POST-HARVEST INCIDENCE OF AFLATOXINS AND FUMONISINS IN MAIZE AND ITS RELATIONSHIP TO CLIMATIC CONDITIONS IN MALAWI

MSc. (ENVIRONMENTAL SCIENCE) THESIS

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UNIVERSITY OF MALAWI
CHANCELLOR COLLEGE

OCTOBER 2020

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MSc. (ENVIRONMENTAL SCIENCE) THESIS

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SUBMITTED TO THE FACULTY OF SCIENCE IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF ENVIRONMETAL SCIENCES

UNIVERSITY OF MALAWI

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OCTOBER 2020

DECLARATION

I, the undersigned, hereby declare that this thesis/dissertation is my own original work which has not been submitted to any other institution for similar purposes. Where other people's work has been used, acknowledgements have been made

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CERTIFICATE OF APPROVAL

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DEDICATION

I dedicate this work to God and my family.

ACKNOWLEDGEMENTS

There are several people to whom I am indebted for their contribution to this study and I wish to express my gratitude and sincerest thanks to them.

Most importantly, I unreservedly thank my supervisor, Dr. M. Monjerezi, for his timely acceptance as my supervisor and for providing me with unquantifiable constructive academic support throughout this study. I also thank Dr. Joseph Atehnkeng for his material support during lab work for this research.

I sincerely thank my friends; Gibson Mkwinda, Thaskani Chipeta, Madalitso Mhango and Grace for their hand in field sample collection.

Many thanks to Modester Khembo, the best friend and soul mate! And Derek, my son. You really sacrificed a lot.

Finally, I would like to thank and praise the Lord for unfailing love, support, guidance and everything.

ABSTRACT

This study provided an assessment of incidence of aflatoxins and fumonisins contamination of maize after harvest under different agro-environmental conditions in Malawi and its relationship with climatic conditions. The maize samples for this study were from the 2015/16 growing season. A total of one thousand two hundred and ninety four (1294) samples of maize for human consumption were collected from rural farmers' households and rural markets, providing a wide geographical coverage of Extension Planning Areas (EPAs) in Malawi's four agro-ecological zones. A questionnaire was used to establish farmers' knowledge on moulds, fumonisins and aflatoxins, processing and consumption patterns. Reveal Q^+ kits were used for quantitation of total aflatoxins and fumonisins. Dietary intake of aflatoxins and fumonisins was estimated using procedures established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Data show most maize samples (> 75%) were widely contaminated with aflatoxins across Malawi's agro-ecological zones. On average, fumonisins were detected in 45% of the samples, and co-occurred with aflatoxins in 38.64% of all maize samples. Maize samples collected from the Lower Shire agro-ecological zone (median 20.8 μg/kg) had relatively higher levels of aflatoxins ($p < 0.05$) than maize samples from the other agro-ecological zones (median: 3.0 μg/kg). Additionally, the majority (75%) of the positive samples from the Lower Shire agro-ecological zone had aflatoxin levels exceeding the EU regulatory limit (4 μg/kg), whereas 25%, 37% and 39% of positive samples exceeded the EU limit in the mid-elevation, lakeshore, upper and middle Shire and highlands agro-ecological zone, respectively. The Lower Shire agro-ecological zone is characterised by high mean monthly temperatures throughout the year and erratic rainfall. Therefore, the results of this study show highest aflatoxin counts in areas with the highest annual mean temperature, supporting the idea that meteorological conditions are the key factors of fungal colonization and mycotoxin production. In contrast to total aflatoxins, levels of total fumonisins in maize did not show a broad variation across the four agro-ecological zones. In general, almost all positive samples from across all agro-ecological zone exceeded the EU regulatory limit for fumonisins in maize products for infants of 0.2

mg/kg but fell short of the regulatory limit for adults of 4 mg/kg, but without discernible differences across the agro-ecological zones. For fumonisins, estimates of daily dietary intake exceeded the JECFA's provisional maximum tolerable daily intake (PMTDI) of 2.0 μg/kg body weight (bw)/day in 40.4%, 50%, 38.6% and 35.2% (median of EPA values) of all samples collected from the highlands, mid-elevation, lakeshore and Lower Shire agro-ecological zones, respectively. In addition, the estimated daily intake of fumonisin exceeded the PMTDI in majority of the fumonisin positive samples, across all agro-ecological zones. Therefore, from a risk assessment perspective, the results of this study may offer a communication tool for stakeholders, including policy makers, to highlight the need for education and awareness for improved mycotoxin risk management in Malawi and possibly the Southern African region.

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LIST OF ACRONYMS AND ABBREVIATIONS

ITCZ	Inter-Tropical Convergence Zone
GoM	Government of Malawi
AFB1	Aflatoxin B1
AFB2	Aflatoxin B2
AFG1	Aflatoxin G1
AFG2	Aflatoxin G2
AEZ	Agricultural Ecological Zones
masl	Meters above sea level
ADD	Average Daily Dose
A. flavus	*Aspergillus flavus*
HBV^{+}	Hepatitis B Virus
MaSSP	Malawi Strategy Support Program
MAPAC	Malawi Program for Aflatoxin control
IARC	International Agency for Research on Cancer
FGR	Fetal Growth Restriction
ppm	Parts Per Million
ppb	Parts Per Billion
WHO	World Health Organisation
JECFA	Joint Expert Committee on Food Additives
ELISA	Enzyme Linked Immunosorbent Assays methods
EPAs	Extension Planning Areas

CHAPTER 1: INTRODUCTION

1.1 Background

In sub-Saharan Africa, maize (*Zea mays* L.) is the primary staple crop accounting for about 73% of the total food demand (Shiferaw et al., 2011), consumed in different forms (Nago et al., 1997). In Malawi, food security is generally equated to sufficiency of maize supply, which accounts for more than 60% of the total food production (Government of Malawi, 2012), more than 60% of energy, and almost 50% of protein, 67% of total iron, 65% of total zinc, and almost 70% of total riboflavin consumption (Ecker and Qaim, 2011). However, maize is susceptible to pre- and post-harvest colonization by fungi that produce mycotoxins, under favourable conditions (Shephard, 2003). In maize, the mycotoxins of greatest concern are aflatoxins and fumonisins, which are produced by certain *Aspergilli* and *Fusaria* species, respectively (Shephard et al., 2013a; Adetunji et al., 2014; Ediage et al., 2014; Matumba et al., 2015a; Hove et al., 2016a; Chilaka et al., 2017). In terms of aflatoxin production, *A. flavus* (B aflatoxins) and *A. parasiticus* (B and G aflatoxins) are the most important species on maize (Molyneux et al., 2007). Fumonisins are produced predominantly by *F. verticillioides* and *F. proliferatum* (Creppy, 2002; Lino et al., 2006) which cause maize ear rot, a common maize fungal disease (Marasas, 2001; Logrieco et al., 2002).

Aflatoxins and fumonisins, as well as other mycotoxins, have elicited interest and concern because they are inimical to public health, food security and trade. Food spoilage caused by mycotoxins leads to economic losses and aggravates food insecurity (Blesa et al., 2004; Sitko et al., 2011; Wu, 2014; Agyekum & Jolly, 2017). Aflatoxin B1 (AFB1), in synergy with hepatitis B virus (HBV) infection, causes human primary liver cancer and International Agency for Research on Cancer (IARC) classified it as a Group I human carcinogen (IARC, 2002; Wu et al., 2014). In high doses, aflatoxins have caused deaths from aflatoxicosis (Williams et al., 2004; Liu & Wu, 2010; Kimanya et al., 2015) such as reported in Kenya, India, and Thailand (CAST, 2003;

Nyikal et al., 2004, Lewis et al., 2005; Probst et al., 2007; Reddy & Raghavender, 2007; Yard et al., 2013). As a result of its ability to suppress the immune system, AFB1 exposure is also associated with increased severity of diseases such as HIV/AIDS (Jiang et al., 2008; Williams et al., 2010; Jolly et al., 2015), malaria (Allen et al., 1992) and tuberculosis (Williams et al., 2004; Keenan et al., 2011). Aflatoxins may also play a role in malnutriution and growth stunting during early childhood and, together with other mycotoxins, accelerate the development of edema in malnourished people and kwashiorkor in malnourished children (IARC, 2015; Kimanya et al., 2015; Harrison et al., 1990). The International Agency for Research on Cancer (IARC) has classified fumonisin B1 (FB1) as Group 2B human carcinogens (IARC, 2002). Fumonisins have been extensively linked to high incidence of esophageal carcinoma in certain parts of South Africa and China (Rheeder et al., 1992; Wagacha and Muthomi, 2008; Mostrom, 2016; Sun, Wang, and Hu, 2007). Fumonisins have also been implicated in the high incidence of neural tube defects (NTDs) in rural populations known to consume contaminated maize, such as the former Transkei region of South Africa (Marasas et al., 2004; Shephard, 2008a).

Generally, mycotoxins are thermally stable, although levels may be reduced to a variable degree through processing (including cooking) (Humpf and Voss, 2004; Park et al., 2004;; Voss et al., 2001). Therefore, human exposure to aflatoxins and fumonisins is primarily through dietary intake (Stoloff, 1983) of contaminated food and "carryover" mycotoxins in products from contaminated grains or their metabolites in milk and meat products (Kotsonis et al., 2001; Yiannikouris & Jouany, 2002; Seglar, 2003). Therefore, frequent presence of aflatoxins and fumonisins in staple food supplies implies that populations are at risk throughout their lives. Pre-natal exposure of foetuses has been reported in Ghana and Nigeria (Lamplugh et al., 1988), Kenya (De Vries et al., 1989) and Gambia (Wild et al., 1991), whereas exposure through mother's milk has been reported in Egypt (El-Sayed et al., 2000), Gambia (Zarba et al., 1992), Ghana (Lamplugh et al., 1988), Kenya (Hendrickse, 1991), Sierra Leone (Jonsyn et al., 1995), Sudan (Coulter et al., 1984) and Zimbabwe (Wild et al., 1987).

1.2 Problem statement

The few available studies that explore the current incidence of mycotoxin contamination in Malawian food have highlighted high prevalence of aflatoxins and fumonisins in maize (Matumba, 2009; Matumba et al., 2009, 2013, 2014b; Mwalwayo and Thole, 2016; Seetha et al., 2018). It is also clear from the periodical surveys that several mycotoxins, with toxicological synergism, co-occur in maize (Matumba et al., 2014) and contamination is persistent over time (Magamba et al. 2017; Seetha et al., 2018) despite intervention efforts (Malawi Nutrition Policy, 2013). Therefore, aflatoxins and fumonisins contamination is a major risk for health and well-being of people, primarily children, in Malawi. The risk is particularly serious for rural subsistence farming communities who, because of lack of economic alternatives, are reliant on the consumption of home-grown crops, with little consideration for quality.

Sufficient and reliable data on the occurrence of aflatoxins and fumonisins in the main staples are required for quantitative exposure assessment, a critical component of risk assessment. However, the issue of food safety, with its emphasis on food quality, is frequently subordinate to issues of food security, with their emphasis on sufficiency of supply, because of chronic shortages of staple foods (Matumba et al., 2017) caused by recurrent droughts and intensified by tenuous agricultural production systems. In Malawi, the mycotoxin surveys have been sporadic and fragmented, often limited geographically and/or to a few samples (Matumba et al., 2017). Therefore, there is a general paucity of nationwide data, available in a comprehensive form, on the pervasiveness of the contamination of maize with aflatoxins and fumonisins. In addition, fungal colonisation and production of aflatoxins and fumonisins is highly dependent on climatic conditions. Variation in incidences of aflatoxins and fumonisins in maize among Malawian agro-ecologies, with micro-climate variations, is underexplored on a national scale. Such information is useful for monitoring programs amongst subsistent populations living in extreme micro-climatic zones (Matumba et al., 2014). Further, routine exposure determinations are necessary for predicting and forestalling mycotoxicoses which are usually difficult to cope with (Lewis et al., 2005; Probst et al., 2007; Yard et al., 2013; IARC, 2015).

To this end, this study expanded the data on aflatoxin and fumonisin incidences in maize across all four agro-ecologies in Malawi (Highlands; Mid-elevation; Lowershire; and Lakeshore), thereby establishing a nationwide geographical pattern. The data on the distribution and concentrations of these mycotoxins was used to assess consumer risk of exposure to aflatoxins and fumonisins. The risk assessment results could form the scientific basis of risk management options or could improve the basis to decide on policy making and further research, in order to reduce health hazards due to aflatoxins and fumonisins.

1.3 Study objectives

This study was carried out to investigate the incidence of aflatoxin and fumonisin contamination in maize after harvest under different agro-environmental conditions in Malawi and its relationship with climatic conditions. The specific objectives were to:

(*i*) determine levels of total aflatoxins and estimated levels of fumonisins in Malawi's maize across the four main agro-ecological zones

(*ii*) estimate dietary intake levels to aflatoxins and fumonisins in Malawi across the four main agro-ecological zones

(*iii*) characterize the extent of variability of levels of contamination and co-occurrence of the two mycotoxins in maize with climatic patterns

CHAPTER 2: LITERATURE REVIEW

2.1 Aflatoxins and fumonisins: Nature and production in maize

Mycotoxins are a large and diverse group of toxic secondary metabolites produced by certain species of filamentous fungi in a wide range of agricultural produce such as cereals, nuts, legumes, spices and fruits (Bhat & Vashanti, 2003). The most studied classes of mycotoxins, for which maximum levels in food are enforced, include aflatoxins, fumonisins, zearalenone, trichothecenes, citrinin, ergot alkaloids, ochratoxin, and patulin (Bennett & Klich, 2003; Driehuis et al., 2008; Binder, 2007). In addition to these known mycotoxins, the so-called "emerging mycotoxins", which include fusaproliferin, beauvericin, enniatins, and moniliformin, and "masked mycotoxins" frequently co-occur in agricultural products (Jestoi, 2008; Gruber-Dorninger et al., 2016; Kovalsky et al., 2016).

Aflatoxins (common saprophytes pathogens) are produced by *Aspegillus* species (Shephard et al, 2010; Marasas & Nelson, 1987) that include mainly *A. flavus* (which produce B aflatoxins only) and *A. parasiticus* (Hussain & Anwar, 2008), which produce both B and G aflatoxins (Molyneux et al., 2007). These two species are ubiquitous in some foods and stored agricultural products (Blandino et al., 2017). Other species reported to produce aflatoxins include *Aspergillus arachidicola*, *Aspergillus minisclerotigenes*, and *Aspergillus* nomius. On the other hand, fumonisins are predominantly produced by *Fusarium verticillioides*, *F. proliferatum* and *F. nygamai* (Blandino et al., 2017), with *Fusarium verticillioides* being the predominant contaminant in food and feeds (Michael & Wyatt, 1993; CAST, 2003). The major source of contamination in cereal grains is the conidia of toxin-producing fungi in the field, which can continuously grow and contaminate the grainsduring both pre-and post-harvest.

Aflatoxins are difuranocoumarin compounds (Bhat, 1991; Jalili, 2015) in which a bifuran group is attached (Figure 2.1) at one side of the coumarin nucleus (Kumar et al., 2017), while a pentanone ring is bonded to the other side in the case of the AFTs-B series, or a six-membered lactone ring is attached in the AFTs-G series (Bennett and Klich, 2003; Nakai et al., 2008). They include aflatoxin B1, B2, G1, G2 (Jalili, 2015; Chen et al., 2013), M1, M2 (Chen et al., 2013), B2a, B3, GM1, G2a M2a GM2, P1, Q1, R0, RB1, RB2, AFL, AFLH, AFLM and methoxy ethoxy and acetoxy derivatives (Bhat, 1991). On the other hand, fumonisins are polyols with a long chain (20 carbons) esterified in the C14 and C15 (Figure 2.1) with two groups of tricarboxylic acids (Fung and Clark, 2004). Fumonisin B1 (FB1), Fumonisin B2 (FB2) and Fumonisin B3 (FB3) are the major naturally occurring (WHO, 2018) fumonisins (Figure 2.1), while aflatoxin (B1, B2, G1, G2 and M1) are the only naturally occurring aflatoxins (Pitt, 2000) with AFB1 being the most abundantly produced and most toxic (IARC, 1993).

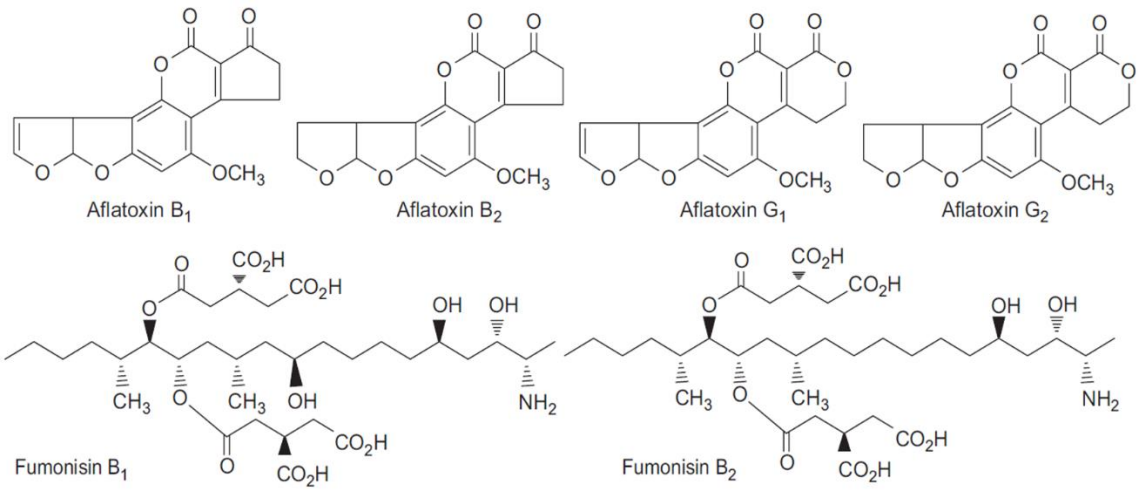

Figure 2.1: Chemical structures of important aflatoxins and fumonisins (adapted from Reddy et al., 2010)

2.2 Factors influencing aflatoxins and fumonisins occurrence in Maize

Maize may initially be contaminated with conidia of *Aspergillus* spp. and *Fusarium* spp. in the field (Kamala et al., 2016; Chulze 2010) and, if conditions are favourable, the fungi may keep growing and metabolizing toxins in the field and during storage (Scheidegger & Payne, 2003; Sanchis & Magan, 2004; Neme & Mohammed, 2017). Figure 2.2 provides a summary of the interaction of the main factors influencing fungal growth and mycotoxin production on agricultural produce.

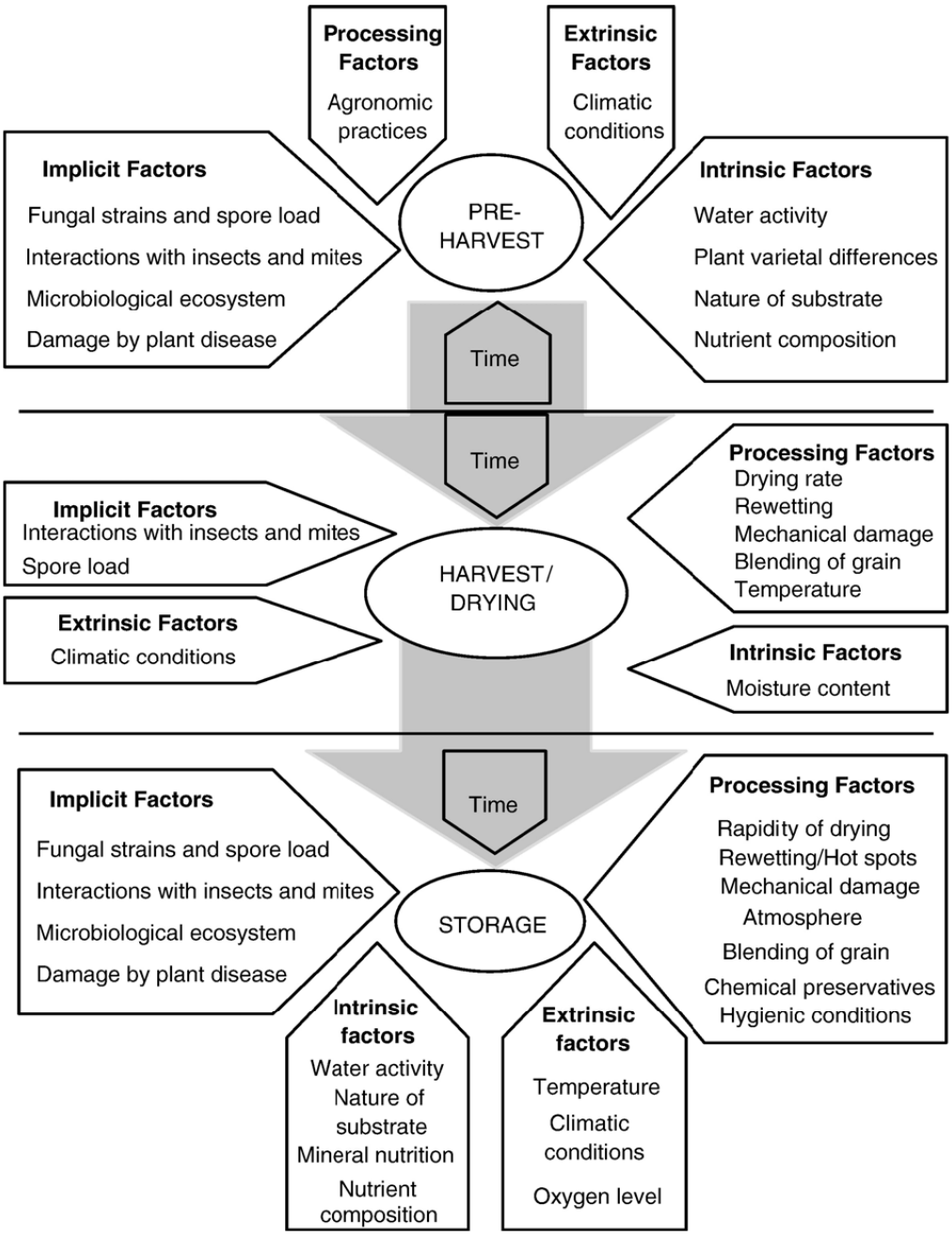

Figure 2.2: Integration of the most important intrinsic and extrinsic factors affecting fungi colonisation and mycotoxin production during pre-harvest and storage period (From Magan et al., 2004).

Several environmental or ecological, biotic and agronomic factors interact and affect the etiology, dominance, and production of mycotoxin by toxigenic fungi (Kim & Manna 2017). Environmental factors include meteorological and other physicochemical conditions conducive to fungal colonization and subsequent mycotoxin production (Sweeney & Dobson, 1998) such as relative humidity,

temperature, water activity, acidity and drought stress (Pitt & Miscamble, 1995; Sanchis & Magan, 2004; Paterson & Lima, 2010, 2011; Magan et al., 2011; Wu et al., 2011). Agronomic factors include agricultural practices such as cropping pattern, variety, level of nitrogenous fertilization, planting date and delayed drying (Jones, 1987), whereas biotic factors include insect and bird damage (McMillian, 1987).

Pre-harvest contamination with aflatoxins is mainly worsened by nitrogen stress during pollination (Jones, 1979), drought stress and high temperature throughout pollination (Jones et al., 1980) and seed maturation (Gorman & Kang, 1991; Payne et al., 1989). Drought stress during crop harvesting results in cracks (Bruns, 2003) that might enhance *Fusarium* and *A. flavus* infection with subsequent mycotoxin development (Smart et al, 1990). Periodic drought stress aggravates aflatoxin and fumonisin contamination in crops by eliminating microbial competitors resulting in the increase of *A. flavus* and *Fusarium* population (Diener, et al., 1987; Sorenson et al., 1984). Several studies have shown that high soil temperature and drought stress are key environmental parameters that are positively correlated with aflatoxin contamination and increased incidence of aflatoxigenic strains or species during the pre-harvest period (Horn & Dorner, 1999; Jaime-Garcia & Cotty, 2010; Orum et al., 1999). High soil temperatures and dry conditions favour dispersal of *A. flavus* conidia (Jones et al., 1980). They contribute to shifts in population structure of *A. flavus* towards high fraction of aflatoxin producers (Horn & Dorner, 2002).

In the stored-grain ecosystem, the main environmental determinants of fungi proliferation and mycotoxin production are air temperature and water availability (Sinha, 1995). Moisture and relative humidity affect both grade and storability of grains (Achglinkame et al., 2017) as it significantly influences microbial growth and toxin production (Wilson & Payne, 1994; Okello et al., 2010). The effect of water availability on grain fungi is usually determined in terms of equilibrium relative humidity (ERH) and water activity (a_w), or in some cases in terms of water potential (Ψ). ERH represents the percent relative humidity of the air between grains in equilibrium with water in the grains (Faraj et al., 1991). Conversely, a_w is the ratio of the vapour pressure of water above the grains to the saturated vapour pressure of water at the same temperature and pressure (Mahmoud et al., 1992), and represents a precise determination of water availability for microbial growth (Achglinkame et al., 2017).

Several studies on the effects of environmental conditions on aflatoxin production by *A. flavus* and *A. parasiticus* show that the interactions between water activity and temperature (a_w x temperature) are more important than the individual parameters (Medina et al., 2017). However, the studies report a wide range of optimum values of these conditions, even within isolates of the same species (Mannaa & Kim, 2017). The reported optimum temperatures and a_w conditions for mycotoxin production of the *Aspergillus* species fall within 24-37 ^{o}C and $a_w > 0.95$ (Abdel-Hadi et al., 2012; Mannaa and Kim, 2017), whereas for and *Fusarium* are reported within 15-30 ^{o}C and $a_w = 0.93$ (Marín et al., 1999). However, during storage, *Aspergillus* spp. may grow and start to produce mycotoxin at grain moisture of about 13 wt% or relative humidity of 65% (Atanda et al., 2011).

Biotic factors, such as insect infestation, promote colonization of *Aspegillus* and *Fusarium* fungi species as they enhance the interaction between the colonizing toxigenic fungal species and substrate (D'Mello & Macdonald, 1997). Insect damage in the field and during crop storage promotes fungal grain contamination (Achglinkame et al., 2017; Udoh et al, 2000) by breaking the testa of grains, which constitutes a natural barrier for fungal growth, through which fungi spores penetrate and produces excreta that acts as pockets of moisture environment conducive for *Aspegillus* and *Fusarium* spp. (Beti et al., 1995; Setamou et al., 1998). Hell et al. (2000) and Kaaya et al. (2006) reported a direct correlation between insect infestation and aflatoxin contamination with shelled maize being more susceptible than maize stored on cob. *A. flavus and Fusarium* infection in crops is further promoted by occurrence of diseases and other pathogens in crops (Bruns, 2003; Alakonya & Monda, 2013), and amplified storage time (Kaaya et al. 2006; Hell et al., 2000). Production of aflatoxins and fumonisin may also be affected by nutritional factors (Liu et al, 2016). These include carbohydrate and nitrogen deficiencies (Jones, 1979; Calvert et al, 1983), sufficient phosphates for plant growth (Bruns, 1991), calcium (Hodges et al, 1991), lipoperoxide, and trace metals (Luchese and Harrigan, 1993). High zinc concentration in soils is associated with aflatoxin contamination (Achiglinkame et al., 2017), however the presence of high levels of phosphate renders zinc unavailable to the *A. flavus* (Lacey & Magan, 1991).

2.3 Patterns of aflatoxins and fumonisins co-occurence in maize for Southern Africa

In southern Africa, maize (*Zea mays* L.) is the primary dietary staple, accounting for about 73% of the total food demand (Shiferaw et al., 2011). It is consumed throughout the region in different forms (Nago et al., 1997) including fresh or processed into milled, cooked or fermented products (Shephard et al., 2013a; Hove et al., 2016a). Household consumption of maize in rural subsistence farming communities in South Africa range from 382 g (Ecker & Qaim, 2011) to reach intake levels of 1–2 kg/person/day (Burger et al., 2010).

However, maize is susceptible to colonisation of *Fusarium* and *Aspergillus* species and subsequent contamination with fumonisins and aflatoxins throughout its growth, harvest, transport and storage (Shephard et al., 2008a). In Africa, climate and poor agricultural and storage practices are the major contributors to the mycotoxin hazard. Therefore, maize and maize products are reported to be frequently contaminated with unacceptable levels of fumonisins and aflatoxins, with FB1 and AFB1 being the most prevalent (Shephard et al., 2011; 2013a). High levels of fumonisin contamination in maize have been reported in several studies in Malawi, South Africa and Zimbabwe (Boutigny et al., 2012; Matumba et al., 2014c; Hove et al., 2016a; Mwalwayo & Thole, 2016). Table 2.1 provides an outline of examples of the wide spectrum of reported occurrence of mycotoxins in maize and maize products in Southern Africa.

Probst et al. (2017) reported 100% (n = 19) of sampled maize samples from Zimbabwe had fumonisins in levels exceeding 10 ppm, while 20% contained aflatoxin levels above 4 ppb. Murashiki et al. (2017) also reported 100% (n = 388) FB1 contamination in maize samples from rural Zimbabwe, although all samples did not exceed EU regulatory. In Malawi, Matumba et al. (2014) reported 75% (n = 12) of samples of locally produced maize puffs with aflatoxins at levels of up to 2 µg/kg. In addition, maize from rural households in Malawi was reported to be highly contaminated with aflatoxins up to 140 µg/kg (Malwayo & Thole, 2016).

Table 2.1: Examples of reported contamination of maize with aflatoxins and fumonisins in Southern Africa

Country	**Mycotoxin**	***n*** **(% pos)**	**Mean/median. (µg/kg)**	**Range (µg/kg)**	**Reference**
Bostwana	Fumonisins	33(85)	247/-	20-1270	Siame et al., 1998
	FB1	30(18)	380/-	9-1146	Mokgatlhe et al., 2011
DRC	Aflatoxins	12(-)	63/-	0-393	Probst et al., 2014
	AFB1	13(31)	0.86/-	0.04-120.09	Manjula et al., 2009
Lesotho	FB1	40(-)	-/-	7-936	Mohale et al., 2013
	AFB1	40(-)	-/-	0-3500	Mohale et al., 2013
Malawi	Aflatoxins	9 (100)	12/-	5-20	Probst et al., 2014
	Fumonisins	9 (-)	2000/-	1000-9000	Probst et al., 2014
	Aflatoxins	90 (100)	8.3/-	0.7-140	Mwalwayo & Thole, 2016
	FB_1 +FB_2	90(-)	900/-	100-7000	Mwalwayo & Thole, 2016
	AFB1	108 (45)	1.71/-	-	Matumba et al., 2009
	Fumonisins	8 (88)	55.6/-	0-135	Doko et al., 1996
Mozambique	AFB1	13(46)	-/70	16-636	Warth et al., 2012
	FB1	13(92)	-/869	159-7615	Warth et al., 2012
	Fumonisins	3(100)	360/-	340-395	Doko et al., 1996
South Africa	AFB1	40(33)	94/-	0-741	Chilaka et al., 2012
	FB1	40(45)	331/-	8-892	Chilaka et al., 2012
	FB1	54(100)	2083/-	56-14990	Shephard et al., 2013
	Fumonisins	261(-)	-/-	0-21800	Ncube et al., 2011
	Aflatoxins	29(21)	-/-	1-149	Mngqawa et al., 2016
	Fumonisins	24(96)	-/-	0-8385	Rheeder et al., 2016
	Fumonisins	201(15)	-/-	>1000	Hove et al., 2016b
Zambia	Aflatoxins	114(100)	5.4/-	0.2-10	Hove et al., 2016b
	Fumonisins	114(100)	73300/-	3700-192000	Hove et al., 2016b
Zimbabwe	FB1	95(95)	242/-	0-1106	Hove et al., 2016b
	FB2	95(31)	120/-	0-334	Hove et al., 2016b
	AFB1	95(1)	11/-	0-11	Hove et al., 2016b
	Aflatoxins	19(-)	9/-	0-123	Probst et al., 2014
	Fumonisins	19(100)	10500/-	3600-15900	Probst et al., 2014
	AFB1	166(22)	-/2.33	0.65-26.6	Murashiki et al., 2017
		166(100)	-/295.15	10.4-432.3	Murashiki et al., 2017
		222(20)	-/2.98	0.577-9.22	Murashiki et al., 2017
		222(100)	-/360.18	13.86-606.6	Murashiki et al., 2017

Further, there has also been evidence of mycotoxin carryover to products and exposure in children in Malawi. Matumba et al. (2014a) reported 89% (n = 9) of traditional Malawian maize-beer samples were contaminated with aflatoxins and all of them (100%) with fumonisins, with median levels of 64 and 2342 µg/kg, respectively, which is significantly above regulation limits worldwide. Seetha et al. (2018) detected AFB1-lys adducts in 67% (n = 230) of blood samples from Malawian individuals, with a mean concentration of 20.5 ± 23.4 pg/mg of albumin, which was attributed to consumption of AFB1 contaminated maize and groundnuts.

Although there is a wide variation in the levels of total aflatoxins in maize samples, the distribution and levels of mycotoxins in maize in the region are influenced by climatic and seasonal variations. Significantly higher exceedance rates and median values for total aflatoxins are associated with sites characterised by higher mean annual temperature and low rainfall (Mukanga et al., 2010; Matumba et al., 2014; Chipinga, 2014; Mwalwayo & Thole, 2016). In Zimbabwe, Hove et al. (2016) reported higher co-occurrence of fumonisins and aflatoxins being attributed to higher rainfall and humidity regions compared to other regions. Mohale et al. (2013) reported seasonal variation in mycotoxin levels of maize samples from Lesotho. Rheeder et al. (2016) reported high fumonisin levels in maize samples, and these fumonisins levels were related to dry and sub-humid microclimates in South Africa. A similar geographical pattern in the occurrence of total aflatoxins and fumonisins in Malawian maize samples was reported in earlier studies (Matumba et al., 2014; Chipinga, 2014; Mwalwayo & Thole, 2016).

2.4 Impacts of aflatoxins and fumonisins

2.4.1 Human and animal health

Occurrence of aflatoxins and fumonisins in food staples and animal feed is an important public health issue (Chhonker et al., 2018; Lizarraga et al., 2011) owing to their detrimental effects on human and animal health (Silva et al., 2018; Hendrickse, 1997; Wild, 2007; Williams et al., 2004; Ali et al., 1998; Sydenham et al., 1990, 1991, 1992; Pittet et al., 1992; Stack & Eppley, 1992; Thiel et al., 1993; Sanchis et al., 1994). In developing countries, the risk of these health effects is aggravated by poorly diversified diets in subsistence rural households (Lizarraga et al., 2011). The chronic health risks of mycotoxins are prevalent in Southern Africa because mycotoxins occur more

frequently under tropical conditions and staple diets in the region are often constituted by mycotoxin susceptible crops (Lizarraga et al., 2011; Shephard, 2004, 2008a).

In humans, mycotoxins have been reported to damage liver and kidney (Majeed et al., 2018; Murphy et al., 2006; Ma et al., 2018; Sun et al., 2015; Zhang et al., 2016). Aflatoxins have been associated with hepatitis in Gujrat and Rajasthan states, India resulting in 106 estimated deaths due to consumption of staple food infected with of *A. flavus* and *fusarium* (Krishnamachari et al., 1975; Bhat & Krishnamachari, 1998); Kenya and Thailand (CAST, 2003; Nyikal et al., 2004; Lewis et al., 2005; Probst et al., 2007; Reddy & Raghavender, 2007; Yard et al., 2013; IARC, 2015; Ngindu et al., 1982), including abdominal pain and diarrhoea (Bhat, et al., 1998). To that effect, multiple aflatoxicosis outbreaks have been reported worldwide, resulting in 500 acute illness and 200 deaths as of 2004 (Center for Disease Control and Prevention [CDCP], 2004; Azziz-Baumgartner et al., 2005). On the other hand, pathogenic effects due to fumonisin ingestion in animals include leukoencephalomalacia (Thiel et al., 1991), pulmonary edema, hepatotoxicity (Riley, 2006), hepatocarcinogenicity and nephrotoxicity (Segvic & Pepeljnjaks, 2001). Therefore, combined infestation of aflatoxins and fumonisins is attributable to various uncongenial mycotoxicoses manifesting as teratogenicity, mutagenicity, hepatotoxicity, birth defects, nephrotoxicity and neurotoxicity (Gao et al., 2017; Takemura et al., 2007; Cole & Cox, 1981).

Human oesophageal cancer has also been ascribed to the pervasiveness of these mycotoxins especially fumonisins as manifested in South Africa and China (Williams, et al., 2011; Kachala, 2010; Voss, et al., 2006; Thiel, et al., 1992) mainly due to the disturbance of sphingolipid metabolism (Okuro et al., 2018; Abnet, et al., 2001; Riley, et al., 2001 ; Voss, et al., 2001) which in turn leads to impairment of cell cycle regulation and cellular differentiation (Wild & Gong, 2010). Malawi is ranked in third position in terms of high prevalence rate of oesophageal cancer (24.2 per 100,000 people) worldwide (Ferlay et al., 2013). High dependency on maize with elevated fumonisin levels (Matumba at. al., 2014) could be an aggravating factor. Oesophageal cancer is the third-most common cancer among Malawian females and the second most important among men (GLOBOCAN, 2008; Mlombe et al., 2009; Wapnick et al., 1972, Msyamboza et al, 2012). Further, fumonisins were associated with neural tube defects

in rural South Africa's Transkei region (Marasas et al., 2004; Shephard, 2008a), whose staple crop is maize (Marasas et al., 2004, IARC, 2015). Fumonisins are also a risk factor for neural tube defects (NTDs) in areas where contaminated maize is a dietary staple (Marasas et al., 2004; Missmer et al., 2006; Voss et al., 2006). Fumonisn B1 (FB1), the most relevant congener of numerous fumonisin analogues, has been associated with liver cancer in China (Ueno et al., 1997; Fan et al., 2013) and abdominal pain and diarrhoea in India (Bhat et al., 1997). However, a causal relationship between fumonisin exposure and human cancer has not been unequivocally established and epidemiological studies suffer from various confounding factors (Braun & Wink, 2018). Correspondingly, FB1 has been classified as possibly carcinogenic to humans (group 2B carcinogen, IARC, 2002).

Studies have also shown that aflatoxins and fumonisins worsen various human health ailments by aggregating oxidative stress, an imbalance between the production of free radicals and the ability of the body to counteract or detoxify their harmful effects through neutralization by antioxidants (Silva et al., 2018). Oxidative stress induced by aflatoxins (Liu and Wang, 2016; Mary et al., 2012; Wang et al., 2017; Abdel-Wahhab and Aly, 2003; Shi et al., 2015) and fumonisins (Abbes et al., 2016; Domijan et al., 2015; Hassan et al., 2015; Mary et al., 2012) may be consequent to reactive oxygen species generation. This in turn lead to damage of DNA (Wang et al., 2016; Zhang et al., 2015; Wang et al., 2017; Kouadio et al, 2005; Galvano et al., 2002), mitochondrial lesions (Liu et al., 2016; Peraica et al., 1999), induced mitochondrial permeability (Liu et al., 2016; Shi et al., 2015). All these impede cell survival and are involved in the regulation of apoptosis (Rumora et al., 2007; Rushing and Selim, 2019) which is a major causative factor in progression of liver cancer (Peraica et al., 1999; Kuniholm et al. 2008) and HBV infection (IARC 2015; Gong, 2012; Eva et al., 2011; Henry et al., 2002) as evidenced in South Africa (Van Rensburg et al., 1985).

Consumption of food contaminated with aflatoxins and fumonisins is also said to promote infant malnutrition, increased morbidity and mortality by negatively impacting immune functions and micronutrient absorption (Katerere et al., 2008). Immune modulation effects of these mycotoxins may intensify health impacts of major diseases troubling Africa such as malaria, kwashiorkor (Gong et al. 2012, 2016; Tchana et al., 2010; Peraica et al., 1999) and HIV/AIDS (Gnonlonfin et al., 2013; Matumba et al.,

2014; IARC, 2015; Misihairabgwi et al., 2017) by modulating cell-mediated immunity (Keenan et al. 2011; Jolly 2014). However, since diseases in developing countries are not fully reported as compared to the developed world, 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 mechanism in resource constrained countries including Malawi. To that end, the burden of disease stemming from long-term aflatoxin and fumonisin exposure (such as hepatocellular carcinoma, impaired growth, immune suppression, oesophageal cancer, etc.) remain extensively undefined (WHO, 2005).

According to WFP (2000), malnutrition is a state in which the physical function of an individual is impaired to the point where adequate bodily functions can no longer be maintained. These functions include growth, pregnancy, lactation, physical work, but also resisting and recovering from disease. In some cases, this condition may be induced by mycotoxins (Castelino et al., 2014, 2015). Mycotoxins have been shown to impair intestines (Shirima et al., 2016; Turner et al., 2012; Dewey & Mayers, 2011; Campbell, Elia, & Lunn 2003; Goto et al. 2009; Humphrey 2009), disrupting absorption of nutrients into the body leading to malnutrition (Etzel et al, 2014). In addition, due to intestine impairment, the toxins can find their way to foetus through the placenta (Wild et al., 1991) and babies through breast milk (Shouman et al., 2012; Magoha et al. 2014b; Adejumo et al. 2013) and weaning foods (Kimanya et al. 2015; Gong et al. 2003, 2004).

Impaired absorption of nutrients may also be attributable to stunting and/or underweight in children (Leroy, Wang, & Jones 2015; Shouman et al., 2012; Shirima et al., 2014; Castelino et al., 2015; Hoffman, Jones & Leroy, 2015; Jiang et al. 2005), impaired glucose-galactose absorption (Shirima et al., 2014), reduced fertility (Eze and Okonofua, 2015; Uriah, Ibeh, and Oluwafemi, 2001) and prevalence of diseases like HIV/AIDS and tuberculosis (Keenan et al. 2011; Williams et. al., 2004; Gong et al., 2004, 2008; Katerere et al., 2008; Khlangwiset et al., 2011; Warth et al., 2012; Kimanya et al., 2015; Jiang et al., 2008). Together with other mycotoxins, aflatoxins are suspected as an exasperating factor in the pathogenesis of kwashiorkor, a frequent condition in African children (Kimanya et al., 2015).

In Malawi, malnutrition remains one of the determining factor of infant and under-five mortality. Chronic malnutrition results in stunting of 47% of Malawian children under the age of five (UNICEF, 2012), while rural children are twice as likely to be malnourished as those in urban areas (UNICEF, 2012). Therefore, with the occurrence of aflatoxins and fumonisins in Malawi's food chains, we should expect that they will aggravate the levels of malnutrition, making children more susceptible to infections which may in turn further lead to weight loss, lowered immunity, mucosal damage, invasion by pathogens, and impaired growth (Castelino et al., 2015; Pitt et al., 2012; Gong et al., 2002; 2004) and development in children.

2.4.2 Agriculture, food security and trade

Growth in agriculture is progressively centered on high value products and access to markets (Swinnen et al., 2013; Gulati et al., 2007). Food safety is an indispensable requirement for participation in such good markets (ReSAKSS, 2015; Van Beuningen & Knorringa, 2009; Ashraf et al., 2009). However, presence of aflatoxins and fumonisins in food chains scotches agricultural trade and food security as they jointly thwart the four pillars of food security (availability, access, utilization, and stability) and agriculture (ReSAKSS, 2015). In this regard, many countries incur high economic losses due to aflatoxin and fumonisin food contamination amongst other mycotoxins (Wu, 2014). For example, USA experience nearly US$500 million annual loss in in connection with these mycotoxins (Vardon et al., 2003) and US$163 million for maize only (Wu, 2006). In Thailand, Indonesia and Philippines, losses in the region of US$1 billion per year have been reported due to a combined market, livestock and poultry mycotoxigenic loses (Lubulwa & Davis, 1994).

In Malawi, aflatoxin and fumonisin contamination has remained a challenge to sustain groundnut export into high value markets with stringent aflatoxin regulations, such as the EU, but also important regional markets such as Tanzania, Kenya and South Africa (which enforce less stringent standards than the EU). According to MAPAC (2013), Malawi used to supply 64.5% of South Africa's groundnut market but that market share drastically declined as a result of aflatoxin contamination. This clearly shows the need of controlling mycotoxins in food chains if smallholder farmers are to access high

paying markets for their commodities. To that end, many countries have established maximum tolerable levels for aflatoxins and fumonisins in foods (Wu & Guclu, 2012). Unfortunately, it is sad to note that even in nations that have respective mycotoxin regulations, many individuals consume their food crops (e.g., maize and groundnuts) that have undergone no regulatory inspection, particularly in nations where subsistence farming is dominant (Wu, 2014).

2.5 Estimating human dietary exposure to aflatoxins and fumonisins.

Exposure assessment is one of the steps in risk assessment which covers (1) hazard identification, (2) hazard characterization, (3) exposure assessment, and (4) risk characterization (Dorne et al., 2011). Exposure assessment entails the qualitative and/or quantitative evaluation of the likely intake of a substance as well as vulnerability from other relevant sources (FAO/WHO, 2008). It mainly estimates the intensity, frequency and duration of the exposure of a population or individuals to a given chemical or group of chemicals (Dellafiora et al., 2018). This helps food safety authorities to develop measures that are friendly to the health of consumers by ensuring that exposure of the population to harmful chemicals is below health-based guidance value (EC., 2007) e.g., acute reference dose or tolerable daily or weekly intake.

Variable levels of hazard/exposure to mycotoxin contamination may be observed and reported from different localities. Such exposure levels may be attributable to the occurrence of toxigenic fungi on crops which varies between geographic regions, climatic conditions and pre- and post-harvest management (De Rijk et al., 2015). The people's dietary exposure also varies between geographic regions due to patchiness in the diets consumed (Brera et al., 2014) and specific individual consumption patterns (Boon et al., 2009; Brera et al., 2014; EFSA, 2014b; WHO/FAO, 2012). Further, industrial and household food preparation processes, such as cleaning or peeling, may furthermore alter the profile and the levels of mycotoxins in consumed food (Cano-Sancho et al., 2013; Meca et al., 2013; Tittlemier et al., 2014; Vidal et al., 2014). Based on estimates of typical maize and groundnuts consumption, contamination levels and body weight, Liu & Wu (2010) estimated aflatoxin exposures in Mozambique (39–180 ng/kg bw per day), South Africa (0–17 ng/kg bw per day) and Zimbabwe (18–43 ng/kg

bw per day). These estimates were much higher than those in Western Europe and North America (0–1 ng/kg bw per day).

When estimating the dietary exposure of humans to mycotoxins, information on prevalence and levels in foods are combined with consumption information (Brera et al., 2014; EFSA, 2014b; Cressey & Reeve, 2013; Dorne et al., 2011). About five common strategies are used when assessing mycotoxins exposure in humans. These are (1) point estimate, (2) observed individual mean, (3) probabilistic approach, (4) dietary exposure using duplicate diet studies and (5) human bio-monitoring studies (Dorne et al., 2011; Sancho, 2013). Each stratagem has advantages and its disadvantages. Depending on the methodology, these strategies may be used to estimate either acute exposure (e.g., during a period of 24 hours or less), or chronic exposure, i.e., over a longer period: from a couple of months up to several years.

2.5.1 Point estimate strategy

In point estimate assessments, a single mycotoxin concentration value is combined with a single input parameter for consumption (Sancho, 2013). The result is one single exposure estimate with a high degree of uncertainty (Nijs et al., 2016; WHO, 2005). The input for concentration often originates from a food monitoring study or a total diet study. The average or maximum consumption level from a food consumption study is used for input for consumption. For multiple exposure from various food items, total exposure is obtained by combining individual exposures from each food item. The Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2011) used this approach to assess acute and chronic exposure of chemicals. In this strategy, conservative estimates of the input variables are applied, resulting in high-end estimation of the exposure. The main benefit of this method is that it is easy to perform and often used for a first conservative risk identification (De Nijs et al., 2016).

2.5.2 Observed individual mean

In this approach, average mycotoxin concentration per food product is combined with the food consumption per day per consumer, averaged over the days available in the survey and, in most cases, divided by the individual's body weight (Boon & Van der Voet, 2015). This results in the average exposure per *kg-bw-per person per day* (De

Nijs et al., 2016). Results from a food monitoring study or a total diet study are used as input for mycotoxin concentration.

For chronic exposure, the mean concentration of mycotoxins is used, because in the long run it is unlikely that a consumer will always consume products with only high or only low levels. With this approach, a distribution of daily average exposures for different individuals within a population or agro-ecological zone can be generated. From this distribution the mean and median chronic exposure and upper percentiles (e.g., 95^{th} or 97.5^{th}) for that population can be estimated. This method is currently used by EFSA to assess the chronic exposure to food contaminants and additives (EFSA, 2012a, c; 2015c). In the case of chronic exposure to mycotoxins it was used for aflatoxins (EFSA, 2007), ochratoxin A (EFSA, 2006), *Alternaria* toxins (EFSA, 2011a), zearalenone (EFSA, 2011c), T2/HT-2 toxins (EFSA, 2011b), ergot alkaloids (EFSA, 2012d), sterigmatocystin (EFSA, 2013b), nivalenol (EFSA, 2013a), DON (EFSA, 2013d), beauvericin/enniatins (EFSA, 2014b) and fumonisins (EFSA, 2014a). Occurrence data from a total diet study were recently used to assess the exposure of consumers in the Netherlands to 48 mycotoxins, including patulin, aflatoxins, ochratoxin A, fumonisins, zearalenone, trichothecenes, ergot alkaloids, *Alternaria* toxins, beauvericin and enniatins (Lopez et al., 2016a; Sprong et al., 2016a, b).

2.5.3 Probabilistic approach

In this approach, both the acute and chronic exposure via food can be estimated. The acute exposure is estimated by combining daily discrete consumption patterns from a food consumption survey with randomly selected levels per food product from a databank with mycotoxin levels in individual samples (De Nijs et al., 2016). It is used to achieve discrete daily exposure estimates that reflect all reasonable combinations of daily consumptions and concentrations in a population. The upper part of the distribution represents consumers with a high intake of the compound (high consumers), which is important for assessing the acute risk. With this approach, population's exposure to mycotoxins is expressed as a range of values (not as a single value), because discrete members of the population may have variable exposure levels according to their age, sex, health, ethnicity, nationality, personal preferences, and region, among other factors (Sancho, 2013).

However, this approach is amenable to high deviations from observed individual mean approach. The resulting long-term exposure distribution are usually less broad than the distribution obtained with the observed individual mean approach, resulting especially in lower estimates of the higher percentiles of exposure and higher estimates of the lower percentiles (De Nijs, 2016). For example, the mean exposure assessed over just two days is more variable than the mean exposure assessed over more (up to hundreds) days that constitute a longer period. Advantage of observed individual mean and probabilistic approaches is that the entire food consumption database, and food concentration data, is considered and it results in more realistic exposure estimates of a given population (in a country of geographic region). This supports the derivation of a health-based guidance value and identifies segments of a population (such as children) with a higher risk. Another advantage is that also the uncertainty in the exposure estimations, caused by a limited size of the database, can be quantified (EFSA, 2012b).

2.5.4 Dietary exposure using duplicate diet studies

In duplicate diet studies, a replicate of the portion consumed by everyone is collected and analysed as consumed (Sancho, 2013), resulting in an actual exposure level per day for that individual. The food consumption data collected in accompanying food diaries can be used to assess the probable sources of exposure. When duplicate portions are collected on only one day per individual, these data can only be used to assess acute exposure. On the other hand, if duplicate portions are collected on multiple days, chronic exposure can be calculated by either averaging the exposure over the collection days per individual as done in *Observed Individual Mean Approach* or by removing the within individual variation using statistical models as done in *Probabilistic approach*.

Duplicate diets have been collected and analysed for estimating the exposure to various mycotoxins, like aflatoxin B1, aflatoxin M1, ochratoxin A, trichothecenes, fumonisins and T-2/HT-2 toxins (Jekel & Van Egmond, 2014; Bakker et al., 2009; Gilbert et al., 2001; Sizoo & Van Egmond, 2005). The effects of food processing and preparation are included in total diet studies and in duplicate diet studies. This is of particular importance for less stable compounds, as otherwise exposure could be largely overestimated. An obvious limitation is the limited number of samples and the high costs and subject burden in duplicate diets studies.

2.5.5 Human biomonitoring studies

Biomarkers may be used to assess exposure to a compound through quantification of internal dose of that commodity in an individual. The measurement of biomarkers of exposure is the only available strategy that integrates exposure from all sources, (e.g., food, inhalation, skin contact) and reflects the internal biological active fraction (Choi et al., 2015). A human biomonitoring study may also give insight in the bioavailability of mycotoxins from unexpected or unknown sources and the presence of modified forms in food (e.g., glucosides, sulphates), which may become available for absorption after conversion to the parent compound in the gastrointestinal tract (EFSA, 2014a; Solfrizzo et al., 2014). Results of epidemiological studies on mycotoxins using biomonitoring have been reported in the past two decades (Duarte et al., 2012; Ediage et al., 2013; Gilbert et al., 2001; Pena et al., 2006; Rubert et al., 2011; Turner et al., 2010; Van der Westhuizen et al., 2011). Biomarkers have previously been used to measure acute exposure to aflatoxins (AFB1) (Groopman et al., 1992a, b) and fumonisins (Wang et al., 1991, 1992; Riley et al., 1993; Morgan et al., 1997; Van der Weshuizen et al., 2001; Kim et al., 2006; Tran et al., 2006; Cai et al., 2007). However, exposure estimates using biomarkers lack accurate validation hence incur high uncertainties (Van der Westhuizen et al., 1999, 2008, 2010; Abnet et al., 2001; Qiu & Liu, 2001; Solfrizzo et al., 2004; Nikiema et al., 2008; Silva et al., 2009).

All in all, it is important to note that exposure assessments to mycotoxins from food are complicated since (1) they are often heterogeneously distributed over the samples; (2) they differ between geographic regions and production years; (3) they can be affected by (food) processing and preparation; and (4) they can be present in modified forms. Therefore, a combination of the different strategies is pivotal for assessing mycotoxins, including the identification of unknown sources or modified forms.

2.6 Estimation of Average Daily Dose (ADD)

To quantify exposure, it is common to estimate an average daily dose (ADD) or intake. This term is usually expressed as the mass of substance in contact with the body per unit body weight (*bw*) per unit time, such as in mg/kg bw/day, for ingestion exposures (IARC 2015), or as the mass of substance per cubic meter of ambient air (e.g., mg/m^3) for inhalation exposures. If the agent being studied is a carcinogen, a lifetime average

daily dose (LADD) is calculated, with an averaging time equal to the expected lifetime of the individual. For example, the United States Environmental Protection Agency (USEPA) assumes an averaging time for cancer risk assessment of 70 years, although the average lifespan in the USA is now longer than this.

To calculate ADD or LADD, the exposure quantity, *E*, must first be estimated. It is the concentration of an agent as a function of time *t*, over an exposure duration. The total exposure quantity (equation 1) during that given time is expressed as the integral (sum) of concentrations *C* over the exposure duration:

$$E = \int C(t)\,dt \tag{1}$$

Alternatively, *E* can be determined using the arithmetic average of the concentration, C_{ave}, over the exposure duration, *ED*, to estimate the total exposure:

$$E = C_{ave} \text{ x } ED \tag{2}$$

The intake rate, *IR*, is the amount of the agent passing through the initial intake barrier (mouth, nose, skin) into the body over a period of time. This can be measured in mg/day or L/day, for example. Then, the ADD is calculated as:

$$ADD = \frac{E \text{ x } IR}{AT \text{ x } bw} \tag{3}$$

where *bw* is body weight and *AT* is the averaging time in days.
LADD is calculated similarly, however, the exposure duration (used to calculate *E*) is the number of years that an individual is exposed to a carcinogen, and the averaging time *AT* is the expected lifetime in years.

2.7 Relationship between population cancer potency and hepatocellular carcinoma (HCC-Virus)

Fumonisins and aflatoxins, especially AFB1, are potent liver carcinogen, causing hepatocellular carcinoma (HCC) (Chhonker, et al., 2018; Wu et al, 2013; Long et al., 2014) in humans. HCC is the most common type of primary liver cancer (Wu, 2015) in relation to aflatoxin and fumonisin contamination (McGlynn & London, 2005; Kuniholm et al., 2008). HCC occurs most often in people with chronic liver diseases, such as cirrhosis caused by hepatitis B or hepatitis C infection. Concomitant exposure to aflatoxin and the hepatitis B virus (HBV) is common in developing countries and

greatly increases HCC risk. Individuals with both (aflatoxins and HBV^+) exposures often have greater risk of developing HCC than those exposed to aflatoxin alone (Chhonker et al., 2018; Bowers et al., 1993; Groopman et al., 2008; Liu et al., 2012; Qian et al., 1994; Yeh et al., 1989). Since the interactions of aflatoxins, fumonisins and hepatitis B virus (HBV) is a high risk for HCC, the country's population cancer potency (as regards to aflatoxin and fumonisin exposures) is assessed by referring to the prevalence of hepatitis B virus (HBV^+) (Wu, 2013). It is calculated by using the following formula (Sancho et al., 2013; Adetunji, Atanda, and Ezekiel, 2017):

$$\text{Population cancer potency} = 0.3p + 0.01(1-p) \quad (4)$$

where p is the proportion of individuals with hepatitis B virus (HBV^+) carriers in each population.

According to WHO (2013), different countries have different prevalence of HBV^+. Several estimates of cancer potency for aflatoxin B1 have been derived from human epidemiological data (Bowers et al., 1993; Hoseyni, 1992; Qian et al., 1994; Wu-Williams et al., 1992; Yeh et al., 1989). Estimates of cancer potency have also been derived from studies in test animals (Henry et al., 1998). However, it was resolved that there is currently insufficient understanding of the differences in metabolism between humans and animals to use animal cancer potencies in human risk assessment (Henry et al., 1998). The Joint Expert Committee on Food Additives (JECFA), a scientific advisory body of FAO and WHO, reviewed cancer potency estimates and selected separate central tendency estimated potencies and ranges for HBV^+ (Henry et al., 1998). The potencies were expressed in terms of an expected increase in the incidence (per 100,000 population) of primary liver cancer per ng aflatoxin B1/kg body weight/day. Potency values of 0.3 cancers/year/100,000 (uncertainty range 0.05-0.5) was derived for HBV^+ individuals.

Using the *Observed Individual Mean or Probabilistic approach* strategies for calculating human exposure to aflatoxins and the prevalence data for HBV^+, different African countries have determined their cancer risk levels due to aflatoxins. These estimates of mycotoxin exposure and cancer potency have been used in subsequent aflatoxin risk assessment (Lee et al., 2009; Liu & Wu, 2010; Shephard, 2008a; Sugita-

Konishi et al., 2010). Table 2.2 shows prevalence of HBV^+ as a factor of HCC cancer risk in selected African countries. It is noteworthy that increased prevalence of HBV^+ and aflatoxin exposures also increases the country's HCC cases per 100, 000 people.

Table 2.2: Prevalence of HBV+ as a factor of HCC cancer risk in selected African countries*

	Prevalence of HBV^+ (%)	**Aflatoxin Exposure (ng/Kg bw/day)**	**Estimated HCC (per 100,000 people) with reference to HBV^+**	**References**
Kenya	11-15	3.5-133	1.05-39.9	Liu & Wu, 2010; Shephard, 2008
Mozambique	4.5-10.6	39-180	11.7-54	Liu & Wu, 2010; Hall & Wild, 1994
South Africa	3.3-10.4	0-17	0-10	Liu & Wu, 2010; Shepherd, 2003
Tanzania	5-9	0.02-50	0.06-15	Liu & Wu, 2010; Manjula et al., 2009
Zimbabwe	10-15	17.5-42.5	5.25-12.8	Liu & Wu, 2010; WHO, 1998
Nigeria	13.2	139-229	41.7-68.1	Liu & Wu, 2010; Bandyopadhay et al., 2007
Ethiopia	6-7	1.4-36	0.42-10.8	Liu & Wu, 2010; Aalew et al., 2006
Malawi	8	**	**	WHO, 2013; Candotti, et al., 2001

*Assuming 60 kg body weight per individual. Aflatoxin exposure was estimated by multiplying aflatoxin concentrations in staple foods by concentration rates in foods (WHO, 2006). Aflatoxin exposure estimates and consequent HCC cases for selected countries in the same WHO region were also calculated. ** Missing data.

Using the same approach, Matumba et al., (2009) determined human aflatoxin exposures and cancer potency through consumption of maize in Mpingu EPA in Lilongwe, Malawi. Assuming 60 kg body weight adult, the mean daily AFB1 exposures from sun-dried flour made from de-hulled and soaked maize, and whole maize flour

were 3.80 and 21.85 ng/kg bw/day respectively, corresponding to cancer potencies of 0.313 ± 0.05 (SE) and 1.8028 ±0.30 (SE) cancer cases per 100 000 people per year in Mpingu area. However, nationwide data for aflatoxin and fumonisin exposure and their cancer potencies in Malawi is still limited.

2.8 Analysis of aflatoxins and fumonisins levels in maize

Different approaches may be used to detect aflatoxins and fumonisins in crops like maize and groundnuts (Krska et al., 2008). The methods vary in purpose and complexity. Mycotoxin detection methods can be grouped into two categories: (1) rapid presumptive tests to identify corn lots that may contain the toxin (2) quantitative methods to determine aflatoxin and fumonisin levels (Siriacha, 1989; Shotwell, 1983).

2.8.1 Rapid presumptive test for aflatoxins and fumonisins

Amongst many methods, the Bright Greenish-Yellow Fluorescent (BGYF) or the "black light" presumptive test for aflatoxins in corn has been extensively used. The basis of the presumptive test for aflatoxin in maize and groundnuts is the characteristic fluorescence under long-wave ultraviolet light (365 µm). The BGYF results from the action of some microorganisms on kojic acid produced by many fungal species including *A. flavus* and *A. parasiticus* (Siriacha, 1989; Marsh et al., 1969). Thus, the BGYF test indicates the growth of the fungi that may have produced aflatoxins.

However, kojic acid (which is detected by the fluorescence) may also be produced by several *Aspegillus species* (e.g., *A. tamari, A. oryzae* and its variant *effusus, A. candidus*, and members of the wenti group) and four groups of *Penicililum* (Moreau, 1979). Therefore, this method may not indicate the exact level of aflatoxins in a substrate when the substrate produces the kojic acid which is not a direct result of aflatoxiginic fungi (Dickens and Whitaker, 1981). Grain infected with *A. flavus* strains that produce kojic acid but do not produce aflatoxin exhibit BGYF and thus are aflatoxin “false positives”. Hence, it has been concluded that the relationship between BGYF and aflatoxin depends on the specific fungal strain on the BGYF grains (Calvert et al., 1983). Several studies have been conducted to assess the relationship between BGYF grains and aflatoxin contamination (Dickens & Whitaker, 1981). The BGYF test was applied to assess 180 maize samples collected from different markets within 12 districts of Malawi

and about 49% of all tested samples had aflatoxin concentrations ranging from 1 to 382 µg/kg (Matumba et al., 2013).

2.8.2 Quantitative methods for determining aflatoxin and fumonisin levels in maize.

Analytical methods for detecting fumonisins and aflatoxins in food crops and their products include thin-layer chromatography (TLC) (Rottinghaus et al., 1992), gas chromatography (Sydenham et al., 1990a), gas chromatography/mass spectroscopy (Plattner et al., 1990), and high-performance liquid chromatography (HPLC) (Shephard et al., 1990). Although thin-layer chromatography and HPLC has been adopted by AOAC International as an official method for aflatoxin and fumonisin determination (Ueno et al., 1993; Sydenham et al., 1996), there are limitations of the need for extensive sample clean-up, time, cost and the requirement of trained staff for analysis.

The acceptance of immunochemical methods is based on their simplicity, which allows use in rapid screening allowing rapid analysis for quality control both in the field and industry (Pestka et al., 1995). Enzyme Linked Immunosorbent Assays (ELISAs) methods for fumonisins have been developed using polyclonal (PAb) and monoclonal antibodies (MAb). Some were prepared against FB1–protein conjugates (Azcona-Olivera et al., 1992a, 1992b; Usleber et al., 1994). Iijima et al. (1996) and developed an extremely sensitive ELISA for fumonisins based on MAbs antibodies against FB1 with a detection limit of 80 ng/g. The sensitivity of this method was 10–60 fold higher than the ELISA previously reported with anti-FB1 MAbs (Azcona-Olivera et al., 1992a, 1992b; Elissalde et al., 1995). Nowadays, more advanced ELISA techniques (e.g., Reveal Q^+ test kits) whose accuracy and precision are like TLC and HPLC (Rodríguez, 2012) have been developed. They are sensitive, quick, easy to use and cheaper than the HPLC method (Chauhan, 2017).

Reveal Q^+ kits are single-step lateral flow immunochromatographic assays, based on a competitive immunoassay format. Lateral flow strips coated with antibodies interact with antigen (mycotoxin) molecules in the sample extract. The Reveal Q^+ assay for aflatoxin is quantitative for total aflatoxins with a linear range of detection of 2–150 ppb. On the other hand, the Reveal Q^+ assay for fumonisin is semi-quantitative for quantification of B1 and B2. The linear range of detection for B1 and B2 is 1–7 mg/kg.

It is important to note that each sample was treated independently. They require minimal training and use ethanol extraction to reduce costs. Several mycotoxin studies have been done using Reveal Q^+ test kits and reported satisfactory results of mycotoxin concentrations (Suleiman et al., 2017; Chauhan et al., 2016).

CHAPTER 3: MATERIALS AND METHODS

3.1 Study area

Maize samples of the 2015/16 growing season were collected randomly from Extension Planning Areas (EPAs) in Malawi's four agro-ecological zones to provide a wide geographical coverage (see Figure 3.1a and Appendex 1). The sample collection was done from September to December of 2016. A total of one thousand two hundred and ninety four (1294) samples of maize for human consumption were collected from rural farmers' households and rural markets from 2015/16 harvest. In Malawi, the agro-ecological zones are categorised, mainly according to elevation, as Lower Shire valley (altitude below 200 m.a.s.l), Lakeshore, middle and upper Shire valley (>200 to 760 m.a.s.l), mid-elevation (>760 to 1300 m.a.s.l) and highlands (>1300 m.a.s.l) (Matchaya et al., 2014) (Figure 3.1a). The spatial variation of climate variables (temperature, humidity and rainfall) depends on elevation (Ravaderkar et al., 2012). Therefore, the agro-ecological zones also represent spatial climatic zonation of the country (Ngongondo et al., 2011; Matumba et al., 2014).

All the agro-ecological zones have a tropical wet and dry "savanna" climate (MetMalawi, 2006), characterised by a distinct rainy season between November and April (Figure 3.2). Annual rainfall varies from 700 mm in the low-lying areas of the Lower Shire valley to 2,500 mm in the highlands (BGS, 2004). The Lower Shire valley is characterised by higher average monthly temperatures (21 – 30 ^{o}C) and lower monthly rainfall than the other agro-ecological zones (Figure 3.1c and 3.1d). Monthly average temperatures are around 16–23 ^{o}C in the highlands, 17-25 ^{o}C in the mid-elevation and 21–28 ^{o}C in the Lakeshore, middle and upper Shire valley agro-ecological zones (Figure 3.2). As reference evapotranspiration and relative humidity levels are tied to temperature and precipitation, they show similar spatial trends.

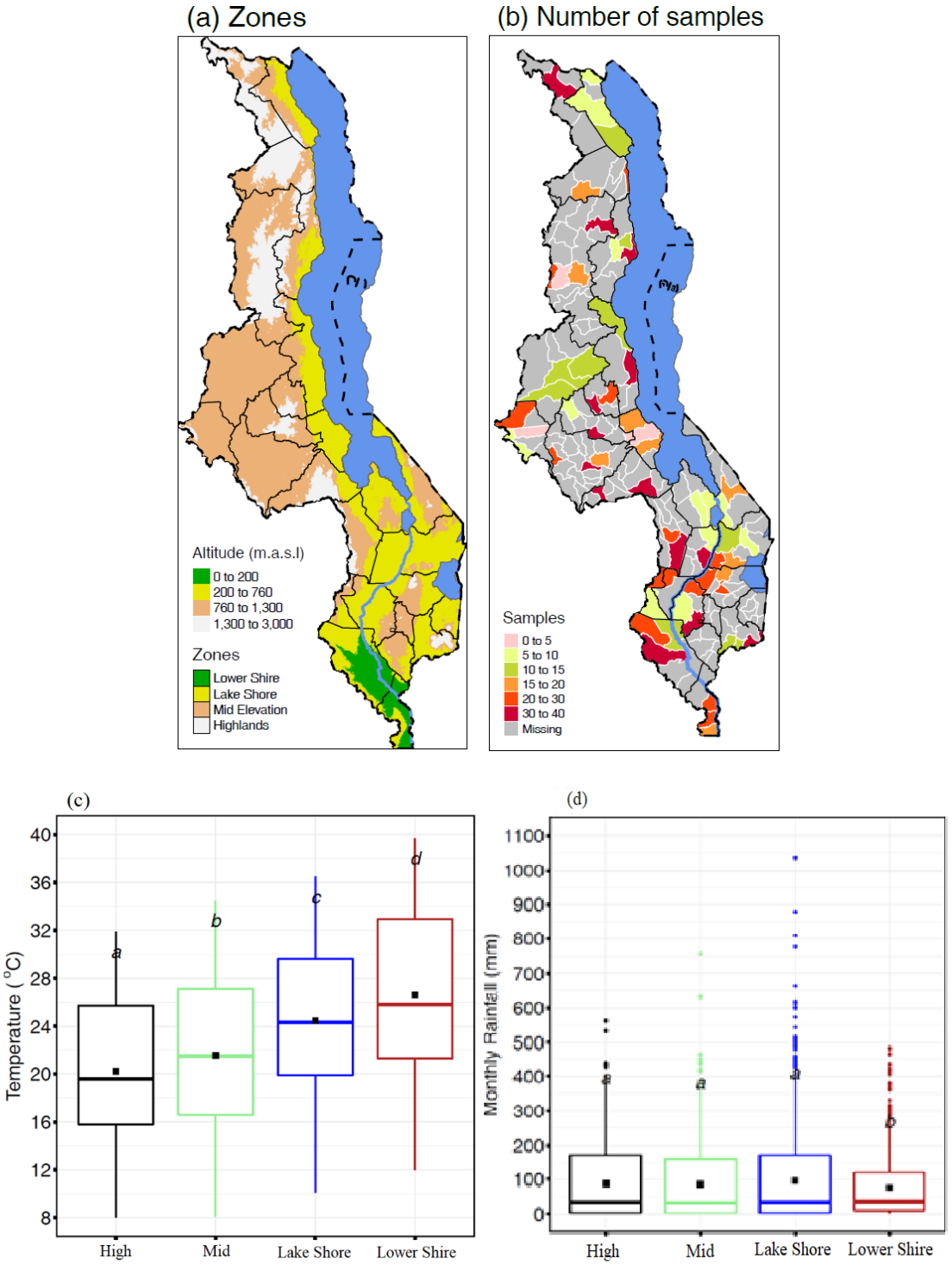

Figure 3.1: (*a*) Map showing agro-ecological zones: Lower Shire valley (altitude below 200 m.a.s.l), Lakeshore, middle an upper Shire (>200 to 760 m.a.s.l), mid-elevation (>760 to 1300 m.a.s.l) and highlands (>1300 m.a.s.l). (*b*) distribution of maize samples in each district depending on agro-ecological zones (elevation). (*c*) monthly average temperatures per agricultural zone (*d*) monthly average rainfall per agricultural zone.

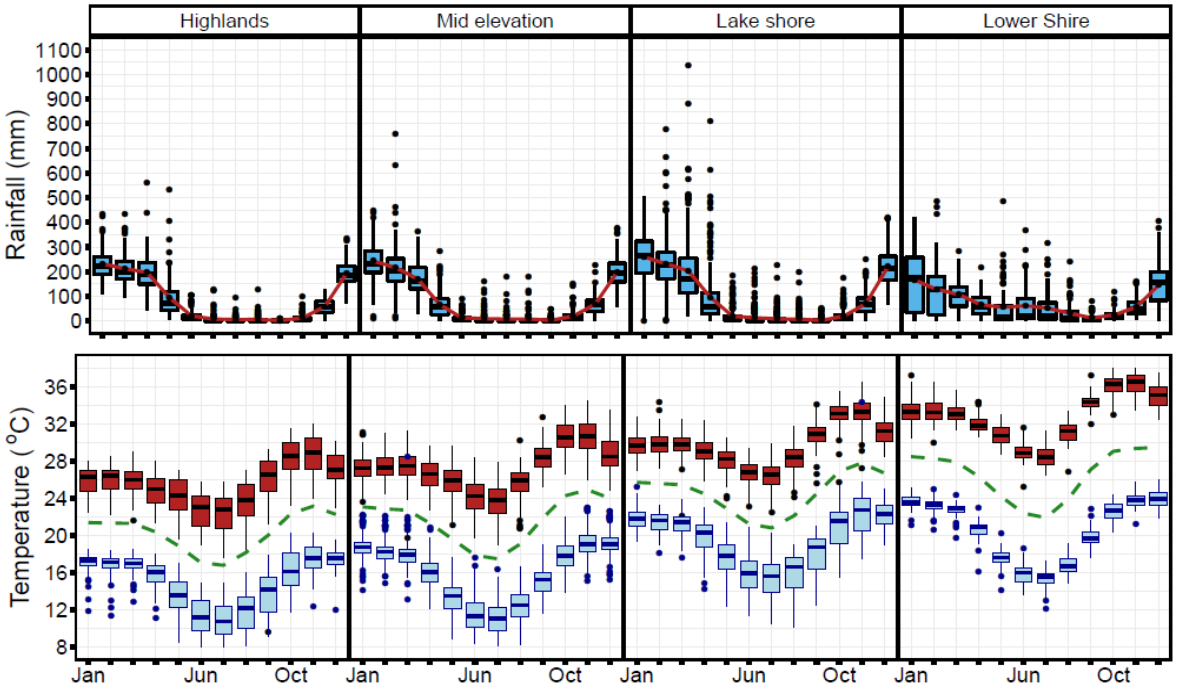

Figure 3.2: Barplots showing monthly distribution of rainfall (upper panbel) and maximum, minimum and mean temperature (lower panel) in each agro-ecological zones (elevation). The red (upper panel) and green (lower panel) lines represent average monthly rainfall and temperature, respectivively. All data based on annual calculations.

3.2 Sample collection

The maize grains were sampled using a cylindrical bag sampler (approximately 1 meter long with 40 mm external diameter). The bag sampler was pushed into a bag twice through both tips of the farmer sown-end (to minimise damaging the bag) and diagonally into the bag placed horizontally to the ground. In order to improve sample homogeneity, the bag sampler was pushed into the bag with the intake aperture facing down and then turned 180^{o} and agitated to fill the bag sampler and then withdrawn from the bag (AHDB, 2018). In the cases where the farmers had stored their maize grains in baskets for their household consumption, they were kindly requested to share approximately 1 kg of maize grain. The samples were packed in thin nylon bags which were in turn put in woven polypropylene sacks (50 kg) and then transported to and stacked in a storeroom at Chitedze Research Station, Lilongwe-Malawi. Farmers' demographic information and their knowledge level on aflatoxins and fumonisins was properly documented using a questionnaire.

3.3 Determination of aflatoxins and fumonisins in maize samples

Levels of total aflatoxins and estimated total fumonisins (in maize samples) were determined according to the manufacturer's directions provided with Reveal Q^+ test kits (Neogen ® Corporation, Lansing, MI, USA). Briefly, the samples (1 kg) were properly mixed, and 500 g of which was ground using a blender (Vitamix 300 professional blender, US), until 75% of its particles could pass through a size 20 mesh sieve. The ground samples were stored in plastic bags in a cool, dry place until time for analysis. For analysis, 20 g of each finely ground sample material was weighed into a 250 ml round-based flask using a top-loading pan analytical balance (Mettler Toledo analytical balance MS104TS/00, Germany). 100 ml of 65% ethanol and 35% double distilled water (v/v) was added to the flask. Aflatoxins and fumonisins were extracted by shaking the mixture using a rotary shaker (GFL 3017; GFL; Burgwedel, Germany) for 3 minutes. The mixture was then filtered through Whatman No. 1 filter paper and both aflatoxin and fumonisin assays were performed on the 65% ethanol extract. For total aflatoxins analysis, 1-part sample extract filtrate was mixed with 5 parts aflatoxin diluents in a sample cup while for total fumonisin analysis, 1-part sample extract filtrate was mixed with 2 parts fumonisin diluent in a sample cup where a respective test strip was inserted with the sample end down.

Reveal Q^+ kits were used for quantitation of total aflatoxins and fumonisins. In order to maintain accuracy, the AccuScan Gold Reader was calibrated by using standard samples provided by the manufacturer (Neogen ® Corporation, Lansing, MI, USA). The developed strip was removed from the sample cup and inserted into a Reveal AccuScan Gold Reader System (AccuScan Gold Reader 9595, Neogen ® Corporation, Lansing, MI, USA) and the reader displayed the aflatoxin or fumonisin content of the sample.

3.4 Estimation of daily dietary intake of aflatoxins and fumonisins.

In this study, the daily mycotoxin exposure was calculated according to the Joint Expert Committee on Food Additives (JECFA), using a per capita consumption of maize. The general equation for dietary exposure (FAO/WHO, 2005, 2006; Shephard, 2008) is presented as (equation 3.1):

$$\text{Dietary exposure} = \sum \frac{\text{consumption x concentration}}{\text{body weight}} \qquad (3.1)$$

where dietary exposure is measured in ng/person/day; consumption = amount of food consumed by individual (kg/day/person); concentration = amount of the chemical of interest in food consumed by an individual (ng/kg); body weight = body weight of individual (kg).

Maize is a main staple food for the Malawian population across the four agro-ecologies, mostly taken as *nsima*, a thick porridge, which is prepared from fine maize flour. The flour may be made from either dehulled or non-dehulled maize. However, because of lack of data on the proportions of which type of flour is used to prepared *nsima*, a conservative exposure was estimated using flour from non-dehulled maize, with an average per capita consumption of 382 g/day (Ecker and Qaim, 2011). This method of estimating dietary mycotoxin exposure has previously been used in aflatoxin risk assessment (Matumba, 2009; Lee et al., 2009; Liu and Wu, 2010; Shephard, 2008; Sugita-Konishi et al., 2010). For an adult person, JECFA uses an average body weight (*bw*) of 60 kg for its estimates (WHO, 1998). For fumonisin, the JECFA established a provisional maximum tolerable daily intake (PMTDI) for fumonisins of 2 μg/kg bw/day based on a no observed adverse effect level (NOAEL) for nephrotoxicity in male rats and an extrapolation factor of 100 (FAO, 2004; Marasas et al., 2004; Bulder et al., 2012; Burger et al., 2010; IARC, 2015). However, no PMTDI for aflatoxins has been developed because of their high toxicity. As such their concentrations in food need to be as low as possible (IARC, 2015).

3.5 Data analysis

All statistical analyses were performed using R: A language and environment for statistical computing version 3.6.3 (R Core Team, 2020). An analysis of variance was performed on levels of fumonisins and aflatoxins by pooling data at Agricultural Extension and Planning Areas (EPA) level in each agro-ecological zone using stat_compare_means (ggpubr package). Thematic maps showing spatial variation of levels of fumonisins and aflatoxins at EPA level were generated using tmap package and all boxplots were implemented in ggplot2 package.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Occurrence of aflatoxins and fumonisins in maize

Levels of total aflatoxins show a wide variation across the four agro-ecological zones as summarised in Figure 4.1(a) and Appendix 1. Total aflatoxins were detected in 78.8% of all maize samples, with a distribution of occurrences across the agro-ecological zones of 76.9%, 78.9%, 83.3% and 76.4% for the mid-elevation, Lower Shire, highlands, lakeshore, upper and middle Shire agro-ecological zones, respectively. On average, maize samples collected from the Lower Shire agro-ecological zone had relatively higher levels of aflatoxins ($p < 0.05$) than maize samples from the other agro-ecological zones (Figure 4.1a). In Lower Shire River valley agro-ecological zone, aflatoxin levels ranged from 0.8 to 1122 μg/kg (Nsanje District), with an average of 99.98 ±23.05 μg/kg (mean ± SEM). Out of the positive samples, 75.6%, 67.8% and 51.1% of the samples exceeded the EU regulatory limit (4 μg/kg; EC, 2010), the median limit in food currently established in legislations worldwide (10 μg/kg FAO, 2004) and the US Food and Drug Administration's (USFDA, 2001) limit for human food (20 μg/kg, total aflatoxin), respectively. In addition, as a proportion of all analysed samples, the Lower Shire River valley agro-ecological zone had a higher percentage of samples ($p < 0.05$) exceeding 4 and 20 μg/kg than the other agro-ecological zones (Figure 4.1a; Figure 4.2d & Figure 4.2e).

For the highlands, total aflatoxin levels ranged from 2 to 1072 μg/kg (Mzuzu), with an average of 11.89 ± 3.78 μg/kg and 38.8%, 9.0% and 6.3% of the positive samples exceeding 4, 10 and 20 μg/kg regulatory limits, respectively. In the lakeshore, upper and middle Shire agro-ecological zone, total aflatoxins ranged from 2 to 358 μg/kg (in Salima), an average of 13.32 ± 1.67 μg/kg), with 36.5%, 19.8% and 15.0% of the positive samples exceeding 4, 10 and 20 μg/kg regulatory limits, respectively. Whereas, for the mid-elevation, values were in the range of 0.9 to 540 μg/kg (Rumphi, average: 8.47 ± 2.21 μg/kg), with 24.6%, 12.5% and 6.34% of the positive samples exceeding 4, 10 and 20 μg/kg regulatory limits, respectively.

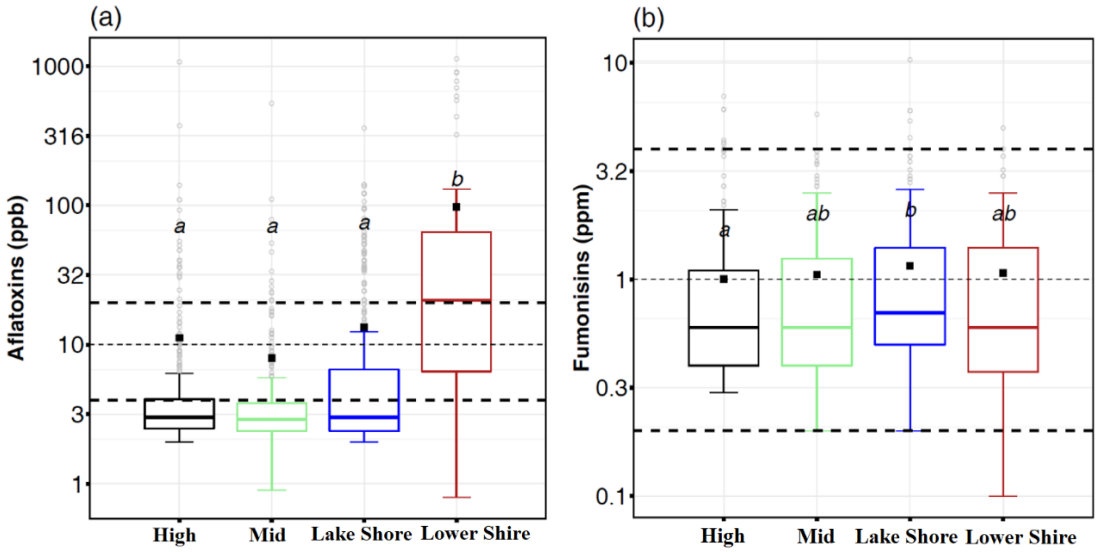

Figure 4.1: Distribution of (*a*) total aflatoxins and (*b*) estimated total fumonisins in maize samples grouped by agro-ecological zones. The horizontal dashed lines indicate guideline values (Aflatoxins: 4, 10 and 20 µg/kg; Fumonisins: 0.2, 1 and 4 mg/kg). Agro-ecological zones with the same letter have insignificant differences (p = 0.05). The small black square in the boxplots indicates a mean for each ecological zone. The dots, error bars and upper and lower ends of the box represent outliers, spread, and first and third quartiles, respectively.

In contrast to total aflatoxins, levels of total fumonisins in maize did not show a broad variation across the four agro-ecological zones (Figure 4.1a; Figure 4.1b). Fumonisins were detected in 48.6%, 47.8%, 38.6% and 44.7% of the samples collected from the highlands, lakeshore, upper and middle Shire River valley, Lower Shire River valley and mid-elevation agro-ecological zones, respectively. Levels of fumonsins ranged from 0.3 to 7.0 mg/kg (average 1.11 ± 0.08 mg/kg), 0.2 to 10.3 mg/kg (average 1.16 ± 0.08 mg/kg), 0.1 to 5.0 mg/kg (average 1.05 ± 0.16 mg/kg) and 0.2 to 5.8 mg/kg (average 1.08 ± 0.08 mg/kg), in the positive samples from the highlands, lakeshore, upper and middle Shire River valley, Lower Shire River valley and mid-elevation agro-ecological zones, respectively (Figure 4.1b). Currently, Malawi does not have a regulatory framework for fumonisins, however, Codex Alimentarius has set the maximum limit for fumonisins in food at 4 mg/kg (Codex, 2015). In general, almost all positive samples from across all agro-ecological zone exceeded the EU regulatory limit (EC 2007, 2010a; Codex, 2015) for maize products for infants (0.2 mg/kg) but fell short of the regulatory limit for adults (4 mg/kg) (Figure 4.1b; Figure 1.2a). The proportion of all analysed samples exceeding 0.2 and 1 ppm fumonisins did not vary significantly across the agro-ecological zones (Figure 4.1b; Figure 4.2b).

Aflatoxins and fumonisins were found to frequently co-occur in maize samples (Figure 4.2). In general, both aflatoxins and fumonisins were detected together in 38.64% of all maize samples, with co-occurrence of 41.26%, 36.28%, 37.67% and 37.85% in the highlands, Lower Shire, Lakeshore, middle and upper Shire and mid-elevation agro-ecological zones. However, the Lower Shire River valley agro-ecological zone had a higher ($p < 0.05$) co-occurrence of aflatoxins and fumonisins at levels exceeding aflatoxin regulatory limits of 4 and 10 µg/kg and fumonisin limit in maize products of 0.2 mg/kg (Figure 4.2c and Figure 4.2f).

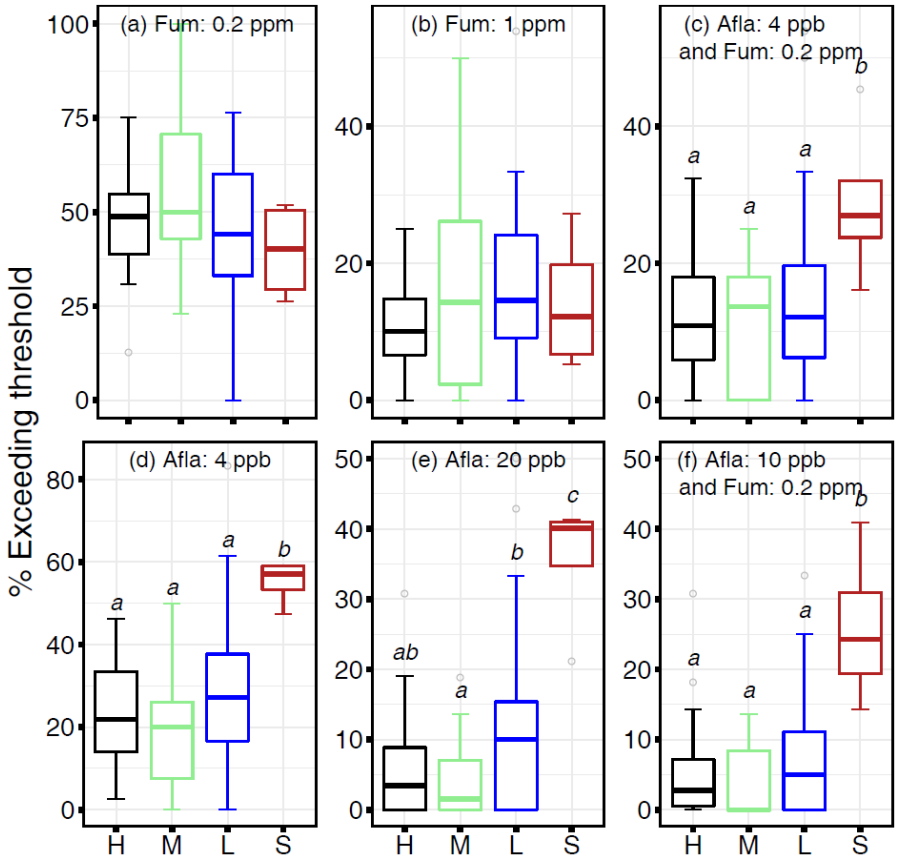

Figure 4.2: Summary of exceedance of aflatoxins and fumonisins limits in foods, grouped by agro-ecological zones as a proportion of all analysed samples: (*a*) 0.2 ppm for fumonisins (the EU regulatory limit), (*b*) 1.0 ppm for fumonisins, (*c*) Co-occurrence of fumonisins and aflatoxins above 0.2 ppm and 4 ppb, respectively, (*d*)) 4 ppb for aflatoxins (EU regulatory limit), (*e*) 20 ppb for aflatoxins (USFDA, 2001), and (*f*) Co-occurrence of fumonisins and aflatoxins above 0.2 ppm and 10 ppb, respectively. Agro-ecological zones with different letters have significant differences ($p = 0.05$). The small square in the boxplots indicates a mean for each ecological zone. The dots, error bars and upper and lower ends of the box represent outliers, spread, and first and third quartiles, respectively. Agro-ecological zones: Highlands (H); Mid-elevation (M); Lakeshore, upper and middle Shire valley (L); Lower Shire valley (S).

4.2 Determinants of geographical variations in aflatoxin and fumonisin levels in maize

The distribution of levels of total aflatoxins and fumonisins, as aggregated at agro-ecological zone (Figure 4.3a), may be skewed by a few heavily contaminated samples (Whitaker and Johansson, 2005; Whitaker, 2006). Figure 4.3b shows the spatial variation of the median values of levels of total aflatoxins in each sampled Extension Planning Area (EPA). Most of the EPAs with median values of levels of total aflatoxins exceeding 4 µg/kg were in the Lower Shire and the Lakeshore agro-ecological zones (Figure 4.3b). In these EPAs, majority of samples had levels of total aflatoxins exceeding 4 µg/kg and a relatively higher proportion of samples exceeding 10 and 20 µg/kg than most of the EPAs in the other agro-ecological zones (Figure 4.4). However, levels of total fumonisins in maize did not show the same zonation as total aflatoxins (Figure 4.3c). Exceedances and median values for total fumonisins were not significantly different across the agro-ecological zones used in this study (Figure 4.4).

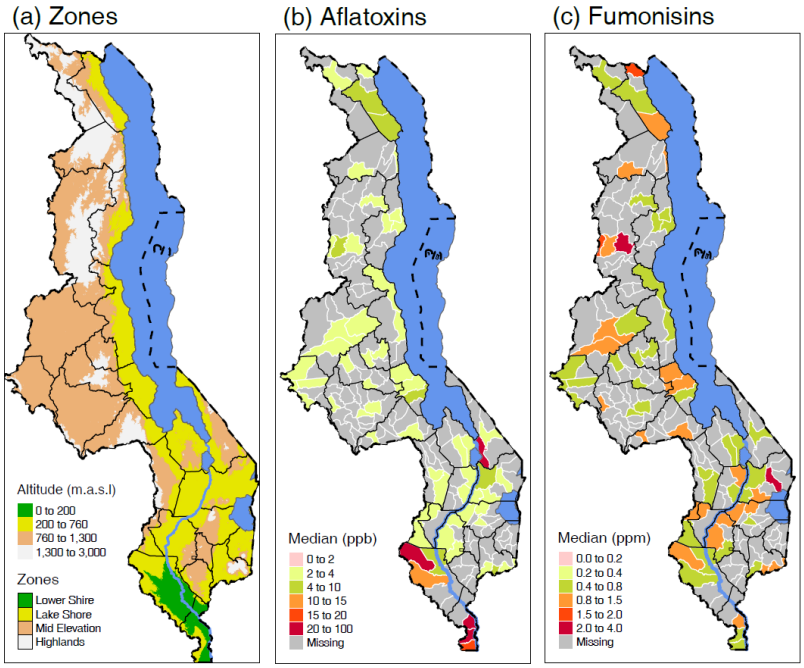

Figure 4.3: Spatial distribution of (*a*) agro-ecological zones, (*b*) median levels of total aflatoxins in maize and (*c*) median levels of total fumonisins in maize at EPA level (*Appendix 2*).

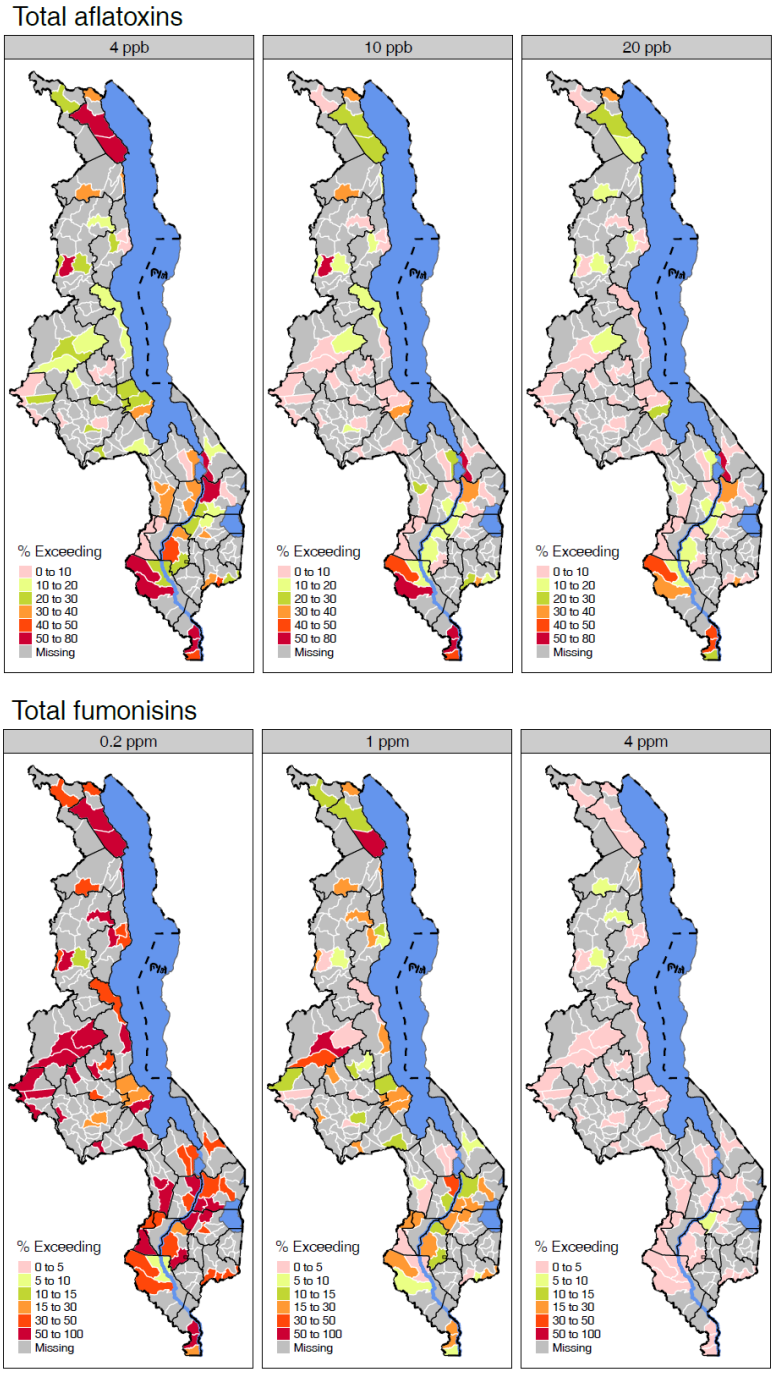

Figure 4.4: Spatial distribution of the proportion of all samples exceeding selected thresholds of total aflatoxins (upper panel) and total fumonisins (lower panel).

Figure 4.5 summarises the variation in total aflatoxins and fumonisins with maize type (either hybrid or local), mean annual temperature (of EPA) and location of sample collection (rural market or household). There are no obvious visible associations between the levels of total fumonisins and maize type, mean annual temperature (of the EPA) and location (Figure 4.5(*a*), (*c*) and (*e*)) or the proportion of samples that exceed the various limits for fumonisins. Median concentrations of total fumonisins are

comparable and mostly below 4 mg/kg for both maize types and sample locations in all the agro-ecological zones (Figure 4.5(*a*) and (*e*)). There are also no obvious associations between levels of total aflatoxins and maize type and sample location (Figure 4.5(*b*) and (*f*)). However, there are higher exceedance levels of total aflatoxins (Figure 4.5*d*) at locations with higher annual average temperature ($\rho = 0.21$, $p < 0.0001$, Spearman's rank correlation). The relationship is particularly clear for EPAs where annual mean temperature exceeds 25 ^{o}C (Figure 4.5*d*).

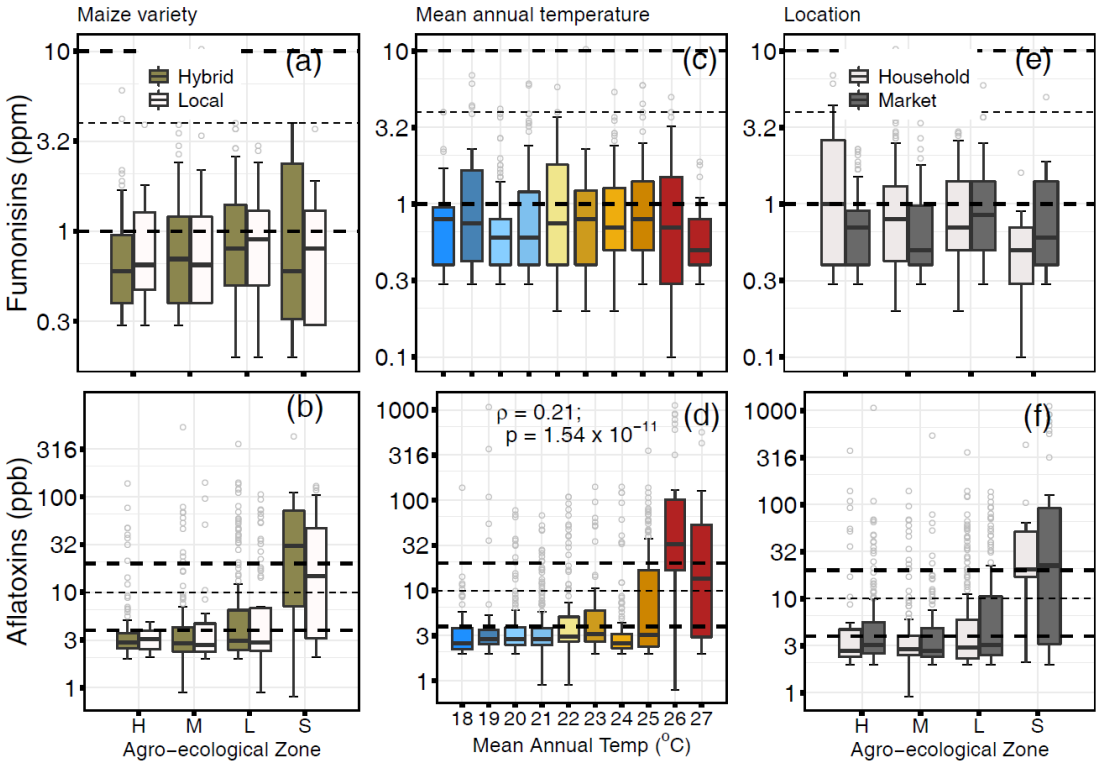

Figure 4.5: Boxplots of total aflatoxins and fumonisins with exceedances grouped by (a and b) maize type (hybrid or local), (c and d) mean annual temperature (based on EPA) and (e and f) sample location (market or household). Dashed lines show guideline value (see text). Spearman's rank correlation coefficient and p value shown for results in plot (*d*).

Several high (outlier) values for total aflatoxins and fumonisins are distributed across the range of climate settings used in this study (Figure 3.2). Total aflatoxin exceedances are observed in all agro-ecological zones, with overall lower counts found in the highlands and mid-elevation agro-ecological zones. These zones are associated with relatively lower annual mean temperature compared to the Lower Shire and Lakeshore agro-ecological zones (Figure 4.5). Highest median total aflatoxins concentrations are found in Lower Shire agro-ecological zone and are higher than those from the Lakeshore agro-ecological zone. This may be highly attributed to high mean annual

temperatures and low annual rainfal. However, outliers > 20 µg/kg are also found in the other agro-ecological zones (Figure 4.3). These outliers may be due to, among other factors, insufficient drying of grains and poor storage conditions of maize.

The results for aflatoxin and fumonisin concentration in maize of this study compare well with previous studies (Matumba et. al., 2014; Williams, 2004; Mwalwayo, 2013; 2016; ICRISAT, 2013). Although there is a wide variation in the levels of total aflatoxins in maize samples, the variation across the agro-ecological zones reflects the micro-climatic differences of the agro-ecological zones (Cotty and Jaime-Garcia, 2010). Significantly higher exceedance rates and median values for total aflatoxins are associated with sites characterised by higher mean annual temperature (Figure 4.5). A similar geographical pattern in the occurrence of total aflatoxins in Malawian maize samples was reported in earlier studies (Matumba et al., 2014; Chipinga, 2014; Mwalwayo and Thole, 2016). The influence of climatic variations on the geographical distribution and levels of fumonisins and aflatoxins has also been reported in the region (e.g., Mukanga et al., 2010; Rheeder et al., 2016; Hove et al., 2016b) and globally (e.g., Shelby et al., 1994). Therefore, this study supports the hypothesis that variations in aflatoxin contamination are related to variations in micro-climatic variables such as temperature.

The proliferation of *Aspergillus* spp. and *Fusarium* spp. and subsequent production of aflatoxins and fumonisins, respectively, depends on multiple drivers including genetic susceptibility of the maize variety, water activity, humidity, temperature, pH, aeration (CO_2/O_2 ratio) fungal spore loading and physical condition of the crop (e.g., drought stress and insect damage) (Lacey & Magan, 1991; Nesic et al., 2015). However, meteorological conditions are the key drivers of fungal colonization and mycotoxin production (Magan et al., 2003), with the other factors contributing to susceptibility and risk. Generally, relative humidity and temperature are the most critical climatic risk factors for *A. flavus* colonization and aflatoxin production during drying and storage (Chauhan et al., 2016). During the pre-harvest period, high temperatures and dry conditions favour growth, conidiation, and dispersal of *A. flavus* and impair growth and development of maize (Jaime and Cotty, 2007; Tola and Kebede, 2016).

Aflatoxins are produced optimally at temperatures between 25 and 35 °C, depending on water activity (O'Brian et al., 2007; Chacha, 2014; Achglinkame et al., 2017; Mannaa and Kim, 2017), which coincide with the diurnal temperature range for low-lying agro-ecological zones of the Lakeshore and Lower Shire River Valley (Figure 3.2). The low-lying agro-ecological zones are associated with high air temperatures and erratic rainfall (Figures 3.1 and Figure 3.2), throughout the year (Met-Malawi, 2006). On the other hand, fumonisins are styled field contaminants (Manna and Kim, 2017) and produced optimally under a relatively wider range of temperatures (15-30 °C) (Alberts et al., 1990; Murphy et al., 1993; Marin et. al., 2004 Samapundo et. al., 2005; Mogensen et. al., 2009; Wu et al., 2011; Medina et. al., 2013). Hence temperatures in all the agro-ecological zones are within the range conducive for *Fusarium* spp. growth and fumonisin production. Therefore, despite significant variations in aflatoxins, there are no significant differences across the agro-ecological zones in fumonisin levels.

Maize may initially be contaminated with conidia of *Aspergillus* spp. and *Fusarium* spp. in the field and if storage conditions are not optimized, the fungi can keep growing and metabolizing toxins during storage (Scheidegger and Payne, 2003; Sanchis and Magan, 2004; Neme and Mohammed, 2017). In Malawi, many farmers practice early harvesting to evade theft of maize in the field. The early harvest is then inadequately dried and stored under sub-optimal conditions without moisture and temperature control for long periods of time. The inadequately dried maize presents a water activity level that when combined with high temperatures create conditions that promote fungal activity leading to mycotoxin production (Giorni et al., 2016).

The results of this study, therefore, highlight the important role of climatic conditions (especially temperature and humidity) in the production (levels and distribution) of aflatoxins and fumonisins in maize. In this regard, climate change may likely lead to higher levels of aflatoxin and fumonisin contamination (Battilani et al., 2016; Paterson and Lima, 2010; Goertz et al., 2010; Magan et al., 2011). Available information suggests that slightly elevated CO_2 concentrations and interactions with temperature and water availability may promote fungal growth and toxin production, especially under water stress (Medina et al., 2017; Stepman, 2018; Van der Fels-Klerx et al., 2019). In Malawi, the temperature is expected to rise and rainfall to become more erratic (Ngongondo et al., 2014; Mittal et al., 2017). Therefore, it is likely that climate change

will impact mycotoxin occurrence in Malawi in terms of levels and geographic distribution (Battilani et al., 2016). These changes may negatively impact the quality and quantity of the maize crop, and therefore raise key issues for human health and food security.

4.3 Estimates of dietary intake of aflatoxins and fumonisins

4.3.1 Common modes of grain consumption in Malawi

The modes of grain consumption are important for quantitative assessment of dietary exposure to mycotoxins (Voss et al., 2001a; Humpf & Voss, 2004; Park et al., 2004), a critical component of risk assessment (FAO-WHO, 2006). In this study, all respondents (100%) acknowledged consuming maize-based meals at least twice a day (as *nsima* for lunch and supper), whereas 73% also indicated consumption of maize-based porridge for breakfast, making it three maize based-meals in a day (Table 4.1). Maize is also consumed in the form of a traditional cake (*chigumu*) (17%) and used in production of opaque beer (8%). In addition, maize is a major ingredient of porridge consumed by children (Table 4.1) as a complementary and weaning food (Hotz and Gibson, 2001).

Overall, discoloured kernels are often encountered in maize, with a small proportion (16%) of traders sorting their maize and most of the respondents understood that discoloured kernels had negative effect on trade and was a risk to human health. However, some respondents (41%) who reported moldy or discoloured maize kernels did not dispose of them, but instead mixed them with good grains for sale or making maize flour (Table 4.1), in agreement with earlier studies by Ambler et al. (2017) and Matumba et al. (2016).

Table 4.1: Responses to questions related to mouldy bad maize kernels, the common forms in which maize is consumed in rural Malawi. The value is the percentage of respondents who acknowledged the item.

Item	**Percentage**
Presence of mouldy/discoloured grains	
Traders sorted grain (*n* =186)	16.13
Visible mould on the grain (*n* = 346)	15.03
Often observe discoloured kernels (*n* = 506)	92.30
Experience bad taste while chewing roasted maize grain (*n* = 485)	98.97
What do you do with discoursed grains (*n* = 557)	
Make maize flour	22.08
Mix with good grain	8.08
Sort and discard	58.53
Knowledge of effect of bad kernels	
Any knowledge about maize rot (*n* = 446)	91.03
Bad kernels affect maize trade (*n* = 440)	93.86
Bad kernels affect health (*n* = 483)	68.53
Common forms of consuming maize (*n* = 449)	
Nsima	100
Porridge	73.05
Traditional cake	16.93
Traditional opaque brew	8.24
Ingredients for baby weaning food (*n* = 408)	
Maize only	18.63
Maize and groundnuts	17.16
Maize and soybean	5.88
Maize, groundnuts and soy	58.33

4.3.2 Estimates of dietary intake

Generally, mycotoxins are thermally stable (Humpf & Voss, 2004; Park et al., 2004; Voss et al., 2001). Therefore, the contaminated maize and maize-based products may constitute a major source of fumonisin and aflatoxin ingestion (Stoloff, 1983; Yiannikouris and Jouany, 2002; Seglar, 2003). In this study, levels of dietary intake of aflatoxins and fumonisins were estimated according to the JECFA procedures. For fumonisins, estimates of daily dietary intake exceeded the JECFA's provisional maximum tolerable daily intake (PMTDI) of 2.0 µg/kg body weight (bw)/day in 40.4%, 50%, 38.6% and 35.2% (median of EPA values) of all samples collected from the highlands, mid-elevation, lakeshore areas and Lower Shire agro-ecological zones, respectively (Figure 4.6c). In addition, the estimated daily intake of fumonsins

exceeded the PMTDI in majority of the fumonsins positive samples across all agro-ecological zones (Figure 4.6d).

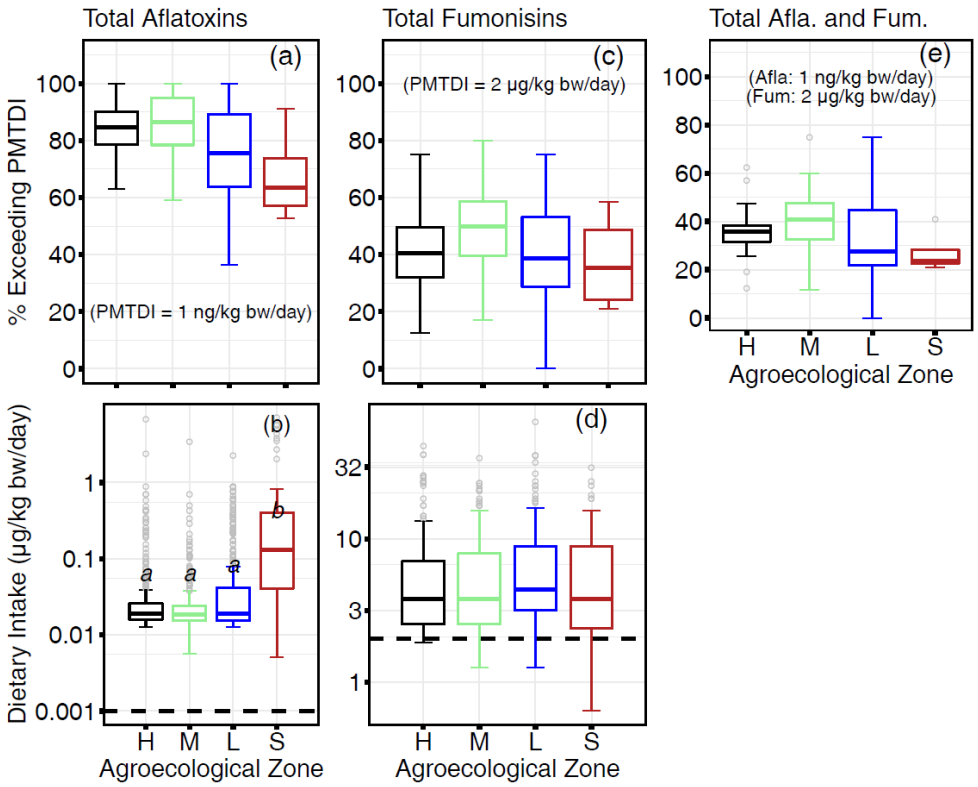

Figure 4.6: Estimated dietary intake of total aflatoxins and fumonisins (µg/kg bw/day) for a 60 kg adult in Malawi's EPAs categorised in their agro-ecological zones. For maize, an ingestion of 382 g of maize flour made from the analysed samples was assumed (Ecker and Qaim, 2011). Broken lines indicate JECFA's provisional maximum for aflatoxins (1 ng/kg bw/day) and for fumonisins (2 µg/kg bw/day). The dots, error bars and upper and lower ends of the box represent outliers, spread, and first and third quartiles, respectively. Agro-ecological zones with different letters, shown for results in plot (*b*), have significant differences ($p = 0.05$). Agro-ecological zones: Highlands (H); Mid-elevation (M); Lakeshore, upper and middle Shire valley (L); Lower Shire valley (S).

4.3.3 Implications of Aflatoxins and Fumonisins exposure to humans

Aflatoxins are among the most potent natural mutagenic and carcinogenic substances known, such that the JECFA has not established a numerical PMTDI for aflatoxins (JECFA, 1999). According to the European Union Scientific Committee for Food (SCF) even exposure to as little as 1 ng/kg bw/day could contribute to a risk of liver cancer (SCF, 1994). The Lower Shire Valley agro-ecological zone posted the highest ($p < 0.05$) estimates of dietary intake of aflatoxins (Figure 4.6b), as a result of high levels of contamination in maize (Figure 4.1a). However, a high proportion of collected samples and all the positive samples had exceeded the JECFA's provisional maximum tolerable daily intake (PMTDI) for aflatoxins across all ecological zones (Figure 4.6a).

The ubiquitous contamination of maize, the main staple crop, with aflatoxins and fumonisins means that the local population is at risk of the chronic health impacts of the mycotoxins (Shephard, 2004, 2008a, b; IARC, 2015; Matumba et al., 2014c, 2015b). Although there have been no reports of severe mycotoxicosis outbreaks in Malawi, there are other reported public health challenges with an epidemiological link to chronic dietary intake of aflatoxins and fumonisins. Aflatoxins (especially aflatoxin B1) cause liver cancer, growth suppression, immune system modulation, and malnutrition in both humans and livestock (Payne & Brown, 1998; Rushing and Selim, 2019). Aflatoxin B1 (AFB1) has been extensively linked to human primary liver cancer in which it acts synergistically with hepatitis B virus (HBV) infection (IARC, 2002; Shephard, 2008b; Wu et al., 2014). Malawi has remarkably high rates of cancers which are known to be linked to mycotoxin exposure (The Global Cancer Observatory, 2019). The Partnership for Aflatoxin Control in Africa (PACA, 2020) estimates that over 2,100 cases of liver cancer and 75,400 healthy life years are lost annually in Malawi due to aflatoxin exposure. However, data on aflatoxin exposure is scanty and the link to cancer or the other health issues, is not well established. As a result of its ability to suppress the immune system, Aflatoxin B1 (AFB1) exposure is also associated with the increased HIV viral load in those infected, and accelerated progression of the HIV infection to AIDS (Jiang et al., 2008; Williams et al., 2010; Jolly et al., 2014, 2015). In addition, it is linked to increase severity of other diseases such as malaria (Allen et al., 1992) and tuberculosis (Williams et al., 2004; Keenan et al., 2011).

Aflatoxins may also play a role in malnutrition and growth stunting during early childhood and, together with other mycotoxins, accelerate the development of edema in malnourished people and kwashiorkor in malnourished children (IARC, 2015; Kimanya et al., 2015). Growth impairment in children is a pervasive public health problem in low- and middle-income countries worldwide and is associated with a wide variety of factors (Black et al., 2008). However, studies show that the consumption of aflatoxin-contaminated food plays a potentially important contributory role (Gong et al., 2002; Shuaib et al., 2012; Khlangwiset et al., 2011) through reduction of nutrient absorption and utilisation (Fink-Gremmels, 2008), disruption of protein, carbohydrate and lipid metabolism (Yarru et al., 2009). In addition, the immunomodulation

associated with aflatoxin exposure (Turner et al., 2003) can also cause recurrent infections in children, which can lead to growth impairment (Gong et al., 2008).

In Malawi, malnutrition remains a determining factor of infant and under-five mortality. Prevalence of stunting, height for age (% of children under 5), in Malawi is 37.10 as of 2015. Thus, aflatoxin exposure and its association with growth impairment in children could contribute a significant public health burden. Childhood growth impairment is associated with increased risk of infectious diseases (e.g., diarrhoea, pneumonia, malaria, and measles) and mortality, and cognitive impairment that can lead to later reduced intellectual ability and economic productivity in adulthood (Caulfield et al., 2004; Black et al., 2008). Children may be exposed to aflatoxins through maternal food intake in utero (Lamplugh et al., 1988; De Vries et al., 1989; Wild et al., 1991; Turner et al., 2007), through breastfeeding (El-Sayed et al., 2000; Zarba et al., 1992) and through weaning and post-weaning foods, particularly where maize and groundnuts are dietary staples (Gong et al., 2002, 2003).

The consumption of maize highly contaminated with fumonisins has been correlated to the high incidence of esophageal carcinoma in certain parts of South Africa and China (Rheeder et al., 1992; Wagacha and Muthomi, 2008; Mostrom, 2016; Sun, Wang, & Hu, 2007). However, environmental risk factors for oesophageal cancer are complex (Schaafsmaet al., 2015) and the etiological role of exposure to fumonisins is mostly limited to ecologic studies (Kamangar et al., 2009; Middleton et al., 2018). Nevertheless, fungus infestation promotes formation of N-nitrosamines, which are known to cause oesophageal cancer, in fungus-infested food (Yang, 1980). Notably, Malawi has one of the highest oesophageal cancer prevalence rates (24.2 per 100,000 people) in the World (Banda et al., 2001; Ferlay et al., 2013; Chasimpha et al., 2017; Nahvijou et al., 2019; Mlombe et al., 2009; Wapnick et al., 1972; Msyamboza et al, 2012), which may be linked to high consumption of fumonisin contaminated maize (Crofts, 2008; Kachala, 2010; Mlomba et al., 2015; Chetwood, et al., 2018). Fumonisins have also been implicated in the high incidence of neural tube defects (NTDs) in rural populations known to consume contaminated maize, such as the former Transkei region of South Africa (Marasas et al., 2004; Shephard, 2008a).

As a staple food, contamination of maize with aflatoxins and fumonisins poses a public health risk in Malawi, which is particularly serious for rural subsistence farming communities and children who may suffer from enhanced exposure (Seetha et al., 2018; Braun and Wink, 2018). In Malawi, the risk is compounded by poorly diversified household food consumption, and limited public awareness of the impacts of ingesting moldy food and the risks associated with mycotoxins (Matumba et al., 2016; Ambler et al., 2017). However, the issue of food safety, with its emphasis on food quality, is frequently subordinate to issues of food security, with their emphasis on sufficiency of supply, because of chronic shortages of staple foods due to tenuous agricultural production systems. Although there are regulations limiting aflatoxins concentrations in food, on their own, they have limited impact as they cannot be strictly applied to the large quantity of subsistence farmers in Malawi (Matumba et al., 2014).

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

This study provided an assessment of a nationwide natural coccurence of aflatoxins and fumonisins in maize stored in rural households and traded in rural markets in Malawi. The maize samples for this study were from the 2015/16 growing season. The results from this study showed that majority of maize samples (> 75%) were widely contaminated with aflatoxins, across Malawi's agro-ecological zones. On average, fumonisins were detected in 45% of the samples, and co-occurred with aflatoxins in 38.64% of all maize samples. Maize samples collected from the Lower Shire agro-ecological zone (median 20.8 μg/kg) had relatively higher levels of aflatoxins ($p < 0.05$) than maize samples from the other agro-ecological zones (median: 3.0 μg/kg). In addition, the majority (75%) of the positive samples from the Lower Shire agro-ecological zone had aflatoxin levels exceeding the EU regulatory limit (4 μg/kg), whereas 25%, 37% and 39% of positive samples exceeded the EU limit in the mid-elevation, lakeshore, upper and middle Shire and highlands agro-ecological zone, respectively. The Lower Shire agro-ecological zone is characterised by high mean monthly temperatures throughout the year and erratic rainfall. Therefore, the results of this study showed highest aflatoxin counts in areas with the highest annual mean temperature, supporting the idea that meteorological conditions are the key factors of fungal colonization and mycotoxin production. In contrast to total aflatoxins, levels of total fumonisins in maize did not show a broad variation across the four agro-ecological zones. In general, almost all positive samples from across all agro-ecological zone exceeded the EU regulatory limit for maize products for infants of 0.2 mg/kg but fell short of the regulatory limit for adults of 4 mg/kg, but without discernible differences across the agro-ecological zones.

Levels of dietary intake of aflatoxins and fumonisins were estimated according to the JECFA procedures. For fumonisins, estimates of daily dietary intake exceeded the

JECFA's provisional maximum tolerable daily intake (PMTDI) of 2.0 μg/kg body weight (bw)/day in 40.4%, 50%, 38.6% and 35.2% (median of EPA values) of all samples collected from the highlands, mid-elevation, lakeshore and Lower Shire agro-ecological zones, respectively. In addition, the estimated daily intake of fumonisin exceeded the PMTDI in majority of the fumonisin positive samples, across all agro-ecological zones. Therefore, as a staple food, contamination of maize with aflatoxins and fumonisins poses a public health risk in Malawi, which is particularly serious for rural subsistence farming communities and children who may suffer from enhanced exposure. Further, the link between climatic conditions and the production (levels and distribution) of aflatoxins and fumonisins in maize, means that this risk is likely to increase with climate change (especially elevated temperatures and humidity). Projected rise in temperature and shortening of growing season is expected to increase contamination of maize with aflatoxins in all agro-ecological zones, unless adaptation or mitigation measures are put in place.

5.2 Recommendations

Further research is required to understand how climate change will impact the contamination risk of aflatoxin and other mycotoxins on maize and other food crops in Malawi. Such a modelling approach with scenario analysis provides possibilities for policy makers and risk managers to study the effects of changes in climate and practices on mycotoxin management. Linked to this, future research is required to explore what mitigation and adaptation measures are available and appropriate for use in Malawi, and the impact these may have on mycotoxin contamination risk of Malawian maize during both pre-and post-harvest. Specifically, the following are recommendations from the study:

1. Efforts should be made to improve grain management at both pre-harvest and post-harvest stages. For example, prevent moisture migration into stored grains through leaking roofs and condensation resulting from inadequate ventilation of the storage room.
2. There is need to use crop varieties which are resistant to mycotoxin development. This may minimize the problem of aflatoxins and fumonisins in Malawi.
3. There is need to use air-tight polypropylene bags when storing maize. It is recommended that grains should be stored in an air-tight environment and placed in an air-dry and clean room.

4. There is need to have clear knowledge of the prevalence of other strands (apart from aflatoxins and fumonisins) since different strands have different interrelationships, among others. Different mycotoxin strands co-occur in Malawi's grains especially maize. Therefore, various mycotoxin prevalence in Malawi's agro-ecological zones may be affected by the presence of the co-occurring strands.

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APPENDICES

Table Appendix 1: Occurrence and comparison of total aflatoxins and fumonisins detected in the samples, percentage of positive samples (Pos), and mean ± standard error (SD), median and percentage exceeding selected regulatory levels.

				Total Aflatoxins					**Total fumonisins**				
Zone	**EPA**	**District**	**n**	**% pos**	**mean±SD (µg/kg)**	**Median (µg/kg)**	**>4 µg/kg (%)**	**>10 µg/kg (%)**	**% pos**	**mean±SD (mg/kg)**	**Median (mg/kg)**	**>0.2 mg/kg (%)**	**>4 mg/kg (%)**
Highlands	Bembeke	Dedza	38	63	4.16±3.566	2.6	21	13	53	0.96±0.855	0.8	100	0
Highlands	Chafumbwa	Dedza	40	75	8.24±24.764	2.7	27	7	68	0.88±0.797	0.8	100	0
Highlands	Chikwatula	Ntchisi	26	85	2.74±0.646	2.6	5	0	31	1.09±1.305	0.6	100	13
Highlands	Chipuka		37	81	3.17±5.229	2.7	3	3	51	0.79±0.413	0.6	100	0
Highlands	Chivala	Dowa	48	94	5.7±11.975	3.2	22	4	35	0.59±0.298	0.5	100	0
Highlands	Kazomba	Mzimba	16	81	8.29±12.836	3.3	31	15	13	3.25±4.03	3.25	100	50
Highlands	Lufita	Chitipa	35	100	5.97±9.606	3.4	23	6	40	0.91±0.629	0.65	100	0
Highlands	Madisi	Dowa	8	88	3.39±0.644	3.4	14	0	75	1.23±1.36	0.65	100	0
Highlands	Manjawila	Ntcheu	37	86	4.87±3.167	3.8	44	6	51	0.53±0.294	0.4	100	0
Highlands	Milonde	Mulanje	39	77	11.84±26.067	3.0	30	17	38	1.49±1.297	1.1	100	0
Highlands	Msikawanjala	Mulanje	11	91	6.42±5.434	3.7	40	30	45	0.64±0.230	0.6	100	0
Highlands	Mulanje boma	Mulanje	13	85	18.00±21.151	6.0	55	45	46	1.03±0.835	0.8	100	0
Highlands	Mzuzu City	Mzuzu	54	78	41.98±173.337	2.9	21	12	56	1.57±1.784	0.75	100	13
Highlands	Nsipe	Ntcheu	21	100	13.26±20.413	3.9	38	24	57	0.73±0.358	0.65	100	0
Mid-elevation	Bolero	Rumphi	16	81	55.84±147.398	2.7	46	38	44	1.94±1.909	0.9	100	14
Mid-elevation	Chileka	Lilongwe	22	59	5.77±7.251	2.8	15	15	55	1.49±1.053	1.05	100	0
Mid-elevation	Chipala	Kasungu	29	76	4.64±6.578	3.0	23	5	48	1.39±0.970	1	93	0

Mid-elevation	Chitsime	Lilongwe	65	77	5.40±5.385	2.9	30	16	23	0.95±1.15	0.4	100	0
Mid-elevation	Lirangwe	Blantyre	1	100	2.60±0.000	2.6	0	0	0		#NUM!	#DIV/0!	#DIV/0!
Mid-elevation	Lisasadzi	Kasungu	10	90	3.17±1.130	2.9	11	0	70	0.93±0.457	0.8	100	0
Mid-elevation	Manyamula	Mzimba	2	100	7.40±6.647	7.4	50	50	50	1.00±0.000	1	100	0
Mid-elevation	Mikundi	Mchinji	4	100	3.60±2.296	2.7	25	0	100	0.93±0.780	0.7	100	0
Mid-elevation	Mjinge	Mzimba	22	86	10.22±17.501	2.6	32	16	41	1.68±1.124	1.7	100	0
Mid-elevation	Mkanda	Mchinji	24	83	2.86±0.764	2.8	5	0	50	0.8±0.609	0.5	100	0
Mid-elevation	Mlonyeni	Mchinji	7	71	2.16±0.811	2.3	0	0	71	0.46±0.167	0.5	100	0
Mid-elevation	Mpenu	Lilongwe	17	76	2.80±0.743	2.6	8	0	29	0.9±0.696	0.5	100	0
Mid-elevation	Nanyumbu	Machinga	7	100	2.93±0.395	2.9	0	0	43	2.57±1.893	3.4	100	0
Mid-elevation	Mtiya	Mangochi	15	53	4.70±5.240	2.5	25	13	40	0.67±0.320	0.6	100	0
Mid-elevation	Ntonda	Blantyre	36	86	4.49±4.799	3.0	23	6	69	0.832±0.570	0.7	100	0
Mid-elevation	Thondwe	Zomba	20	90	4.82±5.086	3.0	33	6	35	0.471±0.095	0.5	100	0
Mid-elevation	Wimbe	Kasungu	10	80	16.56±37.919	3.1	13	13	70	0.39±0.899	0.4	100	0
Lakeshore	Chingale	Zomba	20	70	9.34±14.341	3.3	36	21	65	1.55±1.559	0.9	100	8
Lakeshore	Chinguluwe	Salima	4	50	3.50±1.131	3.5	50	0	25	1.3±0.000	1.3	100	0
Lakeshore	Chiweta	Karonga	20	90	9.77±15.720	2.8	39	17	60	1.98±2.181	0.9	100	25
Lakeshore	Kalambo	Chikwawa	21	76	43.42±42.189	33.4	69	63	43	0.96±0.585	1.1	100	0
Lakeshore	Kombedza		17	65	9.10±16.674	3.7	45	9	18	1.83±1.910	1.2	100	0
Lakeshore	Kunthembwe	Blantyre	9	89	10.98±18.696	3.9	50	13	33	0.87±0.493	1.1	100	0
Lakeshore	Limphasa		41	61	4.54±7.778	2.5	12	8	49	0.84±0.627	0.65	95	0
Lakeshore	Linga	Nkhotakota	51	82	6.97±19.020	2.8	17	7	76	1.11±0.967	0.7	100	3
Lakeshore	Lirangwe	Blantyre	26	85	8.06±12.441	3.3	36	18	23	0.97±0.628	1	83	0
Lakeshore	Lupembe	Karonga	8	100	12.84±16.067	4.9	50	25	75	0.95±0.773	0.65	100	0
Lakeshore	Maiwa	Mangochi	6	83	24.00±12.235	21.0	100	100	0		#NUM!	#DIV/0!	#DIV/0!
Lakeshore	Malosa	Zomba	17	71	3.50±1.927	2.8	25	0	59	1.44±1.111	0.95	100	0
Lakeshore	Mitole	Chikwawa	13	46	38.20±54.441	5.0	50	33	8	0.7±	0.7	100	0
Lakeshore	Mpamba	Nkhatabay	10	60	2.33±0.288	2.2	0	0	40	0.675±0.492	0.5	100	0

Lakeshore	Mpata	Karonga	13	100	17.97±36.858	5.2	62	23	69	1.63±0.93	1.4	100	0
Lakeshore	Mpilisi	Balaka	34	82	16.06±32.924	3.3	46	18	32	1.736±2.393	0.7	100	9
Lakeshore	Nthiramanja	Mangochi	7	71	2.24±0.219	2.4	0	0	43	0.47±0.058	0.5	100	0
Lakeshore	Mtubwi	Machinga	24	88	13.86±32.423	2.5	29	14	63	0.88±0.674	0.5	100	0
Lakeshore	Mwanza	Mwanza	5	40	3.05±1061	3.1	0	0	60	0.7±0.265	0.6	100	0
Lakeshore	Mzenga	Nkhatabay	9	67	8.50±12.241	2.9	33	17	67	1.18±1.377	0.45	100	0
Lakeshore	Mbonechera	Machinga	10	100	19.26±26.257	4.2	50	30	30	0.73±0.351	0.7	100	0
Lakeshore	Nasenga	Mangochi	9	67	16.82±30.348	3.7	50	33	33	0.57±0.115	0.5	100	0
Lakeshore	Nkhunga	Nkhotakota	11	91	5.94±7.012	3.3	20	20	45	0.56±0.230	0.6	100	0
Lakeshore	Nyungwe	Karonga	9	100	13.80±19.731	2.8	33	33	33	1.23±0.814	1.6	100	0
Lakeshore	Tembwe	Salima	16	75	43.76±100.476	5.3	50	42	50	0.95±0.6	0.7	100	0
Lakeshore	Thambwani	Mwanza	22	36	6.39±6.963	2.9	25	25	45	1.11±0.839	0.85	100	0
Lakeshore	Ulongwe	Balaka	6	100	3.83±1.868	3.1	33	0	50	1.2±0.755	1.1	100	0
Lower Shire	Mbewe	Chikwawa	56	91	45.20±98.773	13.6	65	55	30	0.75±0.501	0.5	100	0
Lower Shire	Mpatsa	Nsanje	22	68	47±40.922	33.7	87	87	55	1.6±1.301	1.1	92	0
Lower Shire	Nyachilenda	Nsanje	19	53	24.08±18.648	19.6	90	80	37	0.61±0.518	0.5	71	0
Lower Shire	Zunde	Nsanje	29	59	341.35±396.71	92.3	94	88	55	1.206±1.434	0.5	94	6

Appendix 2: Map of Malawi showing Extension Planning Areas (EPAs) as adapted from department of agriculture extension services (2007)