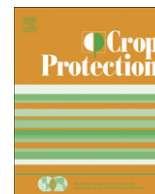




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## Occurrence and distribution of aflatoxin contamination in groundnuts (*Arachis hypogaea* L.) and population density of *Aflatoxigenic Aspergilli* in Malawi

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## ABSTRACT

Groundnut (*Arachis hypogaea* L.) is susceptible to pre- and post-harvest infections by *Aspergillus* spp. Aflatoxin B<sub>1</sub> (AFB<sub>1</sub>), is the contaminant produced by the fungus in infected grains posing a threat to human and animal health. This paper reports of a study undertaken in Malawi to determine the occurrence and distribution of *Aflatoxigenic Aspergilli* in the soil and AFB<sub>1</sub> contamination in groundnuts. A total of 1397 groundnut samples collected from farm homesteads, local markets, warehouses and shops in 2008 and 2009 were analyzed for AFB<sub>1</sub> contamination using the enzyme linked immunosorbent assay (ELISA), and *A. Aspergilli* population densities in 1053 soil samples collected from the same sites were estimated using serial dilutions plated on *A. Aspergilli* medium. Farmer socio-economic profile information was also collected to determine relationships to AFB<sub>1</sub> contamination. The results revealed 46% and 23% of the total samples, from 2008 to 2009, respectively, had AFB<sub>1</sub> contamination levels greater than 4 ppb, and those above 20 ppb were 21% for 2008 and 8% for 2009, respectively. Fitted smooth curve relationships show that there is a clear increase in the chance of groundnut contamination when the population density of *A. Aspergilli* in the soil increased beyond 3000 (log (cfu) > 8). The measured level of *A. Aspergilli* in soil varied by location, as well as ecologies within location. Low-altitude ecologies, which were warmer and experienced low precipitation levels, had the highest densities of *A. Aspergilli*, whereas cooler high-altitude ecologies had the lowest density of these fungi. Similarly high AFB<sub>1</sub> contamination, was recorded across the country with 11–28% of all samples collected from the warm low to mid-altitude ecologies recording contamination ≥20 ppb and low contamination (2–10% of samples) in the mid to high altitude cool ecologies. From a crop management perspective, this study also suggests that both less experienced and older farmers were more likely to produce groundnuts contaminated with aflatoxin. These findings have implications in the design of intervention strategies to avoid short- and long-term human health effects from aflatoxin exposure.

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### 1. Introduction

Groundnut (*Arachis hypogaea* L.) is an important crop worldwide ranking sixth and thirteenth among oil and food crops,

respectively. The crop is widely consumed in Malawi where it provides nutritional security to many households, as a rich source of protein and vitamins, supplementing diets where maize, rice, and cassava are the major energy foods. Groundnut is also important as a source of income when sold locally or exported, particularly to the European Union (EU). However, groundnut exports have declined since 1990, and lost significant market share primarily due to aflatoxin contamination. The European Commission set standard for aflatoxin contamination is 4 ppb in groundnuts intended for direct human consumption (Otsuki et al., 2001). In the US the set

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limit is 20 ppb (FAO Food and Nutrition Paper 81, 2003). As a result, 42% (by volume) of groundnuts from Malawi exported to the European market in 2005 were rejected due to aflatoxin contamination (Diaz-Rios and Jaffe, 2008).

Aflatoxins are a potent class of mycotoxins produced by several *Aspergillus* spp. in the section *Flavi*. These aflatoxigenic fungi are soil inhabiting and are found in both agricultural and non-agricultural environments (Azziz-Baumgartner et al., 2005; Cotty and Bayman, 1993; Jaime-Garcia and Cotty, 2004; Lewis et al., 2005; Sétamou et al., 1997; Waliyar et al., 1994, 2003; Williams et al., 2004). Therefore, infection and contamination of groundnuts can occur both in the field (pre-harvest) and in storage facilities (post-harvest). Pre-harvest contamination by *Aspergillus* sp., however, is more important in the semi-arid tropic regions of the world such as Malawi, especially when crops are exposed to end-of-season drought (Waliyar et al., 1994). Among the mycotoxins produced by the fungi in section *Flavi*, aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) is the most potent and potentially lethal metabolite (Azziz-Baumgartner et al., 2005; Lewis et al., 2005; Lu, 2003; WHO, 2005; Wild, 2007; Williams et al., 2004). *Aspergillus* section *Flavi* includes the species, *Aspergillus parasiticus*, *Aspergillus flavus*, *Aspergillus nomius*, *Aspergillus bombycis*, and *Aspergillus pseudotamarii*, which under certain conditions produce highly toxic and carcinogenic aflatoxins (Cotty and Cardwell, 1999; Egel et al., 1994; Ito et al., 2001; Peterson et al., 2001). In addition, more than 50 other species of filamentous fungi, including several species of *Penicillium* and a distantly related *Chaetomium*, have been reported to synthesize sterigmatocystin and other aflatoxin precursors (Barnes et al., 1994; Frisvad, 1985). Among the 18 potential mycotoxins produced by species in section *Flavi*, the more common toxins are aflatoxin B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>. *A. flavus* typically produces only the B aflatoxins, but may also produce G aflatoxins; *A. parasiticus* produces both B and G aflatoxins. Four other aflatoxins M<sub>1</sub>, M<sub>2</sub>, B<sub>2</sub>A, G<sub>2</sub>A which may be produced in minor amounts have been isolated from cultures of *A. flavus* and *A. parasiticus*. A number of closely related compounds namely aflatoxin GM<sub>1</sub>, parasiticol and aflatoxicol are also produced by *A. flavus*. Aflatoxin M<sub>1</sub> and M<sub>2</sub> are major metabolites of aflatoxin B<sub>1</sub> and B<sub>2</sub> respectively, found in milk of animals that have consumed feed contaminated with aflatoxins (<http://www.icrisat.org/aflatoxin/aflatoxin.asp>).

Human and animal exposure to aflatoxin, through ingestion of contaminated grains may result in several health problems (Hendrickse, 1997; Lu, 2003; Wild, 2007; Williams et al., 2004). In a recent review, Williams et al. (2004) summarized the health effects from aflatoxin exposure stating that i) large doses lead to acute illness and death, usually through liver cirrhosis; ii) chronic sub-lethal doses have nutritional and immunologic effects and iii) all doses have a cumulative effect on cancer. Since diseases in developing countries are not fully reported, aflatoxicosis outbreaks like the one in Kenya (Lewis et al., 2005), are likely to be a more common occurrence than currently realized due to poor detection mechanisms in resource constrained countries including Malawi. The burden of disease stemming from long-term aflatoxin exposure (such as hepatocellular carcinoma, impaired growth, immune suppression, etc) remains undefined (WHO, 2005).

Aflatoxin levels in foods likely provide a good indication of aflatoxin exposure (Azziz-Baumgartner et al., 2005; Lewis et al., 2005; Moss, 1998). Studies have shown that AFB<sub>1</sub> concentration in food above a certain limit is considered hazardous and a threat to food security (Lewis et al., 2005). As a result, countries have adopted various allowable AFB<sub>1</sub> contamination levels for food. In EU and USA safe limits are currently considered to be 4 and 20 ppb respectively, yet many aflatoxin-prone developing countries including Malawi, do not have AFB<sub>1</sub> contamination safety limits. Those in developing countries that do have safety limits, however,

lack skills and resources for detection and enforcement, and populations in these developing countries continue to be exposed to aflatoxins (Wild, 2007; Williams et al., 2004).

Although various reports and personal communications indicate high incidence of aflatoxin on groundnut and other food crops in Malawi (Mkoka, 2007a,b), little evidence based knowledge is available to support this assertion. Due to the mode of infection and transmission of aflatoxin to human beings, this study was designed to provide a broad understanding of the current status of aflatoxin poisoning associated with groundnut consumption in Malawi. Specifically, the study was set up to i) assess the occurrence and distribution of AFB<sub>1</sub> contamination in groundnuts; ii) estimate the distribution of *A. Aspergilli* in the soils of groundnut growing districts; and iii) generate information on perceptions of farmers and traders on aflatoxin.

Raising public awareness about the aflatoxin contamination problem and disseminating information is an important intervention strategy (WHO, 2005). However, the basis of intervention is to know the extent of the problem. Therefore, assessing what farmers and vendors know about aflatoxin is an important first step, coupled with analyzing samples collected from homesteads and markets for aflatoxin. Analyzing farm and market samples has been used in many studies to gauge the extent of aflatoxin contamination (Azziz-Baumgartner et al., 2005; Lewis et al., 2005; Waliyar et al., 2003). In addition, estimation of densities of *Aflatoxigenic Aspergilli* would be useful in efforts aimed at reducing soil populations of the toxigenic *Aspergilli* spp.

## 2. Materials and methods

### 2.1. Ecological investigation

The study was undertaken during the 2007/08 and 2008/09 seasons and covered 11 important groundnut production districts of Malawi. In total, the study involved 75 Extension Planning Areas (EPAs), and 1053 farmers (Malawi has 27 districts subdivided into 150 EPAs). Purposive sampling was used to select the 11 districts (Fig. 1) that were representative of i) important groundnut production areas; ii) clusters of cancer cases reported by Kamuzu Central Hospital; and iii) agro-ecological zones based on altitude. Malawi is located in Southern Africa stretching latitude 09°41'S–16°55'S South and parts of the country lie at longitude 32°58'E–35°40'E East. Malawi's latitude, longitude and topography have created some variation in its climate. In the highland areas the weather is relatively cool but lower elevations are hot. The main climate that prevails in Malawi is of tropical continental nature. Temperature and annual precipitation depends mostly on altitude, ranging from an annual mean of 23–27 °C in the low-altitude areas of Chikwawa, Salima, Phalombe, Nkhhotakhota districts to 18–22 °C in the mid to high-altitude areas of Mzimba, Ntcheu, Dowa, Ntchisi, Lilongwe, Mchinji and Kasungu. In the lower Shire Valley the altitude is as low as 37 m above sea level (m asl), and in the mountainous areas the altitude can be as high as 2000 m asl and influences the weather greatly. Groundnuts in Malawi are produced from 37 m asl to 1500 m asl.

A multistage sampling scheme was used in which major groundnut-producing EPAs were randomly selected from each of the districts, and within each of those EPAs, entrepreneurial groundnut farmers and traders were randomly selected for interviews. To standardize the interviewing process, a three day training workshop was conducted at the outset to train and acquaint enumerators with the data collection instrument and how to collect the data as stated in Section 2.2 below. Training also covered interviewing procedures and mock interviews. Simple random sampling technique was used to select farmers and sample



Fig. 1. Map of Malawi showing study districts and sample points.

collection and interviews was limited to groundnut entrepreneurs. Major groundnut producing EPAs were selected at random as were major producing sections in each EPA and farmers within the sections in each EPA.

## 2.2. Groundnut sample

A total of 1397 samples of fresh and processed groundnuts were collected. Two collection dates were chosen: the first collection was sampled 8–11 months after the 2008 harvest to investigate the effect of long-term storage, and the second collection was sampled 1–2 months after the 2009 harvest. Consideration of the sample size was based on resources, time, and the desired precision of the estimates. Groundnut samples were collected from farmers, traders, supermarkets (including peanut butter), processors, and middlemen who buy crops from farmers. At sampling, information recorded included: date of collection, farmer's name, farmer's age, farmer's years of formal education, farmer's years experience growing groundnuts, district name, EPA, village name, geographical positioning system location. For unprocessed groundnuts the physical condition of sample was noted as healthy or unhealthy, i.e., shriveled, insect damaged, or moldy.

## 2.3. Aflatoxin B<sub>1</sub> assay

Collected groundnut samples were further air dried and brought to a uniform moisture content ( $\leq 7\%$ ) immediately after collection and serologically assayed for AFB<sub>1</sub> contamination within four weeks of collection using the indirect Enzyme Linked Immunosorbent Assay (ELISA). From each 1 kg sample, 100 g of shelled seeds were weighed and blended. 100 ml of 70% methanol (v/v) containing 0.5% KCl, was added to 20 g of the blended groundnut sample and blended further. For assaying AFB<sub>1</sub> contamination in peanut butter, 20 g of peanut butter was blended with methanol and KCl as described in the previous sentence. The mixture was then transferred to a 250 ml conical flask and shaken at 300 rpm for 30 min (Gallenkamp Orbital Shaker). The mixture was then filtered through Whatman No. 41 filter paper and diluted 1:10 in phosphate buffer saline with Tween (1 ml filtrate in 9 ml buffer). Microtiter plates sensitized with aflatoxin B<sub>1</sub>–BSA conjugate were incubated at 37 °C for 1–2 h followed by wash with PBS–Tween. In all the steps, 150  $\mu$ l/well of appropriate wash solution was used. Then the plates were washed with PBS–Tween followed by addition of blocking solution (0.2% Bovine serum albumin) before 30–45 min incubation at 37 °C and washing. The extracts of the samples, or AFB<sub>1</sub> standard solution of 100  $\mu$ l/well, were incubated with 50  $\mu$ l of polyclonal antibody solution in the plate for 60 min. Polyclonal antibodies were cross-absorbed with 0.2% BSA for 30 min at 37 °C prior to addition to the plates. Then diluted anti-rabbit IgG

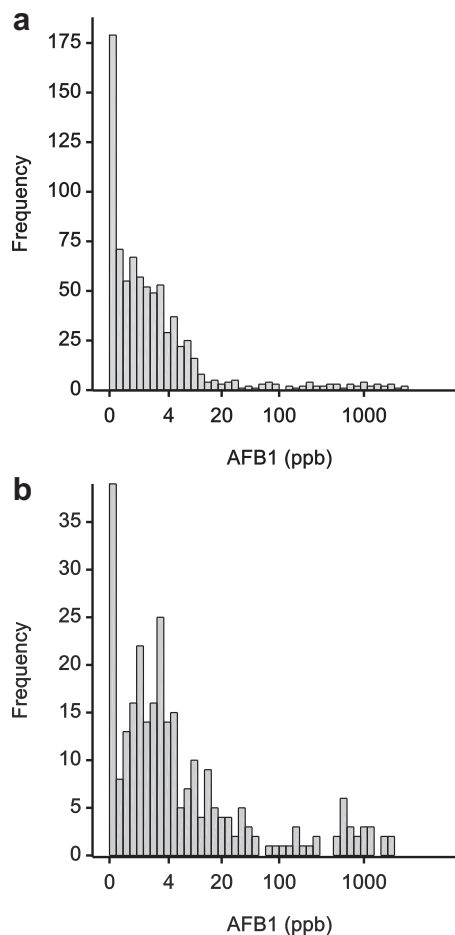


Fig. 2. a: Histogram of AFB<sub>1</sub> levels (ppb) in 1185 fresh groundnut samples measured 1–2 months after harvest. b: Histogram of AFB<sub>1</sub> levels (ppb) in 212 stored groundnut samples measured 8–11 months after harvest.

**Table 1**

Percent of groundnut samples (analyzed within 2 months of harvest and those stored for 8–11 months) with AFB<sub>1</sub> above the acceptable levels of 4 and 20 ppb.

Storage Regime	Percent of samples		
	0–4 ppb	4.1–20 ppb	≥20 ppb
1–2 months (1185 samples)	77	15	8
8–11 months (212 samples)	54	25	21

labeled with Alkaline Phosphatase was added to each well and the plates incubated at 37 °C for 60 min. After washing, p-Nitro phenyl phosphate prepared in 10% di-ethanolamine was added and the plates were read at 405 nm in the Multiskan Plus (Labsystem) ELISA reader. The principle of ELISA lies in immobilizing the antigen onto solid surface capturing antigen by specific antibodies and probing with specific immunoglobulin carrying an enzyme label (Waliyar et al., 2009). The enzyme retained in case of positive reaction is detected by adding suitable substrate. The enzyme converts substrate to a product, which can easily be recognized by its color.

#### 2.4. *A. Aspergilli* spp. population densities

A total of 1053 soil samples were collected from the farms where groundnuts were sampled. From each farmer's field, a composite soil sample of 50 g was obtained by bulking samples taken randomly from five sites, across 0.5 ha, at 3–7 cm deep, and kept in labeled paper bags. Soil samples were taken back to Chitedze Agricultural Research Station (CARS) in Lilongwe for processing. At CARS, soil samples were oven dried at 35 °C for 4 days, ground to a fine powder using pestle and mortar and screened through and No. 20 sieve. 10 g of soil was then suspended in 90 ml of distilled water, and 0.5 ml aliquots of 10<sup>-3</sup> and 10<sup>-4</sup> dilutions were plated on AFPA medium. Petri-dishes were then incubated at 28 °C in the dark for 3 days, and bright yellow to orange colonies were counted as *A. Aspergilli* colony forming units (CFU) on day 4 (Pitt et al., 1983; Steyn, 1980).

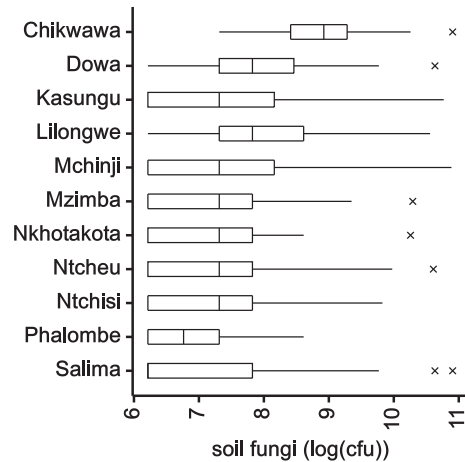
#### 2.5. Statistical methods

The AFB<sub>1</sub> contamination values have an extremely skewed distribution with many zeros. The usual practice of transforming skewed data does not solve the problem when there are large numbers of zeros. Hence we model the probability of a sample being about the limits of 4 ppb and 20 ppb using logistic regression. As the shape of responses of these probabilities to the explanatory variables is unknown, we model them with smooth splines. They are fitted using the generalized additive model framework implemented in Genstat 13 (VSNI 2010). Smoothing splines with 2 degrees of freedom are used to describe the relationships.

**Table 2**

Percent of fresh groundnut samples from different districts with AFB<sub>1</sub> above acceptable levels of 4 and 20 ppb.

District	n	Percent >4 ppb	Percent >20 ppb	Range ppb
Chikwawa	29	52	28	0–2963
Dowa	33	33	12	0–1290
Kasungu	83	37	17	0–1878
Lilongwe	198	18	5	0–2065
Mchinji	155	17	7	0–2020
Mzimba	111	18	2	0–478
Nkhotakota	35	20	3	0–3240
Ntcheu	57	33	9	0–2181
Ntchisi	51	31	10	0–2427
Phalombe	10	30	20	0–493
Salima	35	31	11	0–1340



**Fig. 3.** Distribution by district of soil fungi cfu from the same locations as fresh groundnut samples.

### 3. Results

#### 3.1. Ecological investigation

*A. Aspergilli* in soil varied by district with higher densities recorded in regions with less rainfall. High AFB<sub>1</sub> contamination was recorded across the country with 11–28% of all samples collected from the warm dry low to mid-altitude ecologies like Chikwawa, Phalombe and Salima recording contamination ≥20 ppb and low contamination (2–10% of all samples) from the mid to high altitude cool wet ecologies like Mzimba, Ntcheu, Lilongwe and Mchinji. Across districts, 65% of respondents were aware of aflatoxin, with Mzimba district ranking highest at 81%. However, most respondents associated aflatoxin contamination with groundnut that were rotten. The major source of information regarding aflatoxin was from farmer to farmer (52.6%) followed by radio programs (31.9%), and lastly through other agricultural institutions.

#### 3.2. Aflatoxin B<sub>1</sub> assay

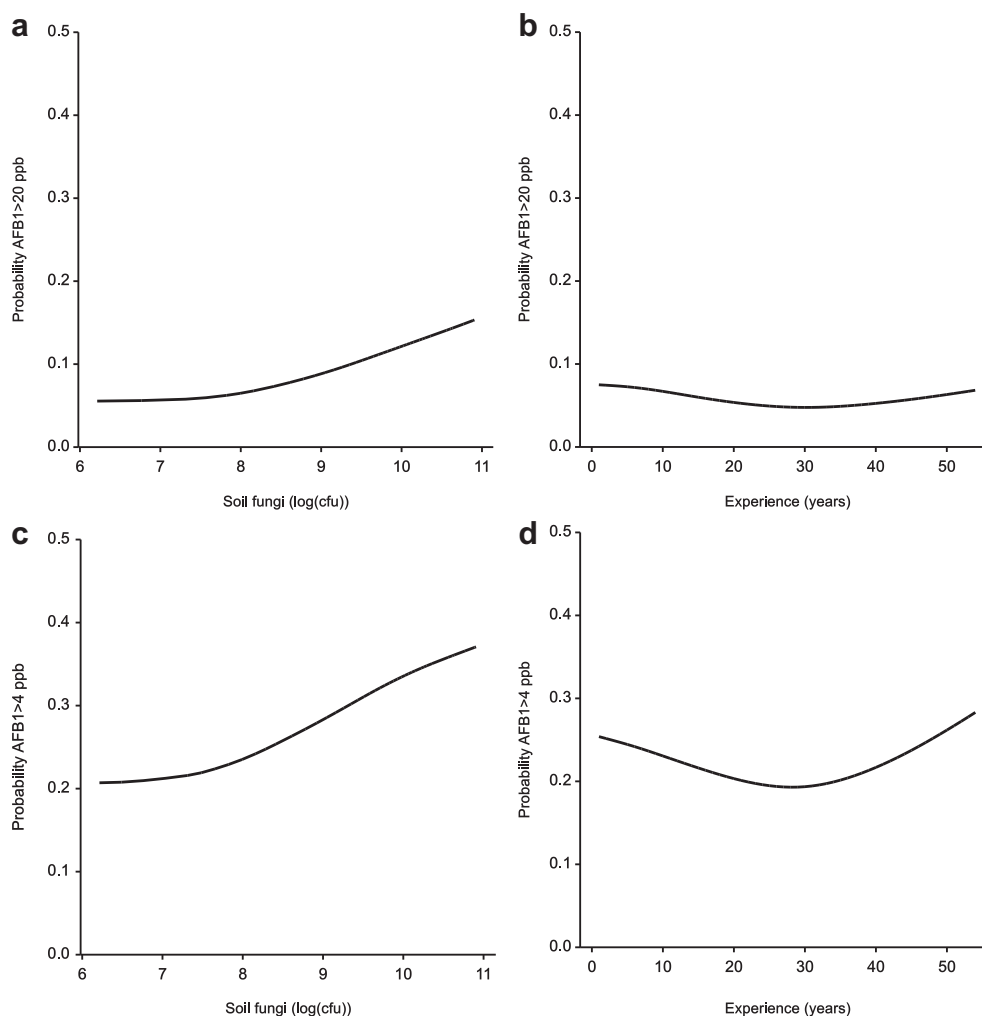
AFB<sub>1</sub> levels were variable, with many samples free of contamination but a few with high concentrations (Fig. 2a and b). 46% of the samples in 2008 and 23% of the samples in 2009, had contamination levels higher than 4 ppb (Table 1) whereas 21% and 8% of the respective lots were above 20 ppb.

High incidences of contamination above 4 ppb (≥30% of all samples) were recorded in Chikwawa, Kasungu, Dowa, Phalombe,

**Table 3**

Analysis of deviance from logistic regression of chance of AFB<sub>1</sub> levels being >4 ppm and >20 ppm, related to soil fungi levels (lcfu), exposure to groundnut farming (exp) and years of education (educ). Note the total sample size is 797 not 1185 due to missing values of some variables.

source	d.f.	Chance of >4 ppb		Chance of >20 ppb	
		Mean deviance	Approx p	Mean deviance	Approx p
lcfu	2	3.712	0.024	4.1347	0.016
exp	2	2.84	0.058	2.0583	0.128
educ	2	0.35	0.705	1.1019	0.332
District	10	3.316	<0.001	2.8564	0.001
lcfu. district	10	1.079	0.374	0.8283	0.601
exp. district	10	1.462	0.147	2.0495	0.025
educ. district	10	0.792	0.636	0.5059	0.887
Residual	750	1.072		0.5043	
Total	796	1.111		0.5719	



**Fig. 4.** a: Fitted probabilities of contamination >20 ppb as influenced by soil fungi (log (cfu)). b: Fitted probabilities of contamination >20 ppb as influenced by exposure to groundnut farming (years). c: Fitted probabilities of contamination >4 ppb as influenced by soil fungi (log (cfu)). d: Fitted probabilities of contamination >4 ppb as influenced by exposure to groundnut farming (years).

Ntcheu, Ntchisi Salima. Of those 30%, 10% exhibited contamination levels above 20 ppb (Table 2).

### 3.3. *A. Aspergilli* population densities

*A. Aspergilli* populations were variable both between and within districts (Fig. 3). Chikwawa district has the highest density of *A. Aspergilli*. Confirmatory analysis (Table 3) shows that the probability of high contamination in groundnuts is related to soil population densities of *A. Aspergilli*. The relationships between farmers experience in groundnut farming and soil cfu do not vary by district, except in the case of the chance of contamination of >20 ppb and farmer experience. In that case the trend varies somewhat by district. The fitted smooth curve relationships in Fig. 4a and 4c show that there is a clear increase in the chance of groundnut contamination when the soil cfu increase beyond about 3000 cfu (log(cfu) > 8). The change in chance of contamination with years of exposure to groundnut production is modest. However, both less experienced and old farmers produced more AFB<sub>1</sub> contaminated groundnuts (Fig. 4b and d). It also shows that number of years of formal education is not as important in AFB<sub>1</sub> contamination as years of exposure (experience farming groundnuts). This is probably because number of years of formal education

at the level of smallholder farmers may not have been as relevant to knowledge on aflatoxin contamination as farmers own experience with groundnut farming.

In Table 4 the values from Table 2 have been adjusted to remove the effects of variation in *A. Aspergilli* and length of experience with farming groundnuts. Plotting these adjusted values against mean

**Table 4**

Percent of fresh groundnut samples from different districts with AFB<sub>1</sub> above acceptable levels of 4 and 20 ppb, adjusted for soil fungi levels and length of experience to groundnut production.

district	Percent >4 ppb	Percent >20 ppb
Chikwawa	44	20
Dowa	32	11
Kasungu	37	17
Lilongwe	17	5
Mchinji	17	7
Mzimba	19	2
Nkhotakota	21	3
Ntcheu	34	9
Ntchisi	32	10
Phalombe	31	20
Salima	32	12

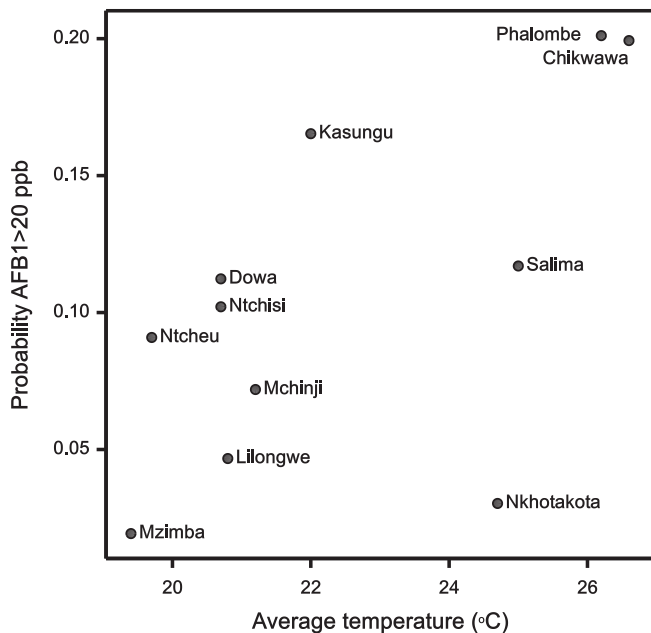


Fig. 5. Adjusted values of proportion of samples >20 ppb for each district plotted against district mean temperature (°C).

Table 5

Percent of stored groundnut samples from different sources with AFB<sub>1</sub> above the acceptable levels of 4 and 20 ppb.

Source	n	Percent ≥4 ppb	Percent ≥20 ppb	Contamination range in ppb
Farm house	213	43	15	0–2197
Local market	152	49	27	0–1643
Local shops	12	54	18	0–594
Super market	16	63	25	0–367
Warehouse	17	41	17	0–804
Others	11	45	38	0–471

growing season temperature, we see evidence of positive correlation (Fig. 5) between warmer temperatures and higher incidence of groundnut contamination. Interestingly, not all districts share this correlation. For example, Nkhotakota has much lower risk of contamination than would be expected from its temperature. Differences in contamination of stored groundnuts from different sources are not significant (Table 5), but this may be due to small sample sizes limiting our ability to detect them. The chance of samples being >20 ppb still varies between 2% and 20% in different districts.

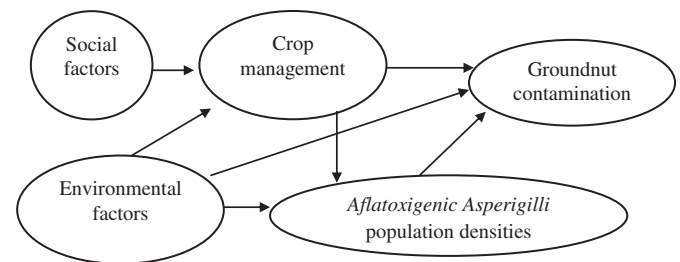
#### 4. Discussion and conclusion

One of the aims of this study was to establish the AFB<sub>1</sub> contamination levels in groundnuts in Malawi. Considering samples above 4 ppb or 20 ppb as contaminated and those below as clean based on the EU import restriction and WHO acceptable safety limit (WHO, 2005), there are significant differences in contamination between districts in Malawi. We observed multiple factors that may affect likelihood of AFB<sub>1</sub> contamination are related to both crop management and field ecology, such as groundnut farming experience and population densities of aflatoxigenic fungi in the soil. Other factors that seem of importance include delayed planting of groundnuts in tobacco-growing regions, and temperature. Delayed planting in Kasungu where farmers normally give early planting priority to tobacco may have exposed the crop to end

of season drought and high temperatures explaining for the higher risk of contamination observed.

AFB<sub>1</sub>, a potent carcinogen, was detected in groundnut samples from all districts. Even though most samples had lower than 20 ppb AFB<sub>1</sub> contamination levels, 21% and 8% of the stored (2008) and fresh (2009) samples had contamination greater than 20 ppb. The levels of contamination found were less than those reported during the aflatoxicosis outbreak in Kenya (Azziz-Baumgartner et al., 2005; Lewis et al., 2005), where aflatoxin concentration as high as 46,000 ppb were found in contaminated maize. In our survey, we report AFB<sub>1</sub> concentrations as high as, 2197 ppb in 2008 and 3240 ppb in 2009. Given the importance of groundnut in the diets of Malawians, this is a significant amount of contamination, and therefore AFB<sub>1</sub> exposure is of concern.

In general, 92% of freshly collected samples in this study are considered safe if we consider 20 ppb as the safety limit. To tackle the aflatoxin problem in groundnuts, it is important to understand the risk factors. In this study we found direct correlation between AFB<sub>1</sub> contamination in groundnuts and the quantity of *A. Aspergilli* in the soil. We also found some evidence of management effects through farmers' experience with groundnuts cultivation. We suggest the model of understanding risk of contamination in fresh groundnuts as shown below:



In this study we were able to show the existence of some of these relationships, though others are demonstrated in the literature. In a study to investigate risk of exposure and mitigation effects of aflatoxin on human health in West Africa, Cardwell and Henry (2004) showed that social factors such as education and access to disposable income determine food sanitation and the variety of foods in the household diet. The environmental variables probably of most relevance (soils and climate) were not available at the scale of the sample data collection, but only as district averages. It is not surprising that observations of contamination levels in a single season are not strongly related to these. There will always be considerable variation between year and between farms within a district. Thus, for example, Nkhotakota may have been unusually cool and damp in the year this data were collected, explaining its risk being less than expected from its mean temperature. Interestingly, population densities of *A. Aspergilli* spp. were highest in Chikwawa district which also had the highest AFB<sub>1</sub> contamination levels. This finding is similar to that of Egal et al. (2005) who showed direct correlation between AFB<sub>1</sub> contamination in maize, concentration of *A. flavus* (in CFU) in the soil and blood toxin levels in children from households where the samples were taken. It will be important to follow through this study and characterize population densities of *A. Aspergilli* for toxigenic and atoxigenic species in the different districts and whether it also translates to contamination in the population in Malawi. The fact that the weather in Chikwawa is generally hotter could partially explain why *A. Aspergilli* were higher, since the fungus, although ubiquitous, is known as thermotolerant and found in warmer areas. Future work should continue to elaborate these relationships so that interventions can be designed. Some

(e.g., the effects of crop and soil management on *A. Aspergilli* levels) may be suitable for experimental investigation. Others will require surveys like this one, but with efforts to collect more relevant explanatory variables (social, environmental and management) at the same scale (farm or field) that the grain data is collected.

For several years Mchinji and Lilongwe districts have had active aflatoxin management projects. These two have among the lowest levels of groundnut contamination. However, they are not particularly low given their average temperature (Fig. 5). This data do not reveal any obvious impacts of these projects. However district level averages are not a sensitive way of detecting the impact of such a project.

This study included Dowa, Ntchisi and Ntcheu districts that reported high incidences of cancer cases (personal communication/Kamuzu Central Hospital surgery registry) to try and assess whether there could have been correlation between cancer patients and aflatoxin incidence. Our findings, however, shows that aflatoxin levels in these districts were not higher than those from other districts. Because our survey is a snap shot of exposure to aflatoxin (2008 and 2009 harvests) and the fact that cancer has been reported to develop from chronic exposure, long-term surveys may be necessary to capture and relate cancer cases in Malawi to aflatoxin loads in food. This study has shown that AFB<sub>1</sub> contamination levels in food have been found to be a significant problem and they are likely to indicate AFB<sub>1</sub> exposure to the public. Intervention studies should be initiated with the aim of developing management strategies for reducing AFB<sub>1</sub> load in food. Low-cost measures like proper drying of groundnuts and storage on raised pallets, if done consistently, reduces AFB<sub>1</sub> in food (Waliyar et al., 1994). These types of intervention strategies are amenable for quick implementation as other intervention methods like breeding for aflatoxin resistance in groundnuts is being developed and tested. Currently, the availability of improved groundnut varieties in Malawi, such as JL 24, CG 7, and ICGV-SM 90704, will continue to favor increased groundnut production, but to sustain this, increased production has to be in tandem with better aflatoxin management methods.

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