI values obtained were less than 1.

1. Introduction

Mycotoxins are secondary metabolites produced by fungi of the genera *Penicillium*, *Aspergillus* and *Fusarium* growing on grains and other agricultural products before harvest, during transportation and in storage [1]. Mycotoxin contamination reduces the quality and nutritional value of food, resulting in economic losses to smallholder farmers, traders and consumers [2]. When ingested, inhaled or absorbed through the skin, mycotoxins may cause liver disease, immune deficiency, toxicity, carcinogenicity, growth retardation and death in animals and humans [3–7].

In sub-Saharan Africa, mycotoxin contamination in foodstuff is a

serious public health issue with over 250,000 hepatocellular carcinoma-related deaths occurring annually as a result of aflatoxin alone [8]. In north-eastern Kenya, 125 deaths from consumption of contaminated maize were recorded in 2004 [9]. Mycotoxin contamination of foods and other agricultural products depends on storage, microclimatic condition and harvesting techniques [10]

Several mycotoxins exist and the ones of public health importance include aflatoxins, deoxynivalenol (DON or vomitoxin), zearalenone, ochratoxins, T-2 toxin, fumonisins, and T-2-like toxins [8]. Currently, there are more than 20 aflatoxins known but the six predominant ones are aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2), aflatoxin M1 (AFM1) and aflatoxin M2 (AFM2)

* Corresponding author at: Department of Biology, Faculty of Science, Gulu University, P. O. Box 166, Gulu, Uganda.

E-mail address: richardechodu2009@gmail.com (R. Echodu).

<https://doi.org/10.1016/j.toxrep.2019.09.002>

Received 2 August 2017; Received in revised form 7 June 2019; Accepted 6 September 2019

Available online 12 September 2019

2214-7500/© 2019 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

[11]. In the developed countries, the tolerance limits for total aflatoxins range from 0 to 50 µg/Kg, while those of aflatoxin B1 in foodstuff is at 0 to 30 µg/Kg [10].

Information on the prevalence and human health risk of mycotoxins in most African countries is still lacking. This is due to combination of limited monitoring systems and lack of public awareness with regard to mycotoxins. Thus, addressing mycotoxin contamination should be given a priority in sub-Saharan Africa. Determination of mycotoxin contamination is an essential first step in understanding and addressing the mycotoxin problem in Africa. This will provide information needed for assessing risks of key mycotoxins and in identifying intervention targets.

In northern Uganda, the civil war that started in the mid 1980s to 2006 forced communities into internally displaced people's (IDP) camps and caused a collapse in the agricultural production system. Today, rural communities are still confronted with the problem of food insecurity and poor agricultural practices. The communities process their foods locally in homes and for quality do not employ checks and good food safety practices. The staple foods produced in northern Uganda, especially millet, maize, sorghum, groundnut and sesame, are highly susceptible to infection with toxigenic fungi such as *Penicillium* spp., *Aspergillus* spp. and *Fusarium* spp. We undertook a cross-sectional study to assess the levels of contamination of aflatoxins, ochratoxins and DON in grain-based foods in the post-conflict districts of Lamwo and Kitgum, northern Uganda, using an enzyme-linked immunosorbent assay. The objective was to gain insights into mycotoxin safety of foods and related health risks. We discuss our results with a view to raising public awareness to mycotoxins risk in the region.

2. Materials and methods

2.1. Study sites

We conducted this study in Kitgum (3°17'20.0"N, 32°52'40.0" E) and Lamwo (3° 32' 0" N, 32° 48'0" E) districts, northern Uganda neighbouring South Sudan. These districts were intensely affected by the Joseph Kony's Lord Resistance Army (LRA) rebellion between the mid 1980's and 2006 [12,13]. The incursion shifted many villagers into internally displaced people's (IDP) camps and distorted social services. The total population in the two districts is 215,904 [14]. The area has rainy and dry seasons with annual rainfall of approximately 1300 mm. With improved security and a reduction in LRA attacks, people in both districts began returning to their villages. Subsistence agriculture is the mainstay of the economy employing up to 98% of the population, with cash crops including tobacco and cotton, and food crops being millet, maize, groundnuts, rice, sorghum, sesame, green vegetables, sunflower, citrus and mangoes, beans, sweet potatoes, cassava and pigeon peas. Livestock such as goats, cattle, pigs, sheep and chickens are also reared and some farmers are involved in bee keeping and fish farming. Majority of households use family labour and rudimentary hand tools such as hoes for cultivation and the foods grown are primarily for home consumption.

2.2. Collection of food grain samples

The grain samples were collected from seven villages in Kitgum and Lamwo districts (Table 1) between November 2014 and July 2015. Food samples collected included millet, maize, sorghum and sesame. We sampled cereal grains produced by farmers and used for household consumption from food storage bags in 75 randomly selected households. From each household, 500 g of each cereal grain was sampled and stored separately in a labelled polyethylene bag. Overall, 105 cereal grain samples were collected from the two districts (Table 1). These samples were transported to the Gulu University Bioscience Research Laboratory and stored at 4 °C until mycotoxin extraction and analyses.

Table 1
Sampling sites for cereal grains.

District	Village	No. of households visited	No. of food grain samples collected
Kitgum	Okidi Central	11	11
	Lamittumangu	19	26
Lamwo	Beyagoya	13	29
	Apeyta South	5	11
	Apeyta West	3	5
	Abam	21	21
	Laraba	3	3
Total		75	105

2.3. Sample preparation and analyses of mycotoxin

Enzyme-linked immunosorbent assay (ELISA) tests were performed using test kit procedures of Romer Labs Singapore Pte Ltd, to determine the mycotoxin levels within cereal grains. 20 g of each grain sample was ground to fine powder in a blender (IKA, Model M20, Germany). Ochratoxin and aflatoxin were extracted from the samples following manufacturers' procedures using methanol and distilled water (for DON). The supernatant was filtered through Whatman No.1 filter paper and the elute was subjected to competitive ELISA analysis.

Mycotoxin concentrations were quantified optically using a spectrophotometer ELISA microplate reader (MULTISKAN FC, model 357, China) with an absorbance and differential filters of 450 nm and 630 nm, respectively, and extrapolated from standard curves generated for each microplate. The kit detection range for ochratoxin was 2–40 ppb, 4–40 ppb for aflatoxin and 0.25–5.0 ppm for DON. To determine the level of co-occurrence of aflatoxin, ochratoxin and DON for each crop type, we used Microsoft Excel 2013 to identify those samples with these mycotoxins.

2.4. Estimation of daily intake and hazard index

The Estimated Daily Intake (EDI) was calculated using the mean levels of aflatoxins, ochratoxin and DON obtained in sorghum, millet, maize and sesame, the daily cereal intakes of 0.82 g/person/day [16] and the mean body weight was 72.3 kg/person as indicated by Kirunda [31]. The EDI (expressed in ng/kg of the body-weight/day (ng/kg bw/day) [17]) was calculated for aflatoxin, ochratoxin and DON as indicated in the formula below:

$$\text{EDI} = \text{Daily Intake (of sample food)} \times \text{mean level of Aflatoxins or ochratoxin or DON} \div \text{average body weight}$$

The Hazard Index (HI) was determined by dividing the EDI by TD50 (the daily dose (ng/kg/body weight/day) at which 50% of test animals would have developed tumors), divided by a safety factor of 50,000 as described by Ishikawa et al. [18], Ismail et al. [19] and Tsakiris et al. [16].

2.5. Statistical analysis

To examine whether the concentrations of each of the three mycotoxins differed among the four crops, we fitted a one-way ANOVA with crop type as fixed factor. Differences among treatment means was assessed using Tukey post-hoc tests, corrected for multiple testing (Bonferroni correction) [20]. Prior to statistical analyses, the concentrations of aflatoxin, ochratoxin, and DON were natural log (x + 1) transformed to improve normality. A *t*-test was used to determine whether or not there are significant differences in the concentrations of aflatoxin, ochratoxin and DON for each crop type between the two districts. All the above analyses were undertaken in SPSS version 23.

Table 2 Mean levels of mycotoxins (\pm SE) in food grain samples from Lamwo and Kitgum districts separately and data pooled for both districts. Grain samples with different letters in a column indicate significant differences in pair-wise tests (one-way ANOVA followed by Bonferroni corrected Tukey post-hoc test).

Grain sample	Kitgum				Lamwo				Pooled data for both districts			
	Total Aflatoxin	Total Ochratoxins	DON	Total Aflatoxin	Total Ochratoxins	DON	Total Aflatoxin	Total Ochratoxins	DON			
Sorghum ($\mu\text{g}/\text{kg}$)	16.0 \pm 3.6b	4.4 \pm 0.8b	253.4 \pm 107.5a	9.0 \pm 1.8c	3.5 \pm 0.7c	361.6 \pm 115.7ab	11.8 \pm 1.8b	3.8 \pm 0.5c	318.8 \pm 81.4ab			
Maize ($\mu\text{g}/\text{kg}$)	1.9 \pm 0.9a	0.6 \pm 0.3a	1092.6 \pm 400.8b	3.3 \pm 0.8ab	0.3 \pm 0.1a	191.4 \pm 76.0ab	2.8 \pm 0.6a	0.9 \pm 0.2a	513.3 \pm 185.2b			
Millet ($\mu\text{g}/\text{kg}$)	2.9 \pm 1.2a	1.1 \pm 0.3a	325.7 \pm 315.9a	4.3 \pm 1.5ab	1.0 \pm 0.3ab	32.0 \pm 31.7a	3.9 \pm 1.1a	1.1 \pm 0.2ab	129.7 \pm 106.5a			
Sesame ($\mu\text{g}/\text{kg}$)	2.4 \pm 1.1a	1.5 \pm 0.3ab	104.8 \pm 48.4a	3.5 \pm 2.9a	1.4 \pm 0.2bc	284.4 \pm 73.6b	3.2 \pm 2.1a	1.4 \pm 0.2b	230.5 \pm 55.2b			

3. Results

3.1. Aflatoxin contamination

All 105 food samples analysed had traceable amounts of total aflatoxin. This was followed by total ochratoxins while DON was least detected (Table 2).

The overall concentration of total aflatoxins varied significantly among the four crops studied (Pooled data, one-way ANOVA $F_{3,101} = 18.1, P < 0.001$; Lamwo, $F_{3,64} = 7.9, P < 0.001$; Kitgum, $F_{3,33} = 14.7, P < 0.001$ (Table 2). According to the pairwise tests (for pooled data), the mean concentrations of total aflatoxins was significantly higher in sorghum ($11.8 \pm 1.8 \mu\text{g}/\text{kg}$) than in millet ($3.9 \pm 1.1 \mu\text{g}/\text{kg}$), sesame ($3.2 \pm 2.1 \mu\text{g}/\text{kg}$) and maize ($2.8 \pm 0.6 \mu\text{g}/\text{kg}$) (all $P < 0.05$, Table 2). The range of aflatoxins in sorghum was nd (not detected)-68.2 $\mu\text{g}/\text{kg}$, millet nd-14.8 $\mu\text{g}/\text{kg}$, maize nd-8.1 $\mu\text{g}/\text{kg}$ and for sesame nd-61.8 $\mu\text{g}/\text{kg}$ (Appendices 1 & 2).

The mean concentration of total aflatoxins in sorghum samples from Kitgum district (mean \pm SE, $16.0 \pm 3.6 \mu\text{g}/\text{kg}$) was slightly higher than the mean concentration of total aflatoxins in sorghum samples from Lamwo district ($9.0 \pm 1.8 \mu\text{g}/\text{kg}$). However, the mean concentration of total aflatoxins in the other grain samples (maize, millet, sesame) was generally higher in samples from Lamwo than in samples from Kitgum district, though the differences were not statistically significant (Table 2). The 46.5% percent of the sorghum consumed in the two districts had a mean total aflatoxin concentration of $16 \mu\text{g}/\text{kg}$ that exceeded the Uganda national maximum tolerable or regulatory limits of $10 \mu\text{g}/\text{kg}$ (Table 3). In comparison to European Union, 86% of the sorghum consumed in the two districts exceeded the tolerable maximum limits of $4 \mu\text{g}/\text{kg}$.

3.2. Ochratoxin contamination

The concentration of pooled total ochratoxins also differed significantly among the four crops (pooled data, $F_{3,101} = 19.7, P < 0.001$; Lamwo, $F_{3,64} = 10.2, P < 0.001$; Kitgum, $F_{3,33} = 9.5, P < 0.001$). Like for total aflatoxins, sorghum had the highest mean concentration of pooled total ochratoxins ($3.8 \mu\text{g}/\text{kg}$), followed by sesame ($1.4 \mu\text{g}/\text{kg}$), millet ($1.1 \mu\text{g}/\text{kg}$) and maize ($0.4 \mu\text{g}/\text{kg}$). The range of the measured total ochratoxins was from nd-16.4 $\mu\text{g}/\text{kg}$ for sorghum, nd-1.8 $\mu\text{g}/\text{kg}$ for maize, nd-3.2 $\mu\text{g}/\text{kg}$ for millet and nd-3.1 $\mu\text{g}/\text{kg}$ for sesame (Appendices 1 and 2).

The mean concentration of ochratoxins in sorghum samples from Kitgum district was slightly higher than for those from Lamwo district (Table 2). The other samples (maize, millet and sesame) showed differences in the mean concentration of ochratoxins, but these differences were not statistically significant (Table 2). In comparison to European Union limits for ochratoxins, 25.6% of the sorghum consumed in the two districts exceeded the maximum levels of $5 \mu\text{g}/\text{kg}$ (Table 3).

3.3. Deoxynivalenol contamination

The concentrations of DON also differed significantly among the four crops studied (pooled data, $F_{3,101} = 4.7, P = 0.004$; Lamwo, $F_{3,64} = 4.7, P = 0.026$; Kitgum, $F_{3,33} = 3.7, P = 0.022$). According to

Table 3 Comparison of maximum tolerable limits of mycotoxin with EU market.

COUNTRY	MYCOTOXIN			Reference
	Total Aflatoxins	Total Ochratoxins	Deoxynivalenol	
EU	4 $\mu\text{g}/\text{kg}$	5.0 $\mu\text{g}/\text{kg}$	750 $\mu\text{g}/\text{kg}$	[21,22]
Uganda	10 $\mu\text{g}/\text{kg}$	-	-	[23]
Current study	nd-68.2 $\mu\text{g}/\text{kg}$	nd-16.5 $\mu\text{g}/\text{kg}$	nd-1904 $\mu\text{g}/\text{kg}$	

Table 4
Daily intakes of the various food grains in Uganda.

Food samples	Daily Intake (Kg/person/day)	References
Sorghum	0.115	[24,25]
Millet	1.8×10^{-4}	[24,25]
Maize	0.106	[24,25]
Sesame	6.0×10^{-6}	[26]

pairwise tests (pooled data), the concentrations of DON was significantly higher in maize (513.3 µg/Kg) and sesame (230.5 µg/Kg) than in millet, 129.7 µg/Kg (Table 2). Generally, low concentrations of DON were detected in all the samples with the highest (2606.2 µg/Kg) being in maize samples from Kitgum (Appendix 1). The highest concentrations of DON in all samples from Lamwo district was less than 1738.2 µg/Kg (Appendix 1). The range of DON in sorghum was nd-1738.2 µg/Kg, maize nd-2606.2 µg/Kg, millet nd-1904.4 µg/Kg and for sesame nd-955.3 µg/Kg (Appendices 1 and 2). In comparison to European Union limits, 18.6% of the sorghum consumed in the two districts exceeded the DON maximum tolerable limits of 750 µg/Kg (Table 3).

3.4. Human risk assessment of exposure to total aflatoxin, Ochratoxin and DON via consumption of cereals

3.4.1. Daily intake

Daily intakes of foods were 0.115 Kg/person/day for sorghum, 1.8×10^{-4} (millet), 0.106 (maize) and 6.0×10^{-6} (sesame), Table 4.

3.4.2. Hazard Index (HI)

The EDI calculated for total aflatoxin, Ochratoxin and DON for northern Ugandan people via consumption of cereals are presented in Table 5. The EDI values ranged from 1.2×10^{-5} –0.125 for total

Table 5
Estimated Daily Intake (EDI) and Hazard Indices (HI) for northern Ugandans via consumption of sorghum, maize, sesame foods.

Food grain	Mean (µg/Kg)	Age	Average body weight (kg)	Estimated Daily Intake (EDI (µg/Kg/.bw/day)	Hazard Index (HI)
Total aflatoxin					
Sorghum	11.8	18 > 65 yrs (adults)	72.3	3.1×10^{-3}	4.7×10^{-8}
		6–59 months (infants)	11.3	0.125	1.9×10^{-6}
Millet	3.9	18 > 65 yrs (adults)	72.3	5.4×10^{-7}	8.3×10^{-12}
		6–59 months (infants)	11.3	2.1×10^{-5}	3.2×10^{-10}
Maize	2.8	18 > 65 yrs (adults)	72.3	1.6×10^{-4}	2.5×10^{-9}
		6–59 months (infants)	11.3	0.007	1.0×10^{-7}
Sesame	3.2	18 > 65 yrs (adults)	72.3	1.2×10^{-5}	1.8×10^{-10}
		6–59 months (infants)	11.3	4.8×10^{-4}	7.4×10^{-9}
Total ochratoxin					
Sorghum	3.8	18 > 65 yrs (adults)	72.3	3.2×10^{-4}	6.1×10^{-8}
		6–59 months (infants)	11.3	0.013	2.5×10^{-6}
Millet	1.1	18 > 65 yrs (adults)	72.3	4.1×10^{-8}	8.0×10^{-12}
		6–59 months (infants)	11.3	1.7×10^{-6}	3.2×10^{-10}
Maize	0.9	18 > 65 yrs (adults)	72.3	1.6×10^{-5}	3.2×10^{-9}
		6–59 months (infants)	11.3	6.7×10^{-4}	1.3×10^{-7}
Sesame	1.4	18 > 65 yrs (adults)	72.3	3.4×10^{-5}	6.6×10^{-9}
		6–59 months (infants)	11.3	9.2×10^{-5}	1.8×10^{-8}
Deoxynivalenol (Vomitoxin)					
Sorghum	318.8	18 > 65 yrs (adults)	72.3	2.236	5.8×10^{-5}
		6–59 months (infants)	11.3	91.521	0.0024
Millet	129.7	18 > 65 yrs (adults)	72.3	0.006	1.5×10^{-7}
		6–59 months (infants)	11.3	0.024	6.3×10^{-7}
Maize	513.3	18 > 65 yrs (adults)	72.3	5.343	1.4×10^{-4}
		6–59 months (infants)	11.3	218.72	0.0057
Sesame	230.5	18 > 65 yrs (adults)	72.3	0.061	1.6×10^{-6}
		6–59 months (infants)	11.3	2.497	6.5×10^{-5}

TD50 of total Aflatoxin = 1.3 µg/kg [29].

TD50 of total Ochratoxin = 0.103 µg/kg [30].

TD50 of Deoxynivalenol (Vomitoxin) = 0.77 µg/kg [26].

Average body weight of an adult in Uganda = 72.3 kg [31].

Average body weight of infants in Uganda = 11.3 kg [32].

aflatoxin, 1.6×10^{-5} –0.013 (total ochratoxin), and 0.06–91.521 (DON). The recorded HI values were in the range of 1.3×10^{-7} to 0.0059 (Tables 4 and 5). In general, it is accepted that an HI ≤ 1 indicates no significant health risk. Nonetheless, the possibility of long-term adverse health effects increases with increasing HI values as an HI between 1.1 and 10 reflects a moderate risk [27], and HI < 10 indicates high risk [28]. HI value for exposure to aflatoxin, Ochratoxin and DON via consumption of sorghum, maize, millet and Sesame, cereal based foods consumed by the northern Ugandan population is less than one. The aforementioned values imply that intake of cereal-based foods will most likely not pose high risk to health of Ugandan population.

3.5. Co-occurrence of mycotoxin

Co-occurrence of the mycotoxins was also observed in the majority of the samples (Table 6). The co-occurrence of aflatoxin and ochratoxin was 86% of sorghum samples, 57.9% of millet samples, 40% of maize samples and 27.6% of sesame samples testing positive for both. Co-occurrence of aflatoxin and DON was found in 34.9% of sorghum, 10.5% of millet, 66.7% of maize and 20% of sesame samples. Co-occurrence of ochratoxin and DON ranged from 37.2% of maize samples, 15.8% in millet, 26.7% of maize to 56.7% of sesame samples. Co-occurrence of all three mycotoxins ranged from not detected in millet samples to as high as 32.6% in sorghum samples (Table 6).

4. Discussion

We assessed mycotoxin contamination levels in food grains in northern Uganda in order to gain insight into food safety and good agricultural practices in post-conflict areas of northern Uganda. Our results indicate high level of mycotoxin contamination. Aflatoxin and ochratoxin levels were high in the cereal grains analyzed in both Lamwo and Kitgum districts. The highest concentration of aflatoxin was

Table 6
Frequency of co-occurrence of mycotoxin.

Sample	Mycotoxin	Co-occurrence (%)
Sorghum	Aflatoxin	86.0
	Ochratoxin	
	Aflatoxin	34.9
	Deoxynivalenol	
	Ochratoxin	37.2
	Deoxynivalenol	
Millet	Aflatoxin	32.6
	Ochratoxin	
	Deoxynivalenol	
	Aflatoxin	57.9
	Ochratoxin	
	Deoxynivalenol	
Maize	Aflatoxin	10.5
	Ochratoxin	
	Deoxynivalenol	15.8
	Aflatoxin	
	Ochratoxin,	0
	Deoxynivalenol	
Sesame	Aflatoxin	40
	Ochratoxin	
	Aflatoxin	66.7
	Deoxynivalenol	
	Ochratoxin	26.7
	Deoxynivalenol	
Sesame	Aflatoxin	20
	Ochratoxin,	
	Deoxynivalenol	
	Aflatoxin	26.7
	Ochratoxin	
	Deoxynivalenol	
Sesame	Aflatoxin	20
	Ochratoxin	
	Deoxynivalenol	56.7
	Ochratoxin	
	Deoxynivalenol	
	Aflatoxin	13.3
Sesame	Ochratoxin	
	Deoxynivalenol	
	Deoxynivalenol	

recorded in sorghum followed by millet, maize and sesame. Our estimates of total aflatoxin in grains are similar to those obtained in Ethiopia [33], Malawi [22,34], Ghana [36] and in the neighbouring Democratic Republic of Congo [37]. In northern Uganda, sorghum is used for food, local alcohol brewing and source of household income. Small-scale farming households in northern Uganda preferentially grow the crop due to its inherent ability to resist drought. Foods are locally processed at homes with no quality checks and communities lack awareness of food safety concerns. The high levels of mycotoxin we found in these foods indicate the use of poor agricultural practices and implies public ignorance about mycotoxins in the region.

The differences between food grain crops contaminations are probably attributable to poor pre- and post-harvest practices, handling and environmental factors. Lack of difference in mycotoxin contamination between Kitgum and Lamwo districts may be due to similar climatic conditions in the two districts (both of them lie in the same agroecological zone) and the shared agricultural practices among farmers in both districts. These similarities in environment and farming methods likely result in more or less similar levels of mycotoxin observed.

Although ochratoxin and DON are not currently regulated in Uganda, aflatoxin is. Overall, 46.5% of the sorghum samples in Lamwo and Kitgum districts exceeded the Ugandan maximum tolerable limit of 10 µg/Kg. If measured against the E.U. standard of 4 µg/Kg, 86.1% of the sorghum samples exceeded the E.U. limit [21]. However, calculation of the HI value indicated that consumption of cereals poses no health risk to northern Uganda consumers. Open sun-drying after harvesting is a traditional method that can reduce mycotoxin contamination. However, communities and commercial grain producers in northern Uganda need to be educated on the importance of good

agricultural practices and food hygiene to ensure better standards are adhered to minimize mycotoxin contamination at every point during grain production. We suggest implementation of an integrated mycotoxin management system at household level in order to lessen mycotoxin contamination in this region. Mycotoxin prevention focusing on pre-harvest management is likely to be beneficial in reducing mycotoxin contamination.

The occurrence of multiple mycotoxins, even in low concentrations, are likely to worsen toxicity and results in serious health outcomes among the people in northern Uganda. Although low levels of mycotoxins may not result in an immediate observable effects, repeated exposures to multiple mycotoxins over a long period of time may result in detrimental health consequences [38]. Additionally, since it is a common practice by the communities in northern Uganda to regularly drink a lot of local brew prepared from sorghum, this increases their chances of daily exposure to high concentrations of different mycotoxins. This might explain the high incidence of oesophageal cancer in northern Uganda as reported in Alema and Iva [39]. Mycotoxins have been shown to cause several cancer related deaths depending on the duration of exposure. For example, high mortality rate are known to occur at an aflatoxin contamination levels of 6.25–15.6 ppm and at a mean daily consumption intake of 2–6 mg per person [42–44]. Northern Uganda also has the highest prevalence of chronic hepatitis B virus (HBV) in the country standing at 17.6% [45]. Two of the major risk factors to hepatocellular carcinoma are HBV infection and dietary exposure to aflatoxin B1 (AFB1) [46]. Aflatoxin B1 causes cancer by binding to and altering the structure of DNA, resulting in genomic mutation [48]. Ochratoxin A has also been linked to serious human health effects, including human endemic nephropathies, pouty ochratoxicosis, porcine nephropathy and urinary tract tumours [5,49].

Overall, our results provide evidence of high levels of mycotoxin contamination and co-occurrence in food grains in northern Uganda where the population is at high risk of exposure, with aflatoxins and ochratoxins at high levels in all the cereal types analyzed. However, the Hazard Indices recorded values less than 1 and so Ugandan consuming these products were not at risk. There is therefore an urgent need for heightened public awareness and both farmers and consumer education on the importance of exposure to mycotoxins as well as implementation of appropriate methods to reduce mycotoxin contamination in food produced and consumed at the household level. This would be best combined with a monitoring system that can provide feedback on the progress in reducing mycotoxin burden.

Declaration of Competing Interest

The authors declare that there is no conflict of interest. The research was conducted with no financial conflict or other factors which is considered to be declared as conflict.

Acknowledgments

This work was supported by VLIR-UOS grant awarded to Prof. Geert Haesaert entitled “Unknown neurotropic virus and mycotoxins: an exploratory study to unravel the cause of Nodding Syndrome”. Special thanks go to the communities in northern Uganda for allowing us collect samples in their households.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.toxrep.2019.09.002>.

References

- [1] FAO, Agriculture food and nutrition for Africa, A Resour. B. Teach. Agric. (1997).
- [2] J.W. Bennett, M. Klich, Mycotoxins, Review Clin. Microbiol. 16 (2003) 497–516.

- [3] D. Fandohan, M.J. Zoumenou, D.J. Hounhouigan, W.F.Q. Marasas, K.H. Wingfield, Fate of aflatoxins and fumonisins during the processing of food products in Benin, *Int. J. Food Microbiol.* 98 (2005) 249–259.
- [4] F.P. Guengerich, Common and uncommon cytochrome P450 reactions related to metabolism and chemical toxicity, *Chem. Res. Toxicol.* 14 (2001) 611–650, <https://doi.org/10.1021/tx0002583>.
- [5] J.I. Pitt, Toxicogenic fungi: which are important? *Med. Mycol.* 38 (2000) 17–22.
- [6] R. Bhat, R.V. Ravishanker, A.A. Karim, Mycotoxins in food and feed: present status and future concerns, *Comp. Rev. J. Food Sci. Food Saf.* 9 (2010) 57–81.
- [7] A. Alborzi, B. Pourabbas, F. Shahian, J. Mardaneh, G.R. Pouladfar, M. Ziyaeyan, Detection of *Leishmania infantum* kinetoplast DNA in the whole blood of asymptomatic individuals by PCR-ELISA and comparison with other infection markers in endemic areas, southern Iran, *Am. J. Trop. Med. Hyg.* 79 (2008) 839–842 doi:79/6/839 [pii].
- [8] J.M. Wagacha, J.W. Muthomi, Mycotoxin problem in Africa: current status, implications to food safety and health and possible management strategies, *Int. J. Food Microbiol.* 124 (2008) 1–12, <https://doi.org/10.1016/j.ijfoodmicro.2008.01.008>.
- [9] Morbidity and Mortality Weekly Report, 53, (2004), pp. 790–793.
- [10] FAO (Food And Agricultural Organisation), FAO Statistical Databases, (1998).
- [11] I.D.F. Inan, M. Pala, Use of ozone in detoxification of aflatoxin B1 in red pepper, *J. Stored Prod. Res. Prod. Res.* 43 (2007) 425–429, <https://doi.org/10.1016/j.jspr.2006.11.004>.
- [12] W. C. C. Wendo, *Lancet* 362 (2003) 1818.
- [13] C.R. Day, “Survival mode”: rebel resilience and the lord’s resistance army, *Terror. Polit. Violence* (2017) 1–21, <https://doi.org/10.1080/09546553.2017.1300580>.
- [14] Uganda Bureau of Statistics, No Title, (2012).
- [15] G.K. Seyedeh Faezeh Taghizadeha, Ramin Rezaeeb, Gholamhossein Davarynejada, Javad Asilic, Seyed Hossein Nematia, Marina Goumenoud, Ioannis Tsakirise, Aristides M. Tsatsakisf, Kobra Shiranig, Risk assessment of exposure to aflatoxin B1 and ochratoxin A through consumption of different Pistachio (*Pistacia vera* L.) cultivars collected from four geographical regions of Iran, *Environ. Toxicol. Pharmacol.* 61 (2018) 61–66, <https://doi.org/10.1016/j.etap.2018.05.010>.
- [16] J. Sifuentes, T. Montagner, E. Yurie, S. Ono, E. Hiromi, M. Carlos, M. Zavariz, D. Miranda, E. Nakagawa, O. Kawamura, E. Yoko, Natural occurrence of deoxynivalenol in wheat from Paraná State, Brazil and estimated daily intake by wheat products, *Food Chem.* 138 (2013) 90–95, <https://doi.org/10.1016/j.foodchem.2012.09.100>.
- [17] E.Y. Hirooka, E.N.I. Angélica, T. Ishikawa, C.ássia R. Takabayashi-Yamashita, Elisabete Y.S. Ono, Artur K. Bagatin, Fabiana F. Rigobello, Osamu Kawamura, Exposure assessment of infants to aflatoxin M1 through consumption of breast milk and infant, *Toxins (Basel)* 8 (2016) 1–11, <https://doi.org/10.3390/toxins8090246>.
- [18] A.H. Amir Ismail, Muhammad Riaz, Robert E. Levin, Saeed Akhtar, Yun YunGong, Seasonal prevalence level of aflatoxin M1 and its estimated daily intake in Pakistan, *Food Control* 60 (2016) 461–465.
- [19] K.M. G.P. Quinn, *Experimental Design and Data Analysis for Biologists*, 1st ed., Cambridge University Press, Cambridge, Cambridge University Press, 2002, www.cambridge.org/9780521811286.
- [20] European Union Commission, European Union, Commission Regulation (EC) No 2174/2003 of 12 December 2003 Amending Regulation (EC) No 466/2001 As Regards Aflatoxins, (2003), p. 12.
- [21] European Commission, Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs, *Off. J. Eur. Union.* (2006) 5–34.
- [22] Uganda National Bureaus of Standards DUS DEAS, (2016).
- [23] Uganda Bureau of Statistics (UBOS), Uganda Bureau of Statistics 2017 Statistical Abstract, (2017).
- [24] P. Harvey, Z.O. Rambelason, O. Dary, Determining the dietary patterns of Ugandan women and children, *Acad. Educ. Dev.* (2010).
- [25] J.F.E.C. On Food, Safety Evaluation of Certain Mycotoxins in Food (No. 74), Food Agric. Organ, United Nations, 2001, p. 505.
- [26] A.D. Lemly, Evaluation of the hazard quotient method for risk assessment of selenium, *Ecotoxicol. Environ. Saf.* 35 (1996) 156–162.
- [27] C.O. Ogunkunle, P.O. Fatoba, Potential Health Risk Assessment for Soil Heavy Metal Contamination of Sagamu, South-West Nigeria Due to Cement Production 3 (2013), pp. 89–96.
- [28] R.H. Li, A.P. Heflich, *Genetic Toxicology*, CRC Press Bost., 1991, p. 350.
- [29] J. Bull, Reckhow R.J, Rotello D.A, Bull V, O.M, Kim, Use of Toxicological and Chemical Models to Prioritize Dbp Research (Water Research Foundation Report), Amwa Res. Found, USA, Washingt. DC (2006), p. 250.
- [30] B.E. Kirunda, Body Weight and Physical Activity of Adults in Rural Uganda, *Univ. Bergen*, (2017), pp. 1–110 <http://bora.uib.no/bitstream/handle/1956/16414/dthesis-2017-Barbara-Eva-Kiruna.pdf?sequence=4&isAllowed=y>.
- [31] M.E. Ralston, M.A. Myatt, Weight estimation tool for children aged 6 to 59 months in limited-resource settings, *PLoS One* 11 (2016) e0159260, <https://doi.org/10.1371/journal.pone.0159260>.
- [32] A. Chala, W. Taye, A. Ayalew, R. Krska, M. Sulyok, A. Logriec, Multimycotoxin analysis of sorghum, (*Sorghum bicolor* L. Moench) and finger millet (*Eleusine coracana* L. Garten) from Ethiopia, *Food Control* 45 (2014) 29–35, <https://doi.org/10.1016/j.foodcont.2014.04.018>.
- [33] L. Matumba, M. Monjerezi, B. Khonga, D. Lakudzala, Aflatoxins in traditional sorghum beer in southern Malawi, *Food Control* 22 (2011) 266–268.
- [34] V.K. Baffour, N. Korley Kortei, A. Akomeah Agyekum, H. Wiisibie Alidu, F. Akuamoaa, Risk assessment and exposure to levels of naturally occurring aflatoxins in some packaged cereals and cereal based foods consumed in Accra, Ghana, *Toxicol. Rep.* 6 (2018) 34–41, <https://doi.org/10.1016/j.toxrep.2018.11.012>.
- [35] P. Udomkun, T. Wossen, N.L. Nabahunu, C. Mutegi, B. Vanlauwe, R. Bandyopadhyay, Incidence and farmers’ knowledge of aflatoxin contamination and control in Eastern Democratic Republic of Congo, *Food Sci. Nutr.* 6 (2018) 1607–1620, <https://doi.org/10.1002/fsn.3.735>.
- [36] G.T. Kuiper, Risk assessment to humans of mycotoxins in animal-derived food products, *Vet. Hum. Toxicol.* 33 (1991) 325–332.
- [37] A. On, B. Iva, Cancer of the esophagus: histopathological sub-types in northern Uganda, *Afr. Health Sci.* 14 (2014) 17–21.
- [38] K. Krishnamachari, Hepatitis due to aflatoxicosis, *Lancet* 1 (1975) 1061–1063.
- [39] K.A. Krishnamachari, R.V. Bhat, V. Nagarajan, T.B. Tilak, Investigations into an outbreak of hepatitis in parts of western India, *Indian J. Med. Res.* 63 (1975) 1036–1049.
- [40] R.F. Keeler, A.T. Tu, Plant and fungal toxins, *Handb. Nat. Toxins*, New York, Marcel Dekker, Inc, (1983), p. 308.
- [41] E. Ochola, P. Ocama, C.G. Orach, K.N. Ziadah, J.N. Kalyango, W. McFarland, C. Karamag, High burden of hepatitis B infection in Northern Uganda: results of a population-based survey, *BMC Public Health* 13 (2013), <https://doi.org/10.1186/1471-2458-13-727>.
- [42] H.C. Wu, R. Santella, The role of aflatoxins in hepatocellular carcinoma, *Hepat. Mon.* 12 (2012) e7238, <https://doi.org/10.5812/hepatmon.7238>.
- [43] J.D. Groopman, P.R. Donahue, J.Q. Zhu, J.S. Chen, G.N. Wogan, Aflatoxin metabolism in humans: detection of metabolites and nucleic acid adducts in urine by affinity chromatography, *Proc. Natl. Acad. Sci. U. S. A.* 82 (1985) 6492–6496, <https://doi.org/10.1073/pnas.82.19.6492>.
- [44] S. Abid, W. Hassen, A. Achour, H. Skhiri, K. Maaroufi, F. Ellouz, E. Creppy, H. Bacha, Ochratoxin A and human chronic nephropathy in Tunisia: is the situation endemic? *Hum. Exp. Toxicol.* 22 (2003) 77–84, <https://doi.org/10.1191/0960327103ht328oa>.