Faculteit Bio-ingenieurswetenschappen

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De invloed van een gewijzigde aanvullende voeding op de blootstelling aan aflatoxines en fumonisines voor kinderen in ruraal Tanzania

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FOREWORD

My stay in Tanzania to collect data for my thesis, was the most unforgettable experience in my life so far. Living and working in Africa was very educational for me. First of all I want to thank Analice Kamala and the other staff for the warm welcome on the Tanzania Food and Drugs Authority institution. I want to thank Analice for making me feel at home in a place far away from home, the guidance in the lab and during the fieldtrip. I also want to thank Mohamed Abdulkadri for his help with the analysis of aflatoxins and fumonisins. I want to thank all the friends I made for the many trips we made and for giving me many beautiful memories.

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ABSTRACT

Keywords: maize, complementary feeding, mycotoxin contamination, mycotoxin exposure, Tanzania

Maize is the main part of cereals used in complementary foods in Tanzania, together with other cereals like sorghum, rice, wheat and finger millet. Unfortunately, maize has a high risk to be contaminated with mycotoxins, such as aflatoxins and fumonisins. Mycotoxins are of great global importance because of their impact on human health with carcinogenic, mutagenic and teratogenic effects. Fumonisin and alfatoxin contamination can lead to growth retardation in children and neural tube defects in developing foetus.

The aim of this study was to analyze maize, sorghum, wheat, rice and finger millet samples from two maize producing Agro Ecological Zones (AEZ) of Tanzania for the detection of fumonisins and aflatoxins. By means of HPLC, the presence and concentration of FB_1, FB_2 total FUMs and AFB_1, AFB_2, AFG_1, AFG_2 and total AFs was detected in all the crops. Afterwards, an exposure assessment was performed to calculate the exposure to each mycotoxin for each crop. The results indicate that maize and sorghum show the highest mycotoxin contamination, while wheat and finger millet are less contaminated with mycotoxins. Of the two AEZ, people in the Eastern lowlands are the most exposed to mycotoxin exposure. Finally, based on the fumonisin and aflatoxin contamination and exposures, alternatives for maize as complementary food could be recommended. Thus, recommendations to change complementary feeding in order to reduce mycotoxin contamination in Tanzania, can be made.

ABSTRACT

Trefwoorden: maïs, aanvullende voeding, mycotoxine contaminatie, mycotoxine blootstelling, Tanzania

Maïs maakt het grootste deel uit van graansoorten gebruikt in aanvullende voeding, samen met sorghum, rijst, tarwe en finger millet. Helaas is het risico groot dat maïs gecontamineerd is met mycotoxines, zoals aflatoxinen en fumonisinen. Mycotoxines zijn wereldwijd heel belangrijk omwille van hun impact op de menselijke gezondheid met kankerverwekkende, mutagene en teratogene effecten. Contaminatie met fumonisinen en aflatoxinen kan leiden tot vertraagde groeiontwikkeling bij kinderen en neurale buisdefecten bij ontwikkelende foetussen. Het doel van deze studie was om stalen van maïs, sorghum, rijst, tarwe en finger millet, afkomstig uit twee maïs producerende Agro-Ecologische Zones (AEZ) in Tanzania, te onderzoeken op de aanwezigheid van fumonisinen en aflatoxinen. Met behulp van HPLC werd de aanwezigheid van FB_1, FB_2, het totale fumonisine gehalte, AFB_1, AFB_2, AFG_1, AFG_2 en het totale aflatoxine gehalte aangetoond in alle gewassen. Daarnaast werd een blootstellingsschatting uitgevoerd om de blootstelling aan ieder mycotoxine per gewas te analyseren. De resultaten tonen aan dat maïs en sorghum de hoogste mycotoxine contaminatie vertonen, terwijl tarwe en finger millet in mindere mate gecontamineerd zijn. Van de twee AEZ, zijn mensen uit de laaglanden in het oosten het meest blootgesteld aan deze mycotoxinen. Uiteindelijk werden, op basis van de contaminatie en blootstelling aan fumonisinen en aflatoxinen, alternatieve gewassen voor maïs voorgesteld voor aanvullende voeding. Op die manier kunnen aanbevelingen worden gemaakt om aanvullende voeding te veranderen in Tanzania om zo mycotoxine contaminatie te reduceren.

TABLE OF CONTENT

ABBREVIATIONS

AEZ	Agro-Ecological Zones
AF	Aflatoxin
AFB_1	Aflatoxin B_1
AFB_2	Aflatoxin B_2
AFG_1	Aflatoxin G_1
AFG_2	Aflatoxin G_2
AFs	Aflatoxins
ALARA	As Low As Reasonable Achievable
Bw	Bodyweight
DON	Deoxynivalenol
FAO	Food and Agricultural Organization of the United Nations
FA_1	Fumonisin A_1
FA_2	Fumonisin A_2
FB_1	Fumonisin B_1
FB_2	Fumonisin B_2
FB_3	Fumonisin B_3
FB_4	Fumonisin B_4
FUM	Fumonisin
FSD	Food Security Department
HPLC	High Performance Liquid Chromatography
IAC	Immuno-Affinity Column
IARC	International Agency for Research on Cancer
JECFA	Joint Expert Committee on Food Additives
LOD	Limit of Detection
LOQ	Limit of Quantification

MTL	Maximum Tolerable Limit
NIV	Nivalenol
NOEL	No Observed Effect Level
OPA	ortho-phtaldehyd
OTA	Ochratoxin A
PBS	Phosphate Buffer Saline
PCD	Post-Column Derivatization
PMTDI	Provisional Maximum Tolerable Daily Intake
SAX	Strong Anion Exchange
TDI	Tolerable Daily Intake
TFNC	Tanzania Food and Nutrition Centre
WHO	World Health Organization
ZEA	Zearalenone
FSD	Food Security Department

FIGURES

TABLES

1. INTRODUCTION

Complementary foods are non-human-milk food-based sources that are given to children when breast milk alone is no longer sufficient to meet the nutritional requirements (WHO, 1998). Complementary foods are an important source of energy, protein and fat for children aged 4-24 months (Dewey & Adu-Afarwuah, 2008; Mbithi-Mwikya et al., 2002; Friedman, 1996). In many parts of Tanzania, maize is the main part of cereals used in complementary foods together with other cereals like sorghum, rice and finger millet. Maize contains high concentrations of energy sources like fermentable carbohydrates and proteins (Alonso et al., 2013; Mamiro et al., 2005). Maize is the main staple food in Tanzania and many other sub-Saharan, developing countries for people living in rural areas who are subsistence or small-scale farmers. According to the food security department (FSD) of Tanzania, maize is grown on 45% of the cultivated land (approximately 2 million hectares). The daily per capita maize consumption on national level ranges on average from 129 g to 308 g (Kimanya et al., 2008a, 2008b, 2009).

Unfortunately, maize has a high risk to be contaminated with mycotoxins, such as aflatoxins and fumonisins (Doko et al., 1996, Miller, 2008). The Food and Agricultural Organization of the United Nations (FAO) has estimated that up to 25% of the world's food crops are significantly contaminated with mycotoxins (WHO, 1999). Mycotoxins are of great global importance because of their impact on human health, animal productivity and the associated economic losses (Darwish et al., 2014; Wagacha & Muthomi, 2008). Out of the 400 mycotoxins produced by more than 100 fungal species, the five most agriculturally important fungal toxins are deoxynivalenol (DON), zearalenone (ZEA), ochratoxins, fumonisins (FUMs) and aflatoxins (AFs) (Miller, 2005, 2008; Wagacha & Muthomi, 2008). Fumonisins are classified as possibly carcinogenic for humans. Consumption of fumonisins has been linked with oesophagael cancer, growth retardation of children, neural tube defects in developing foetus and cardiovascular effects. They are also linked with leukoencephalomalacia in horses and pulmonary oedema in pigs (Kimanya et al., 2012; Marin et al., 2013). Aflatoxins and especially aflatoxin B_1 (AFB_1), are acutely toxic, immunosuppressive, mutagenic, teratogenic and carcinogenic (IARC, 1993). Aflatoxins have been classified as class 1 human carcinogen and consumption of AFB_1 can result in chronic aflatoxicosis with impaired growth and kwashiorkor in children (Gnonlonfin et al., 2013; Perrone et al., 2014; Wagacha & Muthomi, 2008).

Crops in tropical areas are more prone to contamination with mycotoxins than those in more temperate areas, because of both high humidity and high temperature (Wagacha & Muthomi, 2008). Delayed harvesting and intercropping are agricultural practices related to mycotoxin contamination (Gnonlonfin et al., 2013).

Mycotoxin contamination starts in the field where the crop gets infected, the fungal growth increases post-harvest and during storage conditions. Improper storage, transportation and processing facilities in poor hygienic conditions may stimulate fungal growth. As population is rapidly increasing on the African continent, proper storage conditions are necessary (Darwish et al., 2014; Wagacha et al., 2008). *Fusarium* species, which produce fumonisins, are dominant in the field, where water activity (aw value) is not a limiting factor for most of the crop growing period. On the other hand, *Aspergillus* species, which produce aflatoxins, are more xerophilic and because of the low moisture content of grains during storage, they are typically 'storage fungi' (Gnonlonfin et al., 2013; Gregori et al., 2012; Logrieco et al., 2003).

Unfortunately, the presence of mycotoxins in food is often overlooked in Africa due to public ignorance about their existence, lack of regulatory mechanisms, dumping of food products, and the introduction of contaminated commodities into the human food chain during chronic food shortage due to drought, wars, political and economic instability (Wagacha & Muthomi, 2008).

The aim of this study is to analyze maize, wheat, sorghum, rice and finger millet samples from two maize producing Agro Ecological Zones (AEZ) on the presence of fumonisin and aflatoxin . First of all, a field survey has been conducted to collect maize, wheat, sorghum, rice and finger millet samples from farmers in the two AEZ: the Northern highlands and the Eastern lowlands. The samples were used for mycotoxin analysis. By means of High Performance Liquid Chromatography (HPLC), the presence and concentration of Fumonisin B_1 ($FB_{1)}$, Fumonisin B_2 ($FB_{2),}$ total FUMs (FB_1+FB_2), Aflatoxin B_1 (AFB_1), Aflatoxin B_2 (AFB_2), Aflatoxin G_1 (AFG_1), Aflatoxin G_2 (AFG_2) and total AFs (AFB_1+AFB_2+AFG_1+AFG_2) was detected in all the crops. The overall fumonisin and aflatoxin contamination between all five crops was compared, followed by a comparison of the contamination between the two AEZ. Afterwards, an exposure assessment was performed to calculate the exposure to each mycotoxin for each crop. Finally, based on the fumonisin and aflatoxin contamination and exposures, alternatives for maize as complementary food could be recommended. Thus, recommendations to change complementary feeding in order to reduce mycotoxin contamination in Tanzania, can be made.

2. LITERATURE

2.1. MYCOTOXIN PROBLEM IN AFRICA

2.1.1. Introduction

Mycotoxins are natural contaminants formed as secondary metabolites by toxigenic fungi in the field and/or during storage. They are toxic for both humans and animals (Gnonlonfin et al., 2013; Shephard, 2008) and they can also cause chronic toxicity, called mycotoxicosis. Because mycotoxins are also toxic in the absence of the toxin-producing fungi, they can be described as abiotic hazards from biotic origin (Marin et al., 2013). Mycotoxins are of great importance in Africa and other parts of the world because of the significant economic losses associated with their impact on human health, animal productivity and trade (Darwish et al., 2014; Wagacha & Muthomi, 2008). The Food and Agricultural Organization of the United Nations (FAO) has estimated that up to 25% of the world's food crops are significantly contaminated with mycotoxins. Unfortunately, the presence of mycotoxins in food is often overlooked in Africa due to public ignorance about their existence, lack of regulatory mechanisms, dumping of food products, and the introduction of contaminated commodities into the human food chain during chronic food shortage due to drought, wars, political and economic instability. Crops in tropical areas are more prone to contamination with mycotoxins than those in more temperate areas, because of both high humidity and high temperature (Wagacha & Muthomi, 2008).

Out of the 400 mycotoxins produced by more than 100 fungal species, the five most agriculturally-important fungal toxins are deoxynivalenol (DON), zearalenone (ZEA), ochratoxins, fumonisins (FUMs) and aflatoxins (AFs) (Miller, 1995, 2008; Wagacha & Muthomi, 2008). Figure 1 shows the incidence of the different mycotoxins in African countries. Aflatoxins are the most present in African countries (43.5%), followed by fumonisins (21.87%), ochratoxins (12.5%), ZEA (9.375%), DON and beauvericin (both at 6.25%) (Darwish et al., 2014).

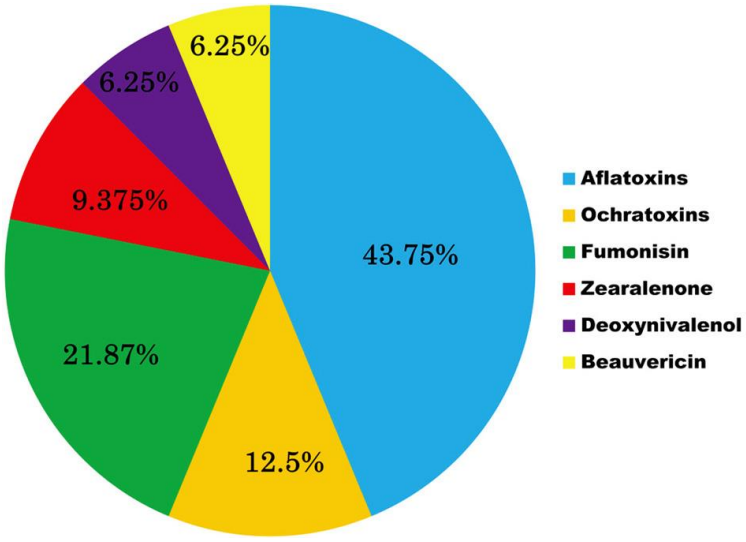

Figure 1: Distribution of mycotoxins in different African countries (Darwish et al., 2014)

The four most important toxigenic fungi belong to the genera *Aspergillus*, *Fusarium*, *Alternaria* and *Penicillium.* The most abundant mycotoxins produced by *Fusarium* species are trichothecenes (e.g. DON, T2, nivalenol, …), fumonisins and ZEA (Gnonlonfin, 2013; Gregori et al., 2013; Logrieco et al., 2003). *F. verticillioides* and *F. proliferatum* produce fumonisins, fusaproliferin and beauvericin. *F. verticillioides* produces also moniliformin and fusarin C (Miller, 1995; Placinta, D'Mello & Macdonald, 1999). Mycotoxins produced by *Aspergillus* include aflatoxins, citrinin, and patulin, while *Penicillium* species produce ochratoxin A (OTA), citrinin, patulin. *Alternaria* species produce alternaric acid, alternariols and aflatoxins. These fungi live in partially overlapping ecological niches and environmental conditions determine which species is dominant (Gnonlonfin, 2013; Gregori et al., 2013). Among the toxigenic fungi, four types can be distinguished: (1) plant pathogens such as *F. graminearum*; (2) fungi that produce mycotoxins on senescent or stressed plants, e.g. *F. verticillioides* and *Aspergillus flavus* on maize; (3) fungi that colonize the plant and predispose the commodity to mycotoxins contamination after harvest such as *A. flavus* in subtropical maize; and (4) fungi that are found in the soil or decaying material that occur on the developing kernels in the field and later proliferate during storage if conditions permit, e.g. *Pencillium verrucosum* on cereals, *A. flavus* on many commodities (Miller, 1995).

2.1.2. Sources of contamination

Mycotoxin production can be caused by environmental factors like high temperature, moisture content and heavy rains. These conditions generally occur in different African countries. Furthermore, insects that feed on plants in the field predispose the kernels to fungal infection through damage, while during storage they open the kernels to fungal invasion. That is why insect damage is a predictor of mycotoxin contamination. Insects carry spores of fungi and transfer these spores from one plant to another. When larvae feed on the kernel of plants, wounds are created, spores can easily enter the plant and mycotoxin contamination is induced (Darwish et al., 2014; Gnonlonfin, 2013; Wagacha & Muthomi, 2008). Another source related to mycotoxin contamination are agricultural practices like intercropping and delayed harvesting. Crops that are consecutively grown in the same field year after year, increase the risk of toxin contamination. Farmers have few knowledge about good agricultural practices such as crop rotation. Drying of maize in the field with humidity conditions, drying maize without husks or on the bare ground is also favorable for fungal populations to grow (Cotty & Jaime-Garcia, 2007; Gnonlonfin et al., 2013).

Once the crop gets infected in the field, the fungal growth increases at post-harvest and storage conditions. Improper storage, transportation and processing facilities in poor hygienic conditions may stimulate fungal growth. Storage in polypropylene bags, which are not airtight, facilitate fungal infection. During transportation, grains have to be covered and aerated. As population is rapidly increasing in the African continent, proper storage conditions are necessary (Darwish et al., 2014; Hell & Mutegi, 2011; Wagacha & Muthomi, 2008). The levels of mycotoxins can vary between seasons and between different growing areas or under different storage conditions (Shephard, 2008). Harvested maize grains in tropics can already contain spores of fungi such as *Fusarium, Aspergillus* and *Penicillium*, that can grow and compete for food if environmental conditions are ideal (Gnonlonfin et al., 2013).

Mycotoxigenic fungi can traditionally be divided into two groups: ‘field’ (plant pathogenic) and ‘storage’ (saprophytic) fungi. ‘Field fungi’ produce mycotoxins before harvest and require high moisture content in the substrate for growth and mycotoxin synthesis (>20%). They are present in pre-harvest or freshly harvested crops that are drying. *Fusarium* and *Alternaria* species are dominant in the field, where water activity (aw value) is not a limiting factor for most of the crop growing period.

‘Storage fungi’ form mycotoxins after being harvested and are able to grow at low moisture content as well. *Aspergillus* species are more xerophilic and are more dominant during storage, because of the low moisture content of grains during storage. These species demand a higher temperature for growth and mycotoxin production unlike *Fusarium* fungi. *Penicillium* species can occur in the field especially on senescent or stressed plants, as well as in storage. However, they are mainly classified as ‘storage fungi’ (Miller, 2008; Gnonlonfin et al., 2013; Gregori et al., 2013; Logrieco et al., 2003; Placinta, D’Mello & Macdonald, 1999).

Mycotoxins can be absorbed by the human body through different routes. One way is via the ingestion of contaminated foods such as cereals, meat, milk or eggs. The metabolism of ingested mycotoxins can lead to accumulation of mycotoxins in different organs or tissues. Processing of cereals like cleaning and sorting can produce dust, which can contain high concentrations of mycotoxins. Inhalation of these contaminated airborne aerosols and dermal absorption can represent an additional route of exposure, which has not yet been widely investigated (Brera et al., 2002; Marin et al., 2013; Marroquín-Cardona et al., 2014).

2.2. MAIZE CONSUMPTION IN TANZANIA

2.2.1. Complementary feeding in Tanzania

Complementary foods are non-human-milk food-based sources that are given to children when breast milk alone is no longer sufficient to meet the nutritional requirements. The process of complementary feeding starts around the age of 4-6 months to 24 months. In many countries in Africa, complementary foods are based on cereal flour, boiled in water. The composite flours that are common in many communities have a cereal/legume ratio of 70:30 (Dewey & Adu-Afarwuah, 2008; Mbithi-Mwikya et al., 2002; WHO, 1998). In Tanzania, the main complementary food consumed by children is a thin porridge prepared from maize flour. The composition of complementary foods depends on the age of children. Children aged 3-5 months consume a very thin maize porridge while older children aged 6-11 months receive a much thicker maize or composite flour porridge. Other cereals used for complementary feeding are sorghum, wheat, rice and finger millet (Mamiro et al., 2005).

The daily average energy requirements from complementary foods for children in developing countries is approximately 200 kcal at 6–8 months, 300 kcal at 9–11 months and 550 kcal at 12–23 months. These values represent 33 %, 45 % and 61 % of total energy needs respectively. Complementary foods are an important source of protein and fat. The amount of protein needed

from complementary foods increases from about 2 g/day at 6–8 months to 5–6 g/day at 12–23 months, with the percentage from complementary foods increasing from 21% to about 50%.
The nutritional value of a food, also known as the protein quality, depends on the concentration and ratios of constituent amino acids making up a specific protein. Availability of amino acids varies with protein source, processing treatment and interaction with other components of the diet (Friedman, 1996). A study in Kilosa pointed out showed that maize contains high concentrations of energy sources like fermentable carbohydrates (on average 69 %) and proteins (12.1 %). However, maize contains also anti-nutritional factors such as phytic acid (88 g/kg protein) (Alonso et al., 2013; Gilani, Xiao & Cockell, 2012; Mamiro et al., 2005).

2.2.2. Maize consumption in Tanzania

Maize is the main staple food in the United Republic of Tanzania and many other sub-Saharan, developing countries for people living in rural areas who are subsistence or small-scale farmers (Kimanya et al., 2009). Cereals and cereal-based products (especially maize) form the main dietary staple food for most people in Eastern and Southern Africa (Doko et al., 1996; Mamiro et al., 2005). According to the food security department (FSD) of Tanzania, maize is grown on 45% of the cultivated land (approximately 2 million hectares). The annual national maize utilization is approximately 3 million tons/year (Kimanya et al., 2008a, 2008b). The Tanzania Food and Nutrition Centre (TFNC), a governmental institution promoting intake of nutritious food, recommends a daily per capita consumption of 771 g for non-dehulled maize flour or rice and 790 g for dehulled maize flour for adequate energy intake. The daily per capita maize consumption on national level ranges on average from 129 g to 308 g. In the high maize consumption regions the daily maize consumption can rise up to 356 g/person. In South Africa similar levels are reported (456 g/person/day) (Kimanya et al., 2008a). Unfortunately, maize has a high risk to be contaminated with mycotoxins, such as aflatoxins and fumonisins. Maize is also exposed to contamination with ZEA and DON (Doko et al., 1996; Miller, 2008).

2.3. MYCOTOXIN CONTAMINATION IN MAIZE

2.3.1. Aflatoxin contamination

Aflatoxins are probably the most studied mycotoxins and the most important mycotoxins with regard to occurrence, toxicity, effect on human health and trade. It is estimated that 40% of the productivity lost to diseases in developing countries is related to AFs intake. The main producers of aflatoxins are the toxigenic fungi *Aspergillus flavus*, *A. parasiticus*, *A. nominus*, *A. pseudotamarii* and *A. bombycis*. These species are found in the soil, air and on crop surface (Cotty & Jaime-Garcia, 2007; Gnonlonfin et al., 2013; Wagacha & Muthomi, 2008). Significant levels of aflatoxins are reported in maize, groundnuts, cashew and other crops. Aflatoxin B_1, B_2, G_1 and G_2 are the most important aflatoxins isolated from foods and feeds. Only *Aspergillus parasiticus*, *A. nominus* and *A. bombycis* produce all four of these aflatoxins. *Aspergillus flavus* and *A. pseudotamarii* produce only B aflatoxins (Gnonlonfin et al., 2013; Wagacha & Muthomi, 2008). The most important agent that causes aflatoxin contamination is *A. flavus*, which exists in complex communities where genetically isolated groups commingle (Cotty & Jaime-Garcia, 2007). This fungus colonizes senescent or stressed plants and introduces contamination after harvest in subtropical maize (Miller, 2008). The fungi can produce these toxins in the field prior to harvest or their growth is stimulated due to poor storage conditions (Shephard, 2008).

Aspergillus infection and aflatoxin contamination in general are associated with warm, humid climates and irrigated hot deserts. They are heat stable and difficult to destroy during processing (Cotty & Jaime-Garcia, 2007; Gnonlonfin et al., 2013; Medina et al., 2014). Each *Aspergillus* species has its own optimum temperature for aflatoxin production. The optimum temperature for *A. bombycis*, *A. nomius* and *A. flavus* is 25°C*. A. parasiticus* is approximately between 27°C and 28°C (Gnonlonfin et al. 2013).

The contamination process is complex and the first phase starts in the field where crops get infected by strains of *Aspergillus* that are found in the soil and on decaying plant material. Plant stress (physiological stress, drought stress), temperatures above 28° favorable for fungal growth and insect damage increase susceptibility of crops for infection. Conidia of *A. flavus* are the most causal agent of maize contamination. Usually, developing crops are very resistant against infection of *A. flavus* and successive aflatoxin contamination if environmental conditions don't stimulate maturation. However, the kernels of developing crops can be infected through damage by insects or birds who carry *Aspergillus* fungi or by stress caused by hot and dry conditions before harvest. Wounding by insects may allow kernels to dry down to moisture content ideal for

growth of *A. flavus,* which form sclerotia, and start to produce aflatoxins. These sclerotia are dispersed in the soil during harvest and survive in soil and produce conidia during the next season. The second phase of contamination takes place at any time from crop maturation until consumption. The toxin of the fungus that was induced during the first phase is now able to increase due to warm and moist conditions during storage. The size of contamination is favored by substrate moisture content when initially dry seeds develop water content under high humidity. Aflatoxin levels may increase and new infections can occur until crops are ultimately consumed (Cotty & Jaime-Garcia, 2007; Gnonlonfin et al., 2013; Probst, Bandyopadhyay & Cotty, 2014; Wagacha & Muthomi, 2008). So, drought-, nutrient- or temperature-stressed plants are more susceptible to colonization by *A. flavus*. Aflatoxin production by *A. flavus* is produced in greater quantity with increasing aw, with an optimum at aw 0,996. Production is minimal at aw 0,85. However, it is previously declared that aflatoxins are xerophilic, so they are able to grow at low moisture content as well. Thus, moisture content of commodities is linked with toxin contamination and this has been reported in Africa for maize, cowpea and groundnut (Gnonlonfin et al., 2013). Studies have showed that insect damage can influence the process of AF contamination. *Aspergillus* spores have been detected from bodies of the corn earworm, *Heliothis zea* Boddie (Lepidoptera: Noctuidae), the European corn borer, *Ostrinia nubilalis* (Hubner)(Lepidoptera: Pyralidae) and the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curcilionidae).

2.3.1.1. Aflatoxin occurrence

Kimanya et al. (2008b) discovered the occurrence of AFB_1 in 12% of the 120 samples, AFG_1 in 9% of the samples, AFB_2 in 8% of the samples and AFG_2 in 10% of the samples in the four main maize-producing regions in Tanzania. AFB_1 was found in the highest concentrations (from 5 to 90 µg/kg), followed by AFG_1 (4 to 89 µg/kg), AFB_2 (from 1 to 20 µg/kg) and AFG_2 (from 1 to 17 µg/kg). Total AFs (AFB_1+AFB_2+AFG_1+AFG_2) were found in 18% of the samples with levels ranging from 1 to 158 µg/kg (table 1). So, maize in Tanzania is highly contaminated with aflatoxins. Aflatoxins were the most widespread in Tabora region (37%), followed by Kilimanjaro (20%). Tabora region is located in the center of Tanzania and is generally warm and dry with low rainfalls. Therefore crops in this region are more vulnerable to drought stress, which are more susceptible to fungal attack and the following possible contamination. In Iringa and Ruvuma only a small fraction (7%) of the samples were contaminated. The regulatory limit of AFB_1 and total AFs in food are set on 5 and 10 µg/kg, respectively. In general, 11% and 12% of the total

samples contaminated exceeded the MTL of AFB_1 and total AFs, respectively. In Tabora, 77% of the samples were contaminated with AFB_1 at levels above 5 µg/kg and 64% of the samples were contaminated with total AFs at levels above 10 µg/kg (table 1) (Kimanya et al., 2008a, 2008b).

Table 1: Occurrence and levels of aflatoxins in maize in Tanzania (Kimanya et al., 2008a)

Region	AFB_1		Total aflatoxins	
	Occurrence (%)	Concentration range (µg/kg)	Occurrence (%)	Range (µg/kg)
Tabora	37	5-90	37	5-158
Kilimanjaro	3	80[1]	20	1-80
Ruvuma	3	15[1]	6	7-26
Iringa	3	58[1]	7	13-58
Overall	12	5-90	18	1-158

[1]One level present.

Surveys in other African countries report the presence of aflatoxins in maize (table 2). In Kenya, between 41% and 51% of the maize samples was contaminated with aflatoxin levels above the regulatory limit of 20 µg/kg in grains for human consumption. Moreover, in Eastern Kenya aflatoxin poisonings associated with eating contaminated maize had been reported with a case-fatality rate of 40%. In Zambia, the concentrations of aflatoxins of 2 districts in Lusaka were 10-fold higher than 2 mg/kg and far higher than the 2 µg/kg maximum daily intake recommended by the FAO/WHO. Another report in Nigeria showed that 33% of the maize samples from different agro-ecological regions were contaminated with aflatoxins. In different AEZ in Benin, the aflatoxin levels before storage exceeded 5 µg/kg and the percentage of contaminated maize was between 9,9% and 32,2%. After 6 months storage, the percentage of contaminated maize increased to between 15% and 32,2% (Darwish et al., 2014).

Table 2: Incidence of total aflatoxin concentrations in maize in different African countries

Country	Concentration range (µg/kg)	Reference
Tanzania	1-158	Kimanya et al., 2008a
Kenya	1–46 400	Darwish et al., 2014; Gnonlonfin et al., 2013
Zambia	20 000[1]	Darwish et al., 2014; Mukanga et al., 2010
Malawi	878[1]	Matumba, 2014
Benin	2–2500	Darwish et al., 2014; Gnonlonfin et al., 2013
Ghana	20-355	Darwish et al., 2014

[1]Only maximum level present

2.3.1.2. Exposure to aflatoxins

Aflatoxins and especially AFB_1, are acutely toxic, immunosuppressive, mutagenic, teratogenic and carcinogenic (IARC, 1993). As previously noted, aflatoxins are hepatocarcinogens and have been classified as class 1 human carcinogen. Exposure to aflatoxins is widespread in many African countries and exposure already starts before birth. Blood tests have showed that a high percentage (98%) of West Africans are exposed to aflatoxins. For example in Benin, 99% of the children had aflatoxin markers in their blood with some of the highest aflatoxin levels ever observed in humans. These symptoms are strongly correlated with the change from breastfeeding to solid foods, such as maize, which is used as the basis for porridge for children. Daily consumption of foods contaminated with low levels of AFB_1 can result in chronic aflatoxicosis with impaired growth and kwashiorkor in children, immune suppression, cancer and reduced life expectancy (Gnonlonfin et al., 2013; Perrone et al., 2014; Wagacha & Muthomi, 2008). Acute toxicity with lethal effects can occur when exposed to large doses. There seems to be an association between the consumption of diets contaminated with aflatoxins and high incidence of liver cancer in Africa. Often up to 1 in 10 of the population in sub-Saharan Africa are infected with Hepatitis B and C, and in combination with AFs exposure, the risk of liver cancer is far more than ten-fold compared to exposure of both Hepatitis alone (Gnonlonfin et al., 2013; Wagacha & Muthomi, 2008). Marroquín-Cardona et al. (2014) noted that between 25 200 and 155 000 cases of new hepatocellular carcinoma out of annual cases worldwide (4.6-28.2 %) are attributed to aflatoxin exposure. In 2004, the largest outbreak of aflatoxicosis occurred in Kenia

where 317 cases and 215 death were reported. The concentration of AFB_1 in maize was approximately 4400 ppb, which is 220 times greater than the provisional limit of 20 ppb, suggested by Kenian authorities (Wagacha & Muthomi, 2008).

Regulations are made on the basis of toxicity of a certain mycotoxin, because different mycotoxins have different toxicities. For a toxin, where adverse effects show a threshold, a tolerable daily intake (TDI) is established. For aflatoxins, where carcinogenity is the basis of concern, TDIs are not applicable. Exposure of as little as <1 ng/kg bw/day to AFB_1 can lead to a risk of liver cancer and because of this a numerical TDI for aflatoxins could not be established. Therefore it is recommended that levels of aflatoxins should be as low as technologically feasible or as low as reasonable achievable (ALARA). Nevertheless, TDIs of <1 ng/kg bw/day have been used in other risk assessments (Matumba, 2014).

However there are a lot of reports of the occurrence of aflatoxins worldwide, few data are available about the exposure of populations to this mycotoxin. This is due to the wide range of left-censored data from occurrence studies, which makes exposure estimation unreliable. Another problem is that a lot of samples can present undetectable levels of aflatoxin contamination, while fewer samples can show high levels of contamination (Marin et al., 2013). Aflatoxin levels in different food products can widely vary, which makes exposure estimates difficult. For example in America, in degermed maize products 5-10 µg/kg of aflatoxin concentrations were found, while in full-fat cornmeal higher levels of 70-80 µg/kg aflatoxins were detected. Estimates of daily intakes of the latter diet ranges between 2.73 and 121 ng/kg bw/day (Moss, 2002).

2.3.2. Fumonisin contamination

In general, the two main fumonisin-producing species mostly isolated from maize are *Fusarium verticillioides* (previously known as *F. moniliforme*) and the related *F. proliferatum*. These fungi have been recovered from leaves, roots, stems and maize kernels (Fandohan et al., 2005b; Kimanya et al., 2009; Miller, 2008). Several studies (e.g. in West Europe and South Africa) showed that *F. verticillioides* is the most prevalent fungus of maize from the field, harvested maize and maize-based commodities (Doko et al., 1996). This is because *F. verticillioides* is prevalent in the warmer maize-growing areas with a temperature above 28°C, while *F. proliferatum* is found in relatively cooler areas (Miller, 2008). According to a study in Benin conducted in four different AEZ, predominance of *F. verticillioides* (68%) and *F. proliferatum* (31%) was found compared to other species of *Fusarium* genera (Fandohan et al., 2005b). The

relationship between the fungus and the maize is mutualistic with the fungus producing metabolites, e.g. fusaric acid, that are beneficial for the plant (Miller, 1995).
Fusarium species are worldwide known as important plant pathogens, saprophytes on debris or opportunistic colonizers of crop commodities. These fungi colonize legumes and cereals, usually before harvest. They are an important cause of storage rot of fruit and vegetables (Logrieco et al., 2003). *F. verticillioides* and *F. proliferatum* are the cause of a disease called fusarium kernel rot. This important ear disease is associated with warm, dry years and insect damage (Miller, 1995). Fumonisins can only be found in stressed or senescing kernel tissue. Fumonisin contamination is caused by environmental conditions, mainly pre-harvest. In regions where hot weather is followed by raining seasons, high levels of fumonisins can be found. Studies committed on fumonisins, concluded that humidity has a very high influence on contamination. Therefore, temperature, drought stress, humidity and rainfall during pre-harvest periods are the most important factors that influence fumonisin contamination (Fandohan et al., 2005b; Miller, 2008).

There are six structurally related fumonisins described: fumonisin B_1(FB_1), fumonisin B_2 (FB_2), fumonisin B_3 (FB_3), fumonisin B_4 (FB_4), fumonisin A_1 (FA_1), and fumonisin A_2(FA_2). Only FB_1, FB_2 and FB_3 are usually isolated from naturally contaminated maize, from which FB_1 and FB_2 are of the most mycotoxicological concern (Doko et al., 1996; Kimanya et al., 2009; Logrieco et al., 2003). *F. proliferatum* and *F. verticillioides* are both linked with the natural co-contamination of maize with FB_1 (Placinta, D'Mello & Macdonald, 1999). The activity of these two fungi and FB_1 production is suppressed by the presence of *F. graminearum*. On the other hand, *F. proliferatum* and *F. verticillioides* are competitive against aflatoxin producers *A. flavus* and *Pencillium spp.*, at aw greater then 0,96 (Gnonlonfin et al., 2013).

2.3.2.1. Fumonisin occurrence

As previously declared, levels of mycotoxins in a country vary from one geographical region to another. Kimanya et al. (2008b) conducted a study towards fumonisin contamination in villages in Iringa region, Tabora region, Ruvuma region and Kilimanjaro region. From 120 maize samples, 52 % (62 samples) were contaminated with total FUMs (FB_1+FB_2) with levels ranging from 61 to 11 048 µg/kg. FB_1 formed 31% of total FUMs with levels up to 6125 µg/kg (median: 206 µg/kg) and FB_2 had levels up to 4923 µg/kg (median: 239 µg/kg). FB_3 was not detected. Like aflatoxins, fumonisins were the most widespread in Tabora (70%), followed by Ruvuma (50%), Kilimanjaro (44%) and Iringa (43%). However Kilimanjaro region is less susceptible to drought, higher levels of fumonisins (ranging from 4000 to 11 048 µg/kg) were found. (Kimanya et al.,

2008b, 2009). The reason is that farmers harvest mature maize during a rain season with a high moisture content. The maize is not dried over a long time and improperly dried maize has a higher risk of fungal infestation. The contamination results in this study were compared with the maximum tolerable limit (MTL) of 1000 µg/kg and it seemed that 15% of the 120 samples exceeded this limit (table 3) (Kimanya et al., 2008b, 2012).

Table 3: Occurrence and levels of total fumonisins in home-grown maize in Tanzania (Kimanya et al., 2008b)

Region	Occurrence (%)	Median (µg/kg)	Range (µg/kg)
Tabora	70	321	71-2763
Ruvuma	47	155	62-3560
Kilimanjaro	47	501	65-11048
Iringa	43	441	61-3353

Fumonisins are also widespread in other regions in Eastern and South Africa, where contamination has been detected in 92,5 % of maize samples, as shown in table 4 (Kimanya et al., 2008a). The concentrations of fumonisins in maize in 6 districts in Lusaka, Zambia, were 20 000 µg/kg and were extremely higher than the recommended daily intake of 2 µg/kg (Mukanga et al, 2010). A survey in Nigeria showed that maize from different AEZ was contaminated with fumonisins. In 78.6% of the samples FB_1 was detected, while FB_2 was detected in 66% of the samples. In the former Transkei region in South Africa similar levels have been found (maximum levels of 7900 µg/kg for FB_1, 3770 µg/kg for FB_2 and 10 140 µg/kg for total FUMs) (Darwish et al., 2014; Kimanya et al., 2008a, 2012; Wagacha & Muthomi, 2008).

Table 4: Incidence of fumonisin levels in maize in different African countries

Country	Concentration range (µg/kg)	reference
Tanzania	61-11 048	Kimanya et al., 2008a, 2012; Darwish et al., 2014
Kenya	39-5000[1]	Alakonya, Monda & Ajanga, 2009
Zambia	70-20 000	Darwish et al., 2014; Doko et al., 1996
Zimbabwe	55-8000[1]	Doko et al., 1996; Kimanya et al., 2008a
Malawi	20-6475	Matumba, 2014
Botswana	35-370	Doko et al., 1996
South Africa	222-10 140	Darwish et al., 2014; Kimanya et al., 2008a
Nigeria	65-1830	Wagacha & Muthomi, 2008

[1] FB_1 levels

2.3.2.2. Exposure to fumonisins

Although there is no strong evidence of adverse effects of fumonisins on human health, consumption of fumonisins has been linked with carcinogenic effects in humans such as oesophageal cancer in various parts of Africa, Central America and Asia. Fumonisins have also an effect on neural tube defects in developing phoetus because fumonisins, via their depletion of sphingolipids, inhibit uptake of folate in different cell lines. This cellular deficiency is known as a cause of neural tube defects. The International Agency for Research on Cancer (IARC) categorized FB_1 as a Group 2B carcinogen and possibly carcinogenic to humans. Further studies in infants in Tanzania have shown that ingestion of fumonisins was associated with growth retardation as measured in infants at 12 months of age. Children with fumonisin exposure above the provisional tolerable daily intake (PMTDI) of 2 µg/kg bw/day were significantly shorter by 1.3 cm and lighter by 328 g than those with exposure below the limit. Furthermore there has been proved that fumonisins induce apoptosis in cultured human cells and in rat kidneys (Fandohan et al., 2005a, 2005b; Kimanya et al., 2009, 2012; Shephard et al., 2013; Wagacha & Muthomi, 2008).

Exposure to fumonisins for an adult of 60 kg bodyweight (bw) in a certain household, is estimated by multiplying the fumonisin content in maize from that household with the daily per capita maize consumption (Kimanya et al., 2008b). The PMTDI for FB_1, FB_2 and FB_3 alone or together recommended by The Joint FAO/WHO Expert Committee on Food Additives (JECFA) is 2 µg/kg bw/day. A study in Tanzania shows the effect of the daily per capita maize consumption on exposure to fumonisins (table 5). Based on the recommended consumption of 771 g/person/day, the range of exposure for individuals in the studied households was 0.78-141.97 µg/kg bw/day. From the households, 38% percent of the individuals exceeded the PMTDI and the total fumonisin contamination in maize in those households exceeded 155 µg/kg. The percentage of households that exceeded the PMTDI decreased from 38% (with maize intake of 771/g/person/day), through 27% (with maize intake of 356 g/person/day) to 16% (with maize intake of 129 g/person/day). Since Tanzania has no MTL for fumonisins, results are compared with the MTL of 1000 µg/kg set for fumonisins in maize flour for human consumption in The European Union countries. The highest limit for unprocessed maize is 2000 µg/kg. Based on a study in South Africa with a high maize consumption, the MTL suggested for people in rural areas is 122 µg/kg and 202 µg/kg for people in urban areas (table 5) (Kimanya et al., 2012).

Table 5: Effect of daily per capita maize consumption on exposure to fumonisins (Kimanya et al., 2008b)

Daily per capita maize intake (g)	Range of exposure (µg/kg bw/day)	Highest concentration in maize (µg/kg)[1]	Percent households exceeding the PMTDI (%)
771	0.78-141.97	155	38
356	0.36-65.55	314	27
308	0.31-56.72	382	25
129	0.13-23.75	870	16

[1] Concentration above which the maize intake results in exposure above the PMTDI.

2.3.3. Co-occurrence of fumonisins and aflatoxins

The co-occurrence of aflatoxins and fumonisins is common and has been detected in maize from Ghana and Benin (Miller, 2008; Gnonlonfin et al., 2013). In Ghana, co-occurrence was detected in 8 out of 15 samples (53%). The highest aflatoxin level in co-contaminated maize was 662 µg/kg and the highest level of fumonisin was 2534 µg/kg (Kpodo, Thrane & Hald, 2000). Kimanya et al. (2008b) also discovered the co-occurrence of the two mycotoxins in maize from Tanzania. In 12 of the 120 samples both aflatoxins and fumonisins were discovered. Total fumonisin levels in the co-contaminated ranged from 111 to 11.048 µg/kg and total AFs from 1 to 151 µg/kg. Fifty-eight percent of the twelve samples showed fumonisin and aflatoxin contamination at levels above the respective MTLs of 1000 and 10 µg/kg. A new study of Kimanya et al. (2014) showed that maize flour samples were co-contaminated with aflatoxins and fumonisins in 29% of the samples. But this higher percentage is probably due to the fact that the flours tested in this study contained other cereals and legumes (e.g. groundnuts) next to maize, while in the previous study maize kernels were studied only. The reason of co-occurrence of these mycotoxins may be due to the fact that the same environmental conditions favour fumonisins production as well as aflatoxin production and that maize is a prevalent host. The exact toxicological implications for humans of the co-occurrence fumonisins and aflatoxins is not known yet and has to be investigated (Kpodo, Thrane & Hald, 2000). Since fumonisins and aflatoxins are produced by *Fusarium* and *Aspergillus,* which infect maize, people consuming maize are at high risk of exposure to multiple mycotoxins. These moulds produce other forms of mycotoxins like nivalenol (NIV), ZEA, DON and OTA in maize (Doko et al., 1996). The interactions between multiple mycotoxins can be additive, synergistic and can vary with exposure time, dose and animal species involved (Doko et al., 1996; Kimanya et al., 2014).

2.4. RISK ANALYSIS FOR MYCOTOXINS IN FOOD

Risk analysis plays an important role in science-based food safety systems and in guiding food safety authorities. It can be used as a tool to detect chemical, physical or microbiological threats to food safety (Shephard, 2008). The process of risk analysis is used to acquire an estimate of the risks to human health and safety, to identify and implement appropriate measures to control the risks, and to communicate with stakeholders about the risks and measures applied. It offers food safety regulators information and evidence they need for effective decision-making, contributing to better food safety outcomes and improvements in public health. In the case of mycotoxins, risk analysis can be used to obtain information and evidence on the level of risk of a

certain mycotoxin in the food supply helping governments to decide which, if any, actions should be taken in response. These actions could include setting or revising a maximum limit for that toxin, increasing testing frequency, review of labelling requirements, provision of advice to a specific population subgroup, issuing a product recall and/or a ban on imports of the product in question. Risk analysis consists of three components: risk assessment, risk management and risk communication (FAO/WHO, 2006).

2.4.1. Risk assessment

Risk assessment of food safety hazards is an objective science-based evaluation of the adverse effects following from human exposure to a risk source. It is a tool used in evaluating the severity and likelihood of potential health implications resulting from mycotoxin exposure (Shephard, 2008). Risk assessment is divided in four stages, namely hazard identification, hazard characterization, exposure assessment and risk characterization (FAO/WHO, 2006; Shephard, 2008). In the context of mycotoxins, hazard identification comprises the identification of mycotoxins capable of causing adverse health effects and which may be present in a particular food or group of foods (FAO/WHO, 2006; Shephard, 2008). In this stage, a 'no-observed-effect-level' (NOEL) in mg/kg of body weight per day is calculated. NOEL is the greatest concentration or amount of that chemical that does not cause detectable effects. For example, the NOEL for renal toxicity is 0.2 mg/kg of bw/day for FUMs (WHO, 2002).

Hazard characterization of a mycotoxin is defined as the qualitative and/or quantitative evaluation of the nature of adverse effects with that toxin, which may be present in that food. A dose response assessment is performed by combining exposure data with toxicity data (WHO, 2002). Furthermore, an estimation of the PMTDI in µg/kg bw equivalent is calculated, based on dividing the NOEL by a safety factor. For FB_1, FB_2 and FB_3, alone or in combination, the PMTDI is 2 µg/kg bw (Shephard, 2008, 2013; WHO, 2002).

2.4.2. Exposure assessment of mycotoxins

Exposure assessment of mycotoxins is a process based on qualitative and/or quantitative evaluation of the likely intake of mycotoxins via food. The extent of exposure is depending on the level of contamination present in the food and on the quantities of contaminated food consumed by individuals. Exposure assessment is a variable across populations and subgroups of populations, unlike hazard identification and characterization, which relate to universal properties of the mycotoxin (Shephard, 2008). Many methods exist for conducting exposure assessments

(Lambe, 2002). A common approach to estimate exposure is the combination of contamination data with consumption data. Contamination data are provided by researchers, while consumption data are in most cases obtained from national dietary surveys (Marin et al., 2013). The most commonly used food consumption data for exposure assessments is food consumption surveys of individuals. Such surveys can be conducted using 24 h recalls, diet histories, food records or food-frequency questionnaires (Lambe, 2002). Individual consumption data and bw of each consumer are used to calculate exposure by multiplying consumption level of food by level of contamination of food, divided by body mass of the subject. Then the individual exposure to each mycotoxin will be compared with health based guide value TDI and PMTDI (Sirot, Fremy & Leblanc, 2013).

Exposure assessment (µg/kg bw/day)

=

$$\frac{\text{Consumption level of food (kg/day)} * \text{Level of contamination of food (µg/kg)}}{\text{Body weight of the subject (kg bw)}}$$

Deterministic exposure assessment uses a single estimate of each variable, a point-estimate approach, by multiplying a fixed value for average food consumption of a population with a fixed value for chemical concentration in that food (usually mean concentration or maximum permitted value) and then sums the intake from all foods. Examples of point estimates of dietary exposure include the theoretical maximum daily intake for food additives. This deterministic approach is widely used as a first step in assessing exposure because it is simple and inexpensive to perform. Inherent to the point-estimate approach are the assumptions that all individuals consume the specified food(s) at the same level, that the food chemical is always present in the food(s) and that it is always present at an average or high level (Lambe, 2002). A disadvantage of this method is that is does not calculate complicated statistics, and quantitative information about variability and uncertainty is not provided.

Another approach, which is more appropriate for sophisticated exposure scenarios, is the probabilistic approach (Marin et al., 2013). Probabilistic models take account of every possible value that each variable can take and weigh each possible scenario by the probability of its occurrence. Thus, every variability and/or uncertainty in variables, including food consumption, are reflected in the model output. The whole distribution of exposure in different communities, from minimum to maximum, is considered (Lambe, 2002; Shephard, 2008). However, the lack of detailed information on contamination levels and consumption patterns in African countries rules

against the use of probabilistic models. On the other hand, single-point determinations based on mean levels can offer insights into the mycotoxin exposure of African populations (Shephard, 2008).

2.5. STRATEGIES TO REDUCE MYCOTOXIN CONTAMINATION

2.5.1. Alternative crops for complementary feeding

Strategies to reduce mycotoxin intake can be based on limiting the level of toxin in the food, limiting the consumption of contaminated food or a combination of both. Since the maximum contamination value for FUMs is 11048 µg/kg and the PMTDI is 150 µg/kg, reduction of contamination is hardly to fulfill in Tanzania with the available technologies. Limiting maize consumption would be more practical by partial replacement of maize with other cereals. Other cereal-based complementary foods given to children in Tanzania include finger millet, wheat, sorghum, rice and peanut composite flour porridge (Kimanya et al., 2012; Mamiro et al., 2005). Non-cereal ingredients of complementary foods include beans, dried sardines, groundnuts and eggs (Mamiro et al., 2005). Cereals are generally low in protein and essential amino acids, like lysine and tryptophan (Osundahunsi & Aworh, 2003).

This is the case for wheat, which has limiting amounts of lysine and threonine (Friedman, 1996). Wheat contains also some antinutritional components such as phytic acid (53 g/kg protein), forming complexes with proteins and reducing amino acid digestibility (Gilani, Xiao & Cockell, 2012). Rice is a major food source for a large number of the world's population. Rice has a protein content between 5% and 7%, which is lower than those found in most other cereals. However, rice is a better quality protein than wheat, because the lysine content of rice proteins (3-4%) is more than 50% greater than that of wheat and the amino acid balance is better (Friedman, 1996). Sorghum is one of the most important staple foods for many people in the semi-arid tropics of Asia and Africa, with the largest cultivated area in Africa (24.5 million ha) and Asia (10.6 million ha) (Elbashir & Ali, 2014; Kaur et al., 2014). The essential amino acid concentrations are low, especially lysine and threonine. Because of the very poor energy and digestibility, sorghum grain flour is not recommended for consumption by small children (Friedman, 1996). Sorghum contains some antinutritional components, like tannins (up to 79 g/kg) and phytic acid (101 g/kg protein) (Gilani, Xiao & Cockell, 2012). Millets, such as finger millet, are considered superior to cereals with respect to some of nutrients especially protein, mineral and fat. However, the presence of various antinutrients, poor digestibility of the protein

and carbohydrates and low palatability greatly affects its utilization as a food (Kaur et al., 2014). Millets contain substantial amounts of antinutritional factors such as tannins (up to 72 g/kg) (Gilani, Xiao & Cockell, 2012). Finger millet is a cereal also used for the preparation of porridges. It has potential health benefits, of which some are contributed to the polyphenol content. The 8-11 % total protein content is better balanced than that of other cereals, with higher amounts of lysine, threonine and valine. Finger millet is more palatable and the mineral content (2.7%), especially calcium, is greater than that of rice (0.6%) or wheat (1.5%) (Mbithi-Mwikya et al., 2002; Saleh et al., 2013).

While cereals are generally low in protein and essential amino acids, legume seeds contain greater amounts of lysine. Grain legumes are generally considered as important sources of food and feed proteins and legume seeds are a necessary supplement to other protein (Duranti & Gius, 1997; Osundahunsi & Aworh, 2003). Mixtures of cereals with locally available legumes that are high in protein and lysine, but low in sulphur amino acids, increases protein content of cereal-legume blends through complementation of their individual amino acids. Locally available cereals and legumes in Nigeria have been used in the production of high protein-energy complementary foods (Osundahunsi & Aworh, 2003).

2.5.1.1. Fumonisin and aflatoxin contamination

Fumonisin levels in sorghum appear to be lower than in maize. A study of five sorghum samples collected from affected households in India, showed FB_1 levels up to 360 µg/kg. FB_1 levels up to 7800 µg/kg in 20 sorghum samples from affected households were found, while FB_1 levels in 12 maize samples were found up to 64 700 µg/kg (Bhat et al., 1997). In Burundi, one sample of sorghum meal, however, showed very high concentration (28 200 µg/kg) (Munimbazi & Bullerman, 1996).

Studies on contamination of finger millets are rare or absent, suggesting that these small cereals might be less vulnerable to fungal infection. A multitoxin analysis of sorghum (70 samples) and finger millet (34 samples) conducted in Ethiopia, discovered the occurrence of fumonisins as well as aflatoxins. After ZEA, FB_1 was the most dominant major mycotoxin in both sorghum and finger millet, with median values of 12.90 µg/kg and 5.26 µg/kg, respectively. All four types of AFs (AFB_1, AFB_2, AFG_1 and AFG_2) were present in sorghum samples, while only AFB_1 and AFG_1 were found in finger millet samples. The maximum aflatoxin levels were higher in sorghum (62.5 and 61.5 µg/kg of AFB_1 and AFG_1, respectively) than in finger millet (1.43 and 3.19 µg/kg of AFB_1 and AFG_1, respectively)(Chala et al., 2014). In Sudan, 43 out of 60 samples were

contaminated with AFB_1 ranged from 0.06 to 12.29 µg/kg. Sorghum samples in Nigeria were contaminated with AFs in a range of 10-80 µg/kg (Elbashir & Ali, 2014). In Uganda, 76% of *F. verticillioides* strains from finger millet produced FB_1 and FB_2, with levels up to 12 400 and 6200 µg/kg, respectively (Saleh et al., 2012).

Mycotoxin contamination in rice is usually lower than in wheat or maize. However, there are some reports that rice has been contaminated with aflatoxins and fumonisins. FB_1, FB_2 and FB_3 were detected in 8, 6 and 5 of 20 samples of rice in the USA, respectively, with maximum concentrations of 4300, 1200 and 600 µg/kg, respectively. Maximum concentrations of 600 µg/kg AFB_1 were detected in all 9 samples from Thailand. In India, maximum concentrations of 317 µg/kg AFB_1, 125 µg/kg AFB_2, 107 µg/kg AFG_1 and 98 µg/kg AFG_2 were found in 13 out of 20 samples. Concentrations up to 77.5 µg/kg AFG_1 and 96.3 µg/kg AFG_2 were found in Malaysia, in two and three out of 84 samples, respectively (Tanaka et al., 2007).

A survey in Asia and Oceania, which tested 98 wheat samples, showed no evidence of aflatoxin contamination above the limit of quantification (LOQ) (1 µg/kg) and low fumonisin contamination, with 4 positive samples (levels up to 646 µg/kg). In Europe and the Mediterranean region, one sample was analysed for fumonisin detection with a concentration of 580 µg/kg and out of 11 samples no aflatoxins were detected above the LOQ (1 µg/kg) (Binder et al., 2007).

2.5.2. Post-harvest measures to lower contamination

Reduction of mycotoxins can be alleviated by a range of post-harvest measures including physical removing of the toxins and detoxification of the food during processing. Physical approaches such as sorting, cleaning, dehulling and milling seem to have a certain effect in reducing mycotoxins in cereals such as maize (Fandohan et al., 2005a). Sorting and winnowing reduced mean aflatoxin level in maize from 6.57 µg/kg to 2.67 µg/kg and mean fumonisin level from 4800 µg/kg to 1500 µg/kg in foods in Benin. However, there has been reported that poor farmers cannot always afford to remove visibly mouldy, insect-damaged and broken grains by hand (Fandohan et al., 2005a; Kimanya et al., 2012). A 91 % aflatoxin reduction was observed after sorting, winnowing and washing of the raw maize. Cleaning of maize resulted in a fumonisin reduction and an aflatoxin reduction of 74% and 61 %, respectively. Dehulling of maize is another tool to reduce mycotoxin contamination. About 91 % of aflatoxins was removed with the discarded hulls and germ in maize food products in Benin, while a 29 % decrease of fumonisins was found (Fandohan et al., 2005a; Hell & Mutegi, 2011). As temperature and moisture increase the growth of toxigenic fungi in stored commodities, freshly harvested

commodities should be dried as quickly as possible to a safe moisture content of 10-13 % for cereals. Although simple sun-drying is not always possible due to high humidity conditions in some parts of Africa, several technologies are used to increase the efficacy of grain drying and to reduce toxin contamination, such as the use of drying platforms, drying outside the fields and drying on mats. Dryers that seem to have a positive effect cannot be used in Africa because of large capital investments required. New storage technologies such as improved hermetic bags, based on triple bagging, which are tested for cowpeas and other commodities, can be used instead of traditional storage methods as polypropylene bags or expensive metal bins (Hell & Mutegi, 2011). Many of these strategies could be useful in Africa as they are simple and do not imply additional cost. Nonetheless, training and awareness campaigns are needed to inform farmers, traders and processors about the risk of toxin contamination and such campaigns have been successfully applied in West Africa (Gnonlonfin et al., 2013).

3. MATERIALS AND METHODS

3.1. FIELD SURVEY IN TANZANIA

3.1.1. Research design and sampling procedures

The aim of this survey was to sample complementary foods (maize, finger millet, rice, sorghum) for determination of fumonisin and aflatoxin contamination levels and food intake from two AEZ. The two AEZ are the Northern highlands (Hanang district in Manyara region), the Eastern lowlands (Kilosa district in Morogoro region) and the South-western highlands (Rungwe in Mbeya region).

Between July and August 2013, a total of 198 maize samples were collected from Kilosa (100 samples) and Hanang (98 samples). In September 2013, 24 samples of wheat, 37 sorghum samples, 10 rice samples and 8 finger millet samples were obtained from 28 villages located in two districts, Hanang and Kilosa. Of the 37 sorghum samples, 12 were collected in Hanang, while 25 were collected in Kilosa. Rice was only collected in Hanang, while finger millet and wheat were only collected in Kilosa. With the guidance of the District crop officers and Village extension officers, villages that cultivate the crops and households that could have some stock of the crops were selected. In Hanang, samples were collected from 15 villages. The villages representing the Northern highland zone in Hanang are: Balangadalaw, Getasam, Wareta, Dirma, Endagaw, Gehandu, Simbay, Dumbeta, Gabadaw, Sirop, Measkron, Endasiwold, Nangwa, Gitting and Galangal. In Kilosa, samples were collected from 13 villages. The villages representing the Eastern lowland zone in Kilosa are: Zombo, Msowero, Kondoa, Madudu, Muungano, Mamoyo, Dumila, Mvumi, Malui, Kivungu, Mvumi, Rudewa batini, Rudewa mbuyuni.

Rice was sampled from 10 villages in Kilosa, while sorghum was sampled from 18 villages (12 in Hanang and 6 in Kilosa). Finger millet and wheat were sampled from 8 and 6 villages in Hanang, respectively. In each village four households were selected. The samples from these households were combined to one sample according to the ‘coning and quartering’ principle. All four of the samples are put together on a pile and afterwards divided in four equal parts. Two parts that were diametrical towards each other were combined together until an average of 1 kg was collected. Thus, the sample of 1 kg represented the composition of the whole village.

3.1.2. Description of research area

The survey was conducted in two main maize producing AEZ in Tanzania, with their own climate characteristics: Manyara region in the Northern highland AEZ and Morogoro region in the Eastern lowland AEZ. In each region a district is selected as study site, based on maize production level and accessibility: Hanang' is chosen for Manyara region and Kilosa for Morogoro region (figure 2).

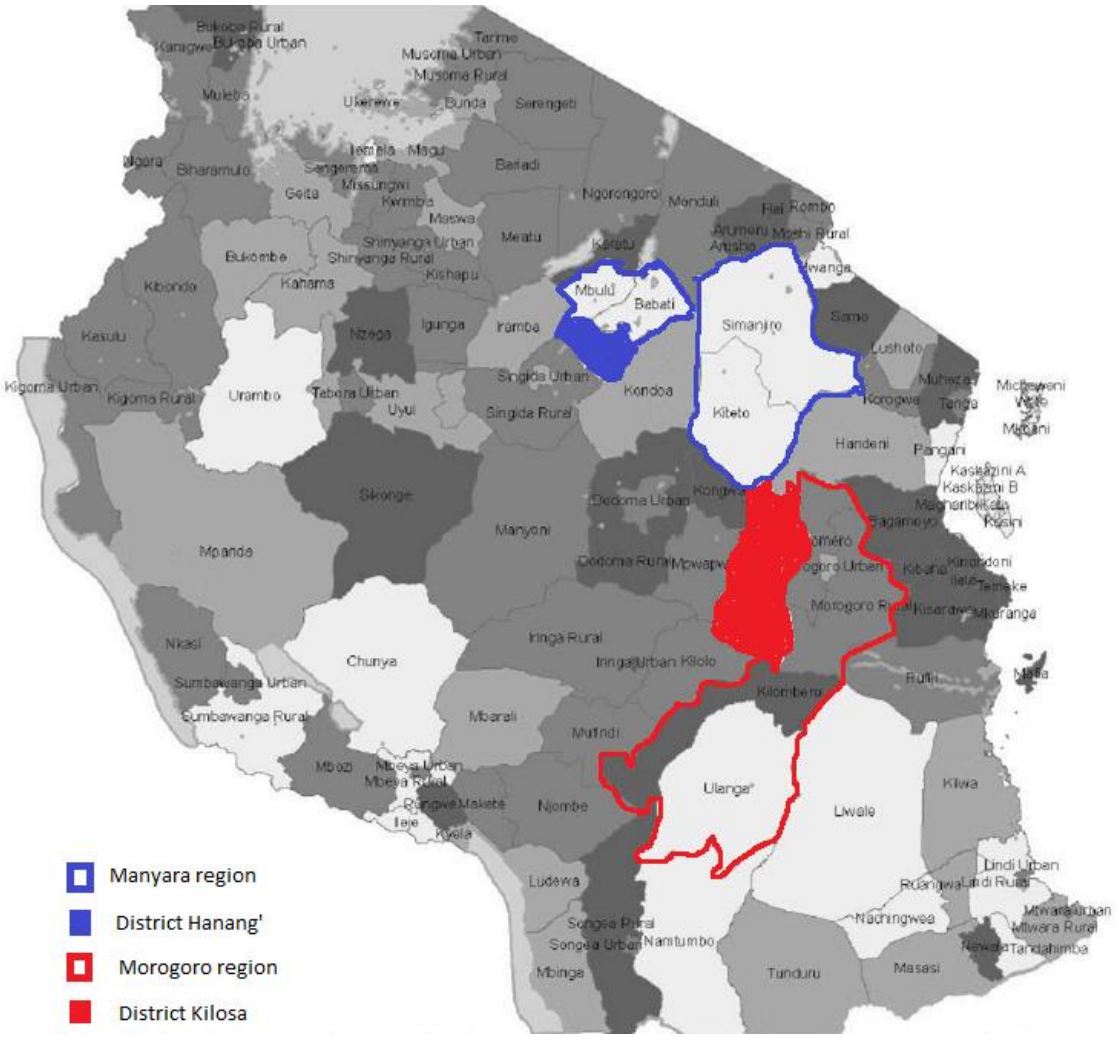

Figure 2: Presentation of research area (Degraeve, 2013)

Hanang is one of the 5 districts located in the Manyara region, in the North-Eastern part of Tanzania. The region lies between latitudes 4° and 5° S and longitudes 35° and 38° E, with altitudes between 1000 m and 2000 m above sea level and Mount Hanang (3676 m above sea level) as the highest peak in the district. Hanang covers an area of 38 141 km^2. According to the

2012 population census, Hanang had about 275 990 inhabitants. The region has two rain seasons, the short rain begins in October and ends in December while the long rain season starts in February and ends in May. The average rainfall ranges between 700 mm and 900 mm. Temperature ranges between 20°C and 25°C (Hanang district council, 2012; Profile Manyara Region, 2013). The main economic activities in the region are agriculture and livestock herding, and, to a lesser extent, fishing and beekeeping. The district's economy suffered from a great drought in 2000 and the economy is depending on agriculture and fluctuations of rainfalls, so incomes are highly seasonal. However, Hanang benefits from rich soil and is known for its high levels of food crop production during years with adequate rainfalls (Chee, Smith & Kapinga, 2002). The most important food crops are maize, millet, wheat, pigeon peas, sorghum, potatoes and beans. Sorghum is a drought resistant crop widely cultivated in the region. Maize and beans are the most popular food crops among the small scale farming communities in the region. Other food and cash crops grown are coffee, sugar cane, sunflower and banana (Chee, Smith & Kapinga, 2002).

Kilosa is one of the 6 districts within the Morogoro region, located approximately 300 km inland from the coast in the Eastern part of Tanzania. It lies between 6°S and 8°S, and 36°30'E and 38°E and covers an area of 14 245 km^2. About 489 513 people are living in the district. The district experiences an average of eight months of rainfall with short rains from October to January, followed by long rains from mid-February to May. The mean annual rainfall ranges from 800 to 1400 mm. The mean annual temperature in Kilosa is about 25°C. The main economic activity is crop cultivation and livestock keeping. A variety of crops is grown in the district including maize, rice, sorghum, millet, cassava, beans, bananas, pigeon peas and cowpeas. Besides food crops, the main cash crops are sisal, cotton, coffee, wheat, cashew nuts, coconuts, sugar cane and tobacco (Kajembe et al., 2013).

3.1.3. Food intake survey

Food intake for maize was obtained from the previous study in Kilosa conducted in 2012. Only consumption data of Kilosa district were collected and are applied to calculate the exposure for Hanang. It is therefore assumed that the consumption of Kilosa is representative for Hanang. For the other crops (sorghum, wheat, rice and finger millet) average food intake values were used from rural areas in Tanzania (Smith & Subandoro, 2007). It is assumed that the consumption in rural areas in Tanzania is representative for Kilosa and Hanang.

3.2. MYCOTOXIN DETERMINATION

3.2.1. Aflatoxins

3.2.1.1. Aflatoxin determination

AFB_1, AFB_2, AFG_1 and AFG_2 were determined in maize, sorghum, wheat, rice and finger millet in accordance with the method described by Stroka et al. (2000).

Aflatoxins were extracted from 12.5 g of each sample, which was grounded and mixed with 50 ml of methanol: water (6:4) in a 100 ml glass bottle. The bottle was fitted on a laboratory magnetic shaker for 60 minutes, followed by filtering of the slurry by using Whatman paper number 4. The total of 10 ml of obtained extract was diluted with 30 ml of phosphate buffer saline (PBS), followed by adjusting the pH to 7.4 by using 0.1M NaOH. The diluted extract was passed through AflaStar immunoaffinity column (IAC) (Romer Lab, Coring System Diagnostix GmbH, Gernsheim, Germany), fitted to a solid-phase extraction manifold (24-Port SPE Vacuum Manifold System, ALLTECH Associates, and Lokeren, Belgium) and allowed to flow. The rinsing of the container with 20 ml of distilled water was done. After rinsing, aflatoxins were eluted by 1.5 ml of methanol (HPLC grade) and transferred to HPLC for analysis.

3.2.1.2. HPLC analysis for aflatoxins

First 10 µl of the eluate was injected into the HPLC for analysis, using a reversed-phase HPLC fluorescence detection system with post-column deriverisation (PCD), involving bromination. The PCD was achieved with an electrochemical cell (Kobra cell) and addition of potassium bromide and nitric acid to the mobile phase. A Shimadzu HPLC system, consisting of a Waters 600 pump and controller, was used. The system was connected to a Shimadzu SIL-10ADvp auto injector, a Shimadzu RF-10AXL florescence detector and a Shimadzu C-R3A chromatopac integrator. Chromatographic separations were performed on a Bondapak ODS column (250 x 4.6mm, 5µm pore size). The methanol:acenitrile:water (15:20:65) solution, containing 119 mg of potassium bromide and 100 µl of 65% nitric acid per litre, was used as mobile phase. The flow rate was set at 1.06 ml/min. The oven temperature was set at 20^oC and end time was 15.5 minutes. Fluorescence of the aflatoxins was recorded at wavelengths of 360 nm (excitation) and 440 nm (emission).

The limit of detection (LOD) defined as the mean value of the blank readings plus three standard deviations of the analytical method for each matrix i.e. maize, rice, sorghum, wheat and

fingermillet, was determined (table 6). To evaluate suitability of the method, blank samples of each matrix were spiked with AFB_1 and AFG_1, each at 0.76, 3.81 and 6.85 µg/kg. The blank samples of each matrix were also spiked with AFB_2 and AFG_2, each at 0.56, 1.31 and 1.675 mg/kg. Average recovery values obtained are shown in table 7. All the results were corrected for recovery.

Table 6: LOD of aflatoxins per matrix (µg/kg)

	Maize	Wheat	Sorghum	Rice	Finger millet
AFB_1	0.53	0.01	0.01	0.05	0.01
AFB_2	0.15	0.50	0.12	0.05	0.50
AFG_1	0.24	0.02	0.51	0.40	0.02
AFG_2	0.01	0.10	0.43	0.04	0.10

Table 7: Recovery values of aflatoxins per matrix (%)

	Maize	Wheat	Sorghum	Rice	Finger millet
AFB_1	89	101	76.2	81	78
AFB_2	87	87	79	93	86
AFG_1	107	101	77	87	98
AFG_2	87	87	88	97	197

3.2.2. Fumonisins

3.2.2.1. Fumonisin determination

High Performance Liquid Chromatographic (HPLC) method based on the work of Sydenham, Shephard & Thiel (1992) and slight modification done by Samapundo et al. (2006) was used for quantification of the fumonisins in maize, wheat, sorghum, rice and finger millet.

Fifteen gram of each grounded sample was used to determine fumonisins, whereby extraction of fumonisins was done by mixing 40 ml of methanol:water (3:1, v/v) in a 100 ml glass bottle, fitted on laboratory shaker for one hour. Whatman paper no.1 was used to filter the slurry and the bottle was rinsed with 10 ml of mixture of methanol:water. The 10 ml of extract was applied to a strong anion exchange (SAX) cartridge (Varian, Bond-Elut LRC, 500 mg, Varian Belgium NV/SA, Sint-Katelijne-Waver Belgium), fitted to solid-phase exchange manifold (Alltech, 24-Port SPE Vacuum Manifold System, Alltech Associates, Inc., Lokeren, Belgium). Before applying the

extract, the SAX cartridge was conditioned with 5 ml of methanol, followed by 5 ml of a methanol:water mix (3:1 v/v). Then the SAX cartridge was washed with 8 ml of methanol:water mix (3:1 v/v) ,followed by 3 ml methanol, after application of the extract. The elution of fumonisins from the cartridge with 10 ml of 1% (v/v) glacial acetic acid in methanol was done. The eluant was collected, followed with evaporation to dryness at 60^{o}C under a gentle stream of nitrogen, using a nitrogen evaporator (Pierce model 18780, Reacti-Vap coupled with a dry bath; Pierce Reacti-Therm, Rockford, IL, USA).

3.2.2.2. HPLC analysis

The dried fumonisins were dissolved in 200 µl of methanol and thoroughly mixed with 200 µl of derivatizing reagent. This derivatizing reagent was prepared by dissolving 40 mg of ortho-phthaldehyde in a mixture of 1 ml of methanol, 5 ml of 0.1M $Na_2B_4O_7.10H_2O$ (Borax) and 50 µl of β-mercaptoethanol. Afterwards 20 µl of the mixture (20 ml) was injected into the HPLC for analysis within 8 minutes by using a reversed-phase HPLC fluorescence detection system. A Shimadzu HPLC system consisting of a Shimadzu 20A pump and a CBM 20A controller was used. The system was connected to a Shimadzu SIL-20A auto injector. Chromatographic separations were performed on a Discovery C8 column (100_4.6 mm, 5 mm; Supelco, Bellefonte, PA, USA). The mobile phase used was methanol:0.1M $Na_2H_2PO_4$ (75:25, v/v) mixture adjusted to pH 3.35 with ortho-phosphoric acid. The mobile phase was set at a flow rate of 1 ml/min and fluorescence of the fumonisin ortho-phtaldehyd (OPA) derivatives was detected at wavelengths of 335 nm (excitation) and 400 nm (emission), using a Shimadzu fluorescence detector model RF-10XL.

The LOD of the analytical method for each matrix i.e. maize, rice, sorghum, wheat and fingermillet, was determined (Table 8). To evaluate suitability of the method, blank samples of each matrix were spiked with FB_1 and FB_2 each at 100, 200, 300, 400 and 500 µg/kg. Average recovery values obtained are shown in Table 9. All the results were corrected for recovery.

Table 8: LOD of fumonisins per matrix (µg/kg)

	Maize	**Wheat**	**Sorghum**	**Rice**	**Finger millet**
FB_1	53	47.2	50	47	56
FB_2	47	51.2	43	35	48

Table 9: Recovery values of fumonsins per matrix (%)

	Maize	Wheat	Sorghum	Rice	Finger millet
FB_1	106	84	88	95	91
FB_2	92	83	94	91	87

3.2 EXPOSURE ASSESSMENT

For the consumption data of all the crops except maize, only mean values were available. Therefore, only a deterministic analysis could be performed. Contamination data were presented based on LOD value, but some data were below LOD or not detected. Therefore, all data below the LOD, for each mycotoxin respectively, were categorized into three scenarios for the purpose of analysis as follows. In the first 'lower bound' scenario, all undetected data were replaced by 0. In the second 'medium bound' scenario, all undetected data were replaced by ½ LOD, for each mycotoxin respectively. In the third 'higher bound' scenario, all undetected values were replaced by the respective LOD value (Marin et al., 2013). Individual consumption data and bodyweight of each consumer are used to calculate exposure by multiplying consumption level of food by level of contamination of food, divided by body mass of the subject. Then the individual exposure to each mycotoxin will be compared with health based guide value PMTDI (Sirot, Fremy & Leblanc, 2013).

Exposure assessment (µg/kg Bw/day)

=

$$\frac{\text{Consumption level of food (kg/day)} * \text{Level of contamination of food (µg/kg)}]}{\text{Body weight of the subject (kg bw)}}$$

4. RESULTS

4.1. OVERALL MYCOTOXIN CONTAMINATION PER CROP

Maize, wheat, sorghum, rice and finger millet samples have been analysed for aflatoxines and fumonisins. By means of HPLC, the presence of AFB_1, AFB_2, AFG_1, AFG_2 and total AFs (AFB_1+AFB_2+AFG_1+AFG_2), FB_1, FB_2, total FUMs (FB_1 + FB_2), is detected.

Table 10: Percentage of samples with detected mycotoxin concentrations above LOD for each matrix (%)

	FB_1	**FB_2**	**Total FUMs**	**AFB_1**	**AFB_2**	**AFG_1**	**AFG_2**	**Total AFs**
Maize	65	44	59	30	22	13	13	24
Sorghum	41	3	30	41	24	32	41	41
Wheat	54	29	54	8	25	29	33	38
Rice	100	0	80	60	60	30	30	70
Finger millet	38	0	25	13	0	25	13	13

In sorghum (37 samples), wheat (24 samples) and finger millet (8 samples), fumonisin and aflatoxin concentrations were detected in 50 % or less than 50 % of the samples, except for FB_1 (54 %) and total FUMs (54 %) in wheat. FB_2 was not detected in rice and finger millet, while in sorghum only one sample (3 %) showed FB_2 contamination. Finger millet showed no AFB_2 contamination, while AFB_1, AFG_2 and total AFs were detected in only one sample each (13 %) (table 10).

4.1.1. Overall occurrence of aflatoxins

The results for aflatoxin analysis in maize, wheat, sorghum, rice and finger millet are represented in figure 3.

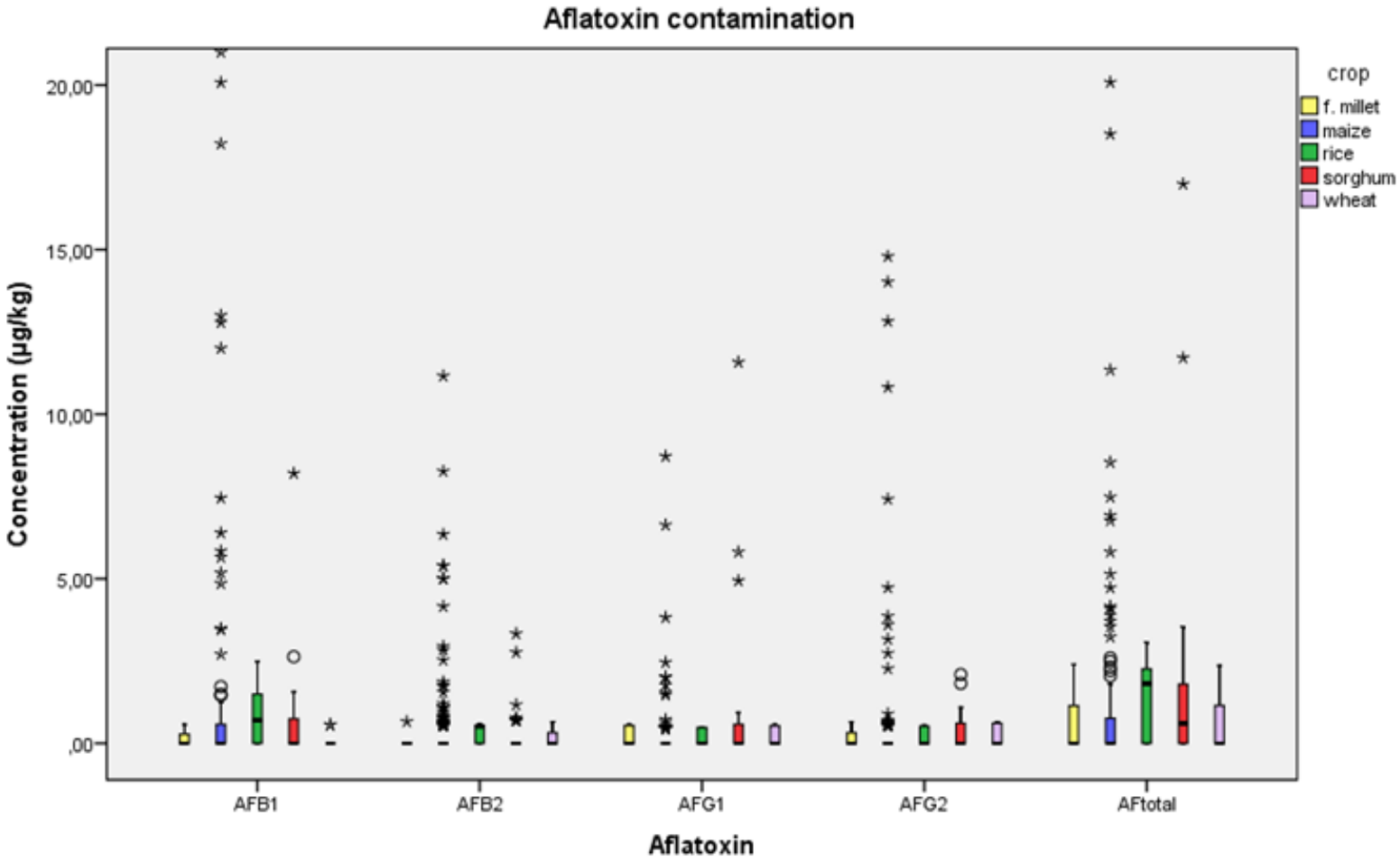

Figure 3: Overall occurrence of aflatoxins in maize, wheat, sorghum, rice and finger millet

In general, all the crops have a low prevalence (maximum median level of 1.82 µg/kg for rice), maize has the most outliers. Maize has the widest range in AFB_1 (0.00-94.23 µg/kg), AFB_2 (0.00-114.19 µg/kg), AFG_2 (0.00-67.87 µg/kg) and total AFs concentration (0.00-219.45 µg/kg). Sorghum has the highest AFG_1 concentration (11.58 µg/kg), followed by maize (8.72 µg/kg). For all the other AFs, sorghum has the second highest maximum concentrations, with maximum AFB_1 concentration of 35.08 µg/kg and maximum total AFs concentration of 51.80 µg/kg. Wheat and finger millet have similar contaminations and have the lowest maximum concentrations for AFB_1 (0.56 µg/kg and 0.57 µg/kg, respectively) and total AFs (2.36 µg/kg and 2.42 µg/kg, respectively). Rice has the highest prevalence for AFB_1 (median level = 0.60 µg/kg) and for total AFs (median level = 1.82 µg/kg). Rice is the least contaminated crop for AFB_2 (0.59 µg/kg), AFG_1 (0.50 µg/kg) and AFG_2 (0.56 µg/kg). Only maize and sorghum have aflatoxin concentrations above the regulatory limit of 5 µg/kg and 10 µg/kg for AFB_1 and total AFs, respectively (table 11) (Kimanya et al., 2008a, 2008b).

Table 11: Percentage of maize and sorghum samples with AFB_1 and total AFs concentrations above the MTLs of 5 µg/kg and 10 µg/kg, respectively

Crop	AFB_1	AFs total
Maize	10	9
Sorghum	5	8

Since all sample data did not meet all assumptions for normality and equal variances, instead of using ANOVA test, a non-parametric test (Kruskal-Wallis rank sum test) was used. For cases where significant differences were found, the Wilcoxon (Mann-Whitney U) rank-sum test was used to determine substantial differences.

The Kruskall-Wallis test shows no significant difference in AFB_1 and total AFs contamination for all the crops ($p \geq 0.05$), but for total AFs the difference is bigger ($p=0.0575$). AFB_2 and total AFs contamination in maize is almost significantly different than in rice ($p = 0.50$ and $p = 0.058$, respectively). Between rice and finger millet, there is no significant difference ($p \geq 0.05$). On the other hand, in the case of AFB_1 contamination the difference is almost significant ($p=0.051$). AFG_1 and AFG_2 contamination in maize is significantly different than in sorghum($p \leq 0.05$). Sorghum has a significantly different AFB_1 contamination than wheat ($p \leq 0.05$), but only two wheat samples showed AFB_1 contamination (table 9). Rice has a significantly different AFB_1 and total AFs contamination than wheat ($p \leq 0.05$). Maize has a significantly different AFB_1, AFG_1

and AFG_2 contamination than wheat ($p \leq 0.05$). Maize has a significantly different AFG_1 contamination than finger millet ($p \leq 0.05$). AFB_1, AFB_2, AFG_1 and total AFs contamination of maize in Kilosa was significantly higher than in Hanang ($p \leq 0.05$). AFG_2 contamination of maize in Kilosa was almost significantly higher than in Hanang ($p=0.051$). AFB_1 contamination in sorghum in Kilosa was significantly higher than in sorghum in Hanang ($p \leq 0.05$).

4.1.2. Overall occurrence of fumonisins

The results for fumonisin analysis are represented in figure 4. Figure 4 represents FB_1, FB_2 and total FUMs contamination of maize, wheat, sorghum, rice and finger millet.

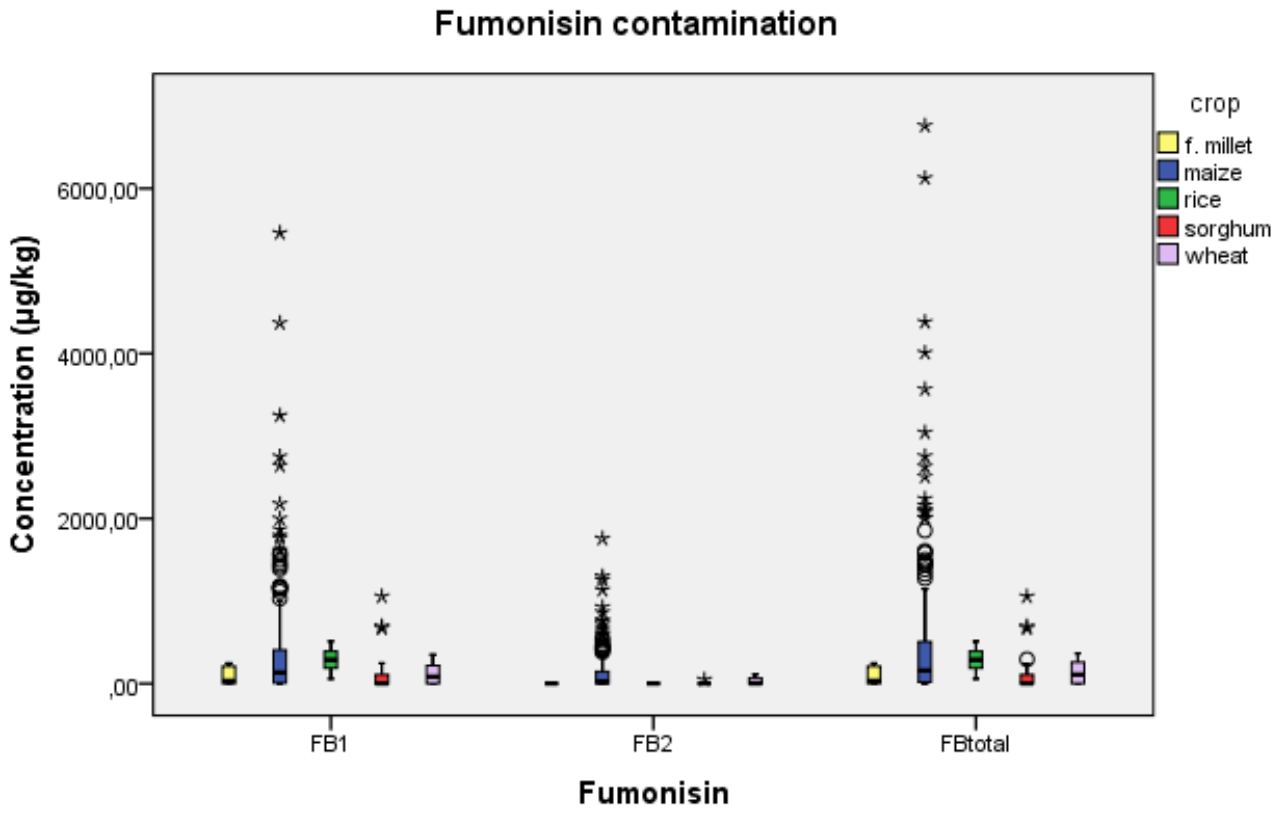

Figure 4: Overall occurrence of fumonisins in maize, wheat, sorghum, rice and finger millet

Maize, rice and wheat have a higher prevalence for FB_1 (median level of 132.50 µg/kg, 287.46 µg/kg and 81.62 µg/kg, respectively) than sorghum and finger millet (median level of 0.00 µg/kg and 31.96 µg/kg, respectively). In general, maize has the widest range in FB_1 (0.00-5461.00 µg/kg), FB_2 (0.00-1756.80 µg/kg) and total FUMs (0.00-6761.00 µg/kg) concentration. For FB_1,

sorghum has the second widest range (0.00-1055.68 µg/kg), followed by rice (58.63-511.51 µg/kg), wheat (0.00-351.28 µg/kg) and finger millet (0.00-245.82 µg/kg). For FB_2, wheat has a higher range (0.00-111.60 µg/kg) than sorghum (0.00-47.56 µg/kg). For total FUMs, the same trend in prevalence like for FB_1 is observed. Rice, finger millet and sorghum have the same median levels and ranges for total FUMs as for FB_1. Maize and wheat have median levels of 159.18 µg/kg and 109.08 µg/kg, respectively. Maize and sorghum are the only samples with fumonisin concentrations above the regulatory limit of 1000 µg/kg set for fumonisins in European countries (Kimanya et al., 2008b) (table 12).

Table 12: Percentage of maize and sorghum samples with fumonisin concentrations above MTL of 1000 µg/kg

Crop	FB_1	FB_2	Total FUMs
Maize	14	2	16
Sorghum	3	0	3

The difference in fumonisin concentration between the five crops is compared to determine whether there were significant differences or not. FB_1, FB_2 and total FUMs contamination in maize was significantly higher than contamination in sorghum, in wheat and in finger millet ($p \leq 0.05$). FB_1 and total FUMs in rice is significantly higher than in wheat, in sorghum and in finger millet ($p \leq 0.05$). Wheat has a significantly different FB_2 contamination than sorghum ($p \leq 0.05$). Maize in Kilosa shows a significantly higher FB_2 and total FUMs contamination than maize in Hanang ($p \leq 0.05$). For sorghum in Kilosa and Hanang, there is no significant difference at all for fumonisin contamination ($p \geq 0.05$).

4.2. CONTAMINATION PER REGION

In Kilosa, contamination data were only collected from maize, sorghum and rice samples. In Hanang, contamination data were collected from maize, sorghum, wheat and finger millet. In table 13, the percentage of maize and sorghum samples with detected mycotoxin concentration above the LOD per region is presented. In general, more samples in Kilosa above the LOD were detected than in Hanang.

Table 13: Percentage of maize and sorghum samples per region with detected mycotoxin concentration above LOD

Region	Sample	FB_1	FB_2	FBs total	AFB_1	AFB_2	AFG_1	AFG_2	AFs total
Kilosa	Maize	71	53	65	46	32	18	17	34
	Sorghum	48	4	40	52	32	40	44	52
Hanang	Maize	60	36	54	14	12	7	8	13
	sorghum	25	0	8	17	8	17	33	17

4.2.1. Contamination in Kilosa

In figure 5 and 6, overall occurrence of aflatoxins and fumonisins in Kilosa are represented, respectively.

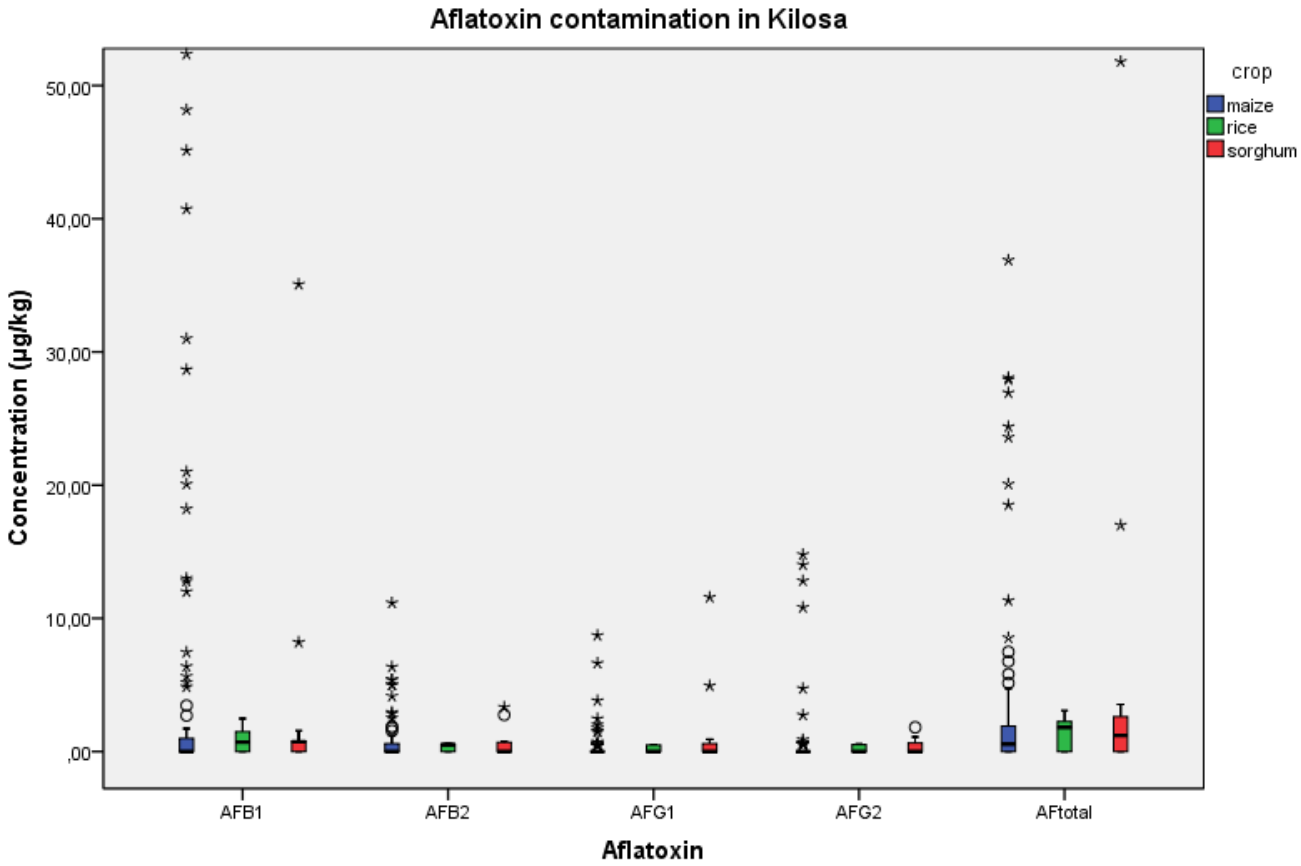

Figure 5: Occurrence of aflatoxins in maize, sorghum and rice in Kilosa

Maize has the highest outliers of aflatoxins, with AFB_1 concentrations up to 94.23 µg/kg, AFB_2 concentration up to 114.19 µg/kg, AFG_2 concentrations up to 67.87 µg/kg and total AFs concentration up to 219.45 µg/kg. Rice has a low spread of AFB_1 (0.00-2.48 µg/kg) and total AFs (0.00-3.07 µg/kg). Sorghum has a low prevalence of AFB_1 (median level = 0.00 µg/kg), however one outlier showed a concentration of 35.08 µg/kg. The prevalence of total AFs (median level = 0.60 µg/kg) is also low, with outliers of total AFs up to 51.80 µg/kg. Sorghum has AFG_1 concentrations up to 11.58 µg/kg. With the outliers left out of consideration, rice has the highest prevalence for AFB_1 and total AFs (median level of 0.70 µg/kg and 1.82 µg/kg, respectively), followed by sorghum (median level of 0.73 µg/kg and 1.20 µg/kg, respectively) and maize (median level of 0.70 µg/kg and 0.57 µg/kg, respectively).

All three samples have a very low prevalence for AFB_2 (rice has a median level of 0.51 µg/kg, maize and sorghum have median levels of 0.00 µg/kg), AFG1 and AFG_2 (median levels of 0.00 µg/kg)(figure 5). The MTL of 5 µg/kg for AFB_1 and 10 µg/kg for total AFs is exceeded in 8 % and 12 % of the sorghum samples, respectively. While for maize, 17 % of the samples exceeded the

regulatory limit of 5 µg/kg for AFB_1 and 15 % exceeded the regulatory limit of 10 µg/kg for total AFs.

For aflatoxin contamination in Kilosa, there is no significant difference for AFB_1, AFB_2 and total aflatoxin contamination between maize, sorghum and rice ($p \geq 0.05$). AFG_1 contamination in maize is significantly different than in rice as well as in sorghum ($p \leq 0.05$). AFG_2 contamination is significantly different in sorghum than in maize ($p \leq 0.05$).

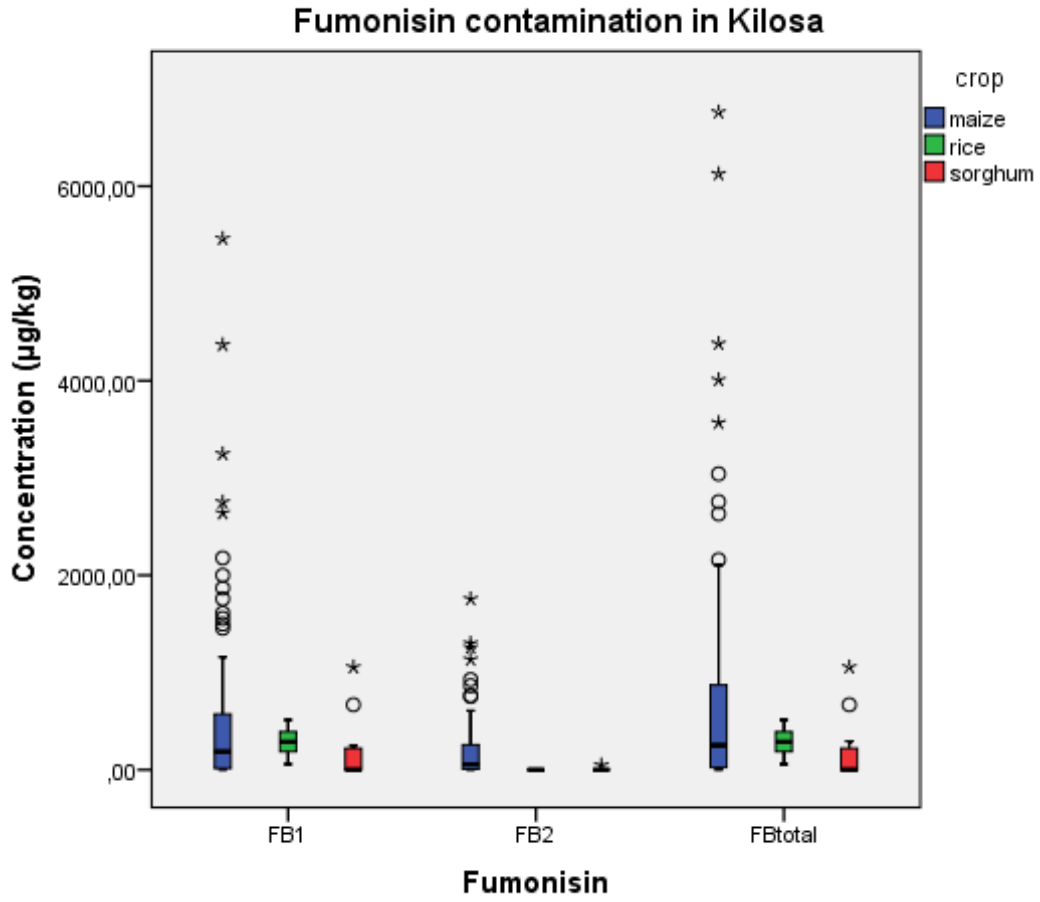

Figure 6: Occurrence of fumonisins in maize, sorghum and rice in Kilosa

Rice has a high prevalence of FB_1 and total FBs concentration (median level = 287.46 µg/kg), which is higher than maize and sorghum. Maize has the most outliers, with FB_1 concentrations up to 5461 µg/kg, FB_2 concentrations up to 1756.80 µg/kg and total maximum FUMs concentrations of 6761.00 µg/kg. With the outliers left out of consideration, maize has also an average prevalence of FB_1 concentration (median=187.50 µg/kg) and total FUMs concentration (median=252 µg/kg). In sorghum only one FB_2 concentration is detected (47.56 µg/kg). Sorghum

has a low prevalence of fumonisins (median level=0.00 µg/kg for FB_1 and total FUMs) (figure 6). The percentage of maize samples exceeding the MTL of 1000 µg/kg was 19% for FB_1, 4 % for FB_2 and 21 % for total FUMs. The MTL of 1000 µg/kg is exceeded in 4 % of the sorghum samples for both FB_1 and total FUMs (one outlier of 1055.68 µg/kg).

FB_1, FB_2 and total FBs contamination is significantly higher in maize than in sorghum ($p \leq 0.05$). FB_1 and total FUMs contamination in rice is significantly higher than in sorghum ($p \leq 0.05$). FB_2 contamination is significantly higher ($p \leq 0.05$) in maize than in rice.

4.2.2. Contamination in Hanang

Figure 7 and 8 represent occurrence of aflatoxins and fumonisins in Hanang, respectively.

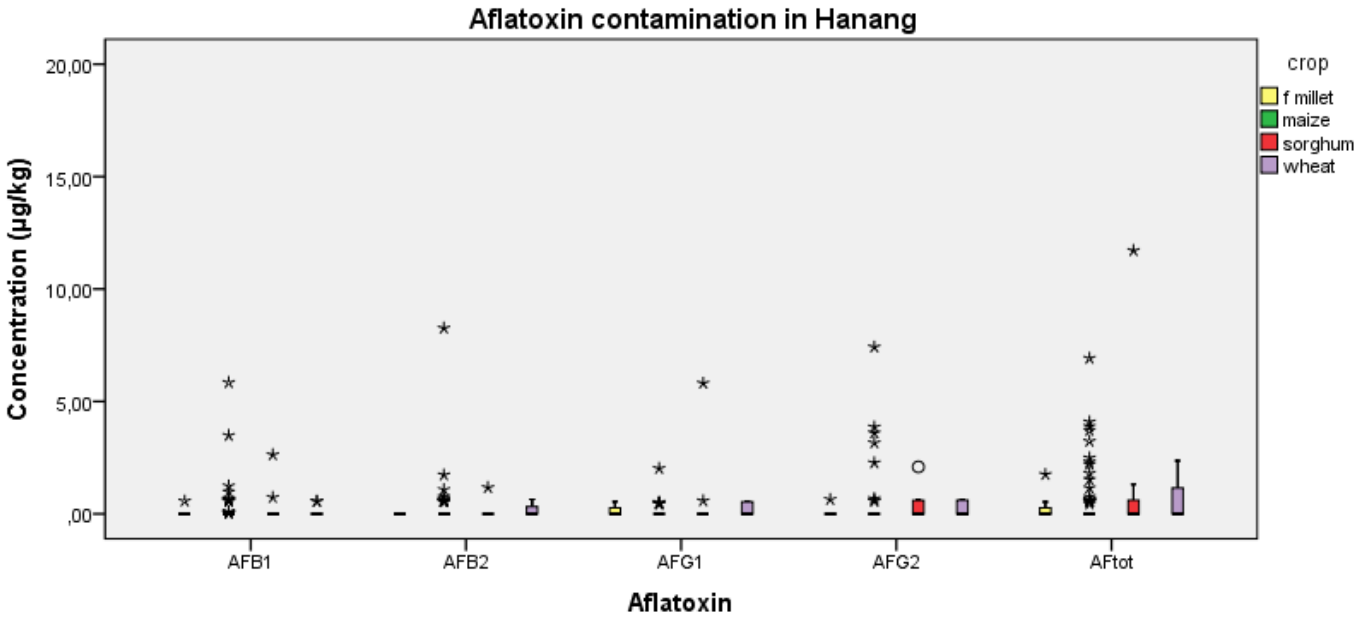

Figure 7: Occurrence of aflatoxins in maize, sorghum, wheat and finger millet in Hanang

Maize has a wide spread of aflatoxin contamination. AFB_1 concentrations are measured up to 73.90 µg/kg and total AFs concentrations up to 91.60 µg/kg. Of the maize samples, 3 % strongly exceeds the regulatory limit of 5 µg/kg for AFB_1 and 1 % exceeds the regulatory limit of 10 µg/kg for total AFs. Finger millet and wheat have comparable low contaminations for all aflatoxins except AFB_2, which was not detected in finger millet and total AFs, which is higher in wheat (2.36 µg/kg). Finger millet showed only one AFB_1 contaminated and one AFG_2 contaminated sample. Sorghum has a low contamination of aflatoxins, only one sample is contaminated with

AFB_2 and maximum total AFs concentration is 11.71 µg/kg, which exceeds the regulatory limit of 10 µg/kg (figure 7). Wheat has the highest interquartile range for total AFs (0.00-1.16 µg/kg), followed by sorghum (0.00-0.61 µg/kg).

There are not many significant differences observed between the crops. Between maize and wheat, there is a significant difference in AFG_1 and AFG_2 contamination ($p \leq 0.05$). Maize has a significant different AFG_1 contamination than sorghum ($p \leq 0.05$).

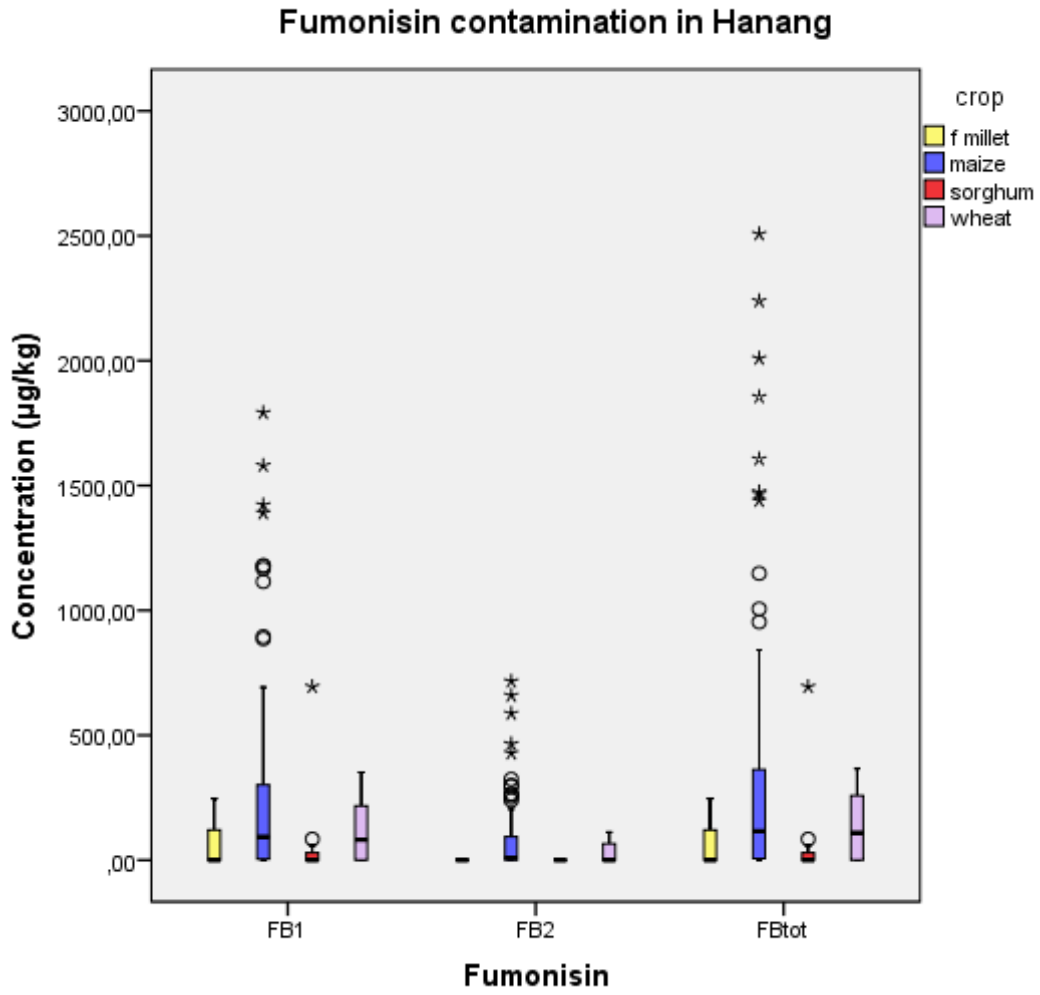

Figure 8: Occurrence of fumonisins in maize, sorghum, wheat and finger millet in Hanang

Maize has the widest range in FB_1 concentration (0.00-1791.60 µg/kg), FB_2 concentration (0.00-715.80 µg/kg) and total FUMs concentration (0.00-2507.40 µg/kg). For FB_1 and total FBs, 8 % and 10 % of the maize samples exceeds the MTL of 1000 µg/kg, respectively. Sorghum and finger millet have a low prevalence for FB_1 (median levels=0.00 µg/kg), with one outlier of 694.49 µg/kg for sorghum. Wheat and maize have an average prevalence of FB_1 concentration

(median level of 81.62 µg/kg and 91.70 µg/kg, respectively). Sorghum and finger millet showed no FB_2 contamination, while maize and wheat have a low prevalence (median levels of 12.00 µg/kg and 0.00 µg/kg, respectively).

For total FUMs, without the outliers, wheat has the second widest interquartile range (0.00-366.54 µg/kg), followed by finger millet (0.00-176.71 µg/kg) and sorghum (0.00-44.10 µg/kg). Maize and wheat have high, equivalent prevalences for total FUMs (median level of 121.70 µg/kg and 109.08 µg/kg, respectively) (figure 8). None of the sorghum samples exceeded the MTL of 1000 µg/kg.

Maize has a significantly higher FB_1, FB_2 and total fumonisin concentration than sorghum, wheat as well as finger millet ($p \leq 0.05$). Wheat has a significantly higher FB_1, FB_2 and total FUMs contamination than sorghum ($p \leq 0.05$).

4.3. EXPOSURE ASSESSMENT

4.3.1. Overall exposure assessment per crop

Deterministic results are presented below and present overall mean exposure to each mycotoxin by consumption of the five different complementary foods (table 14, 15 and 16). The consumption data for maize from the previous study in Kilosa in 2012 are used for calculations. For the other crops the average food intake in different rural areas is used for calculations (Smith & Subandoro, 2007). It is assumed that these intakes are representative for Kilosa as well as Hanang. All dietary exposure values which exceeds the TDI (<1.0 ng/kg for aflatoxins) or PMTDI (2.0 µg/kg for fumonisins) are marked red (Kimanya et al., 2009; Matumba, 2014) (table 14, 15, 16, 17, 18 and 19).

In general, the risk of exposure to aflatoxins as well as to fumonisins is the highest in maize. The mean consumption value (for Kilosa) used in the calculations was 0.0037 kg/kg bw/day, which is almost the half of the mean food intake in the different rural areas in Tanzania (0.0068 kg/kg bw/day) (Smith & Subandoro, 2007). This could indicate that the consumption of maize in Kilosa is lower than in other rural areas. If the consumption of maize in all rural areas would be considered, the exposure would be almost twice as high. Rice has the second highest risk of FB_1 and total FUMs exposure, followed by sorghum, wheat and finger millet. The exposure to aflatoxins in wheat and finger millet is much lower than in rice and sorghum. This could be due to the consumption of rice (0.000957 kg/kg bw/day) which is much higher than that of wheat (0.000187 kg/kg bw/day) and finger millet (0.000072 kg/kg bw/day) in different rural areas in

Tanzania (Smith & Subandoro, 2007). The exposure to aflatoxins is higher in sorghum than in rice.

For maize, all aflatoxins exceed the TDI and the difference is greater between low, medium and higher bound than in sorghum and rice. In sorghum only AFB_1 and total AFs exceed the TDI and in rice only total AFs. The exposure of total AFs in sorghum (0.0029 µg/kg bw/day) is higher than the exposure in rice (0.0015 µg/kg bw/way). For fumonisins, only total FUMs exposure in maize in higher bound scenario (2.0627 µg/kg bw/day) exceeds the PMTDI of 2 µg/kg. For aflatoxins, only maize, sorghum and rice exceed the TDI of 1 ng/kg in lower, medium and higher bound scenario.

Table 14 : Mean exposure to mycotoxins by consumption of five complementary foods (µg/kg bw/day) in lower bound scenario

Mycotoxin	Maize	Sorghum	Wheat	Rice	Finger millet
	MEAN	MEAN	MEAN	MEAN	MEAN
FB_1	1.45	0.0960	0.0209	0.2647	0.0065
FB_2	0.48	0.0011	0.0048	0.0000	0.0000
Total FUMs	1.91	0.0905	0.0242	0.2527	0.0059
AFB_1	0.01	0.0013	0.0000	0.0008	0.0000
AFB_2	0.00	0.0003	0.0000	0.0003	0.0000
AFG_1	0.00	0.0006	0.0000	0.0001	0.0000
AFG_2	0.00	0.0003	0.0000	0.0002	0.0000
Total AFs	0.02	0.0024	0.0001	0.0014	0.0000

Table 15 : Mean exposure to mycotoxins by consumption of five complementary foods (µg/kg bw/day) in medium bound scenario

Mycotoxin	Maize	Sorghum	Wheat	Rice	Finger millet
	MEAN	MEAN	MEAN	MEAN	MEAN
FB_1	1.4840	0.1085	0.0229	0.2647	0.0075
FB_2	0.5304	0.0187	0.0081	0.0167	0.0017
Total FUMs	1.9952	0.1180	0.0285	0.2605	0.0083
AFB_1	0.0119	0.0013	0.0000	0.0008	0.0000
AFB_2	0.0039	0.0003	0.0001	0.0003	0.0000
AFG_1	0.0011	0.0008	0.0000	0.0003	0.0000
AFG_2	0.0039	0.0004	0.0000	0.0002	0.0000
Total AFs	0.0206	0.0027	0.0001	0.0015	0.0001

Table 16: Mean exposure to mycotoxins by consumption of five complementary foods (µg/kg bw/day) in higher bound scenario

Mycotoxin	Maize	Sorghum	Wheat	Rice	Finger millet
	MEAN	MEAN	MEAN	MEAN	MEAN
FB_1	1.5171	0.1210	0.0249	0.2647	0.0085
FB_2	0.5758	0.0362	0.0115	0.0335	0.0034
Total FUMs	2.0627	0.1454	0.0327	0.2683	0.0106
AFB_1	0.0127	0.0013	0.0000	0.0008	0.0000
AFB_2	0.0042	0.0003	0.0001	0.0003	0.0000
AFG_1	0.0014	0.0009	0.0000	0.0004	0.0000
AFG_2	0.0040	0.0005	0.0001	0.0002	0.0000
Total AFs	0.0219	0.0029	0.0002	0.0015	0.0001

4.3.2. Exposure assessment per region

If the exposure to mycotoxins is considered in the two regions apart from each other, it is noticeable that there are more exposure values that exceed the TDI of 1 ng/kg bw/day for aflatoxins or PMTDI of 2 µg/kg bw/day for fumonisins (tables 17, 18 and 19). The exposure risk to all of the mycotoxins is much higher in Kilosa than in Hanang, for both consumption of maize and sorghum. Exposure values by consumption of rice (Kilosa) are also much higher than

exposures by consumption of wheat and finger millet (Hanang) (tables 14, 15 and 16). For maize in Kilosa, all exposure values to aflatoxins exceed the TDI. Only maize in Kilosa exceeds the PMTDI to FB_1 and total FUMs. For sorghum in Kilosa, only exposures to AFB_1 and total AFs exceed the TDI.

Table 17: Mean exposure to mycotoxins (µg/kg bw/day) by consumption of maize and sorghum in two districts in Tanzania in lower bound scenario

		FB_1	FB_2	Total FUMs	AFB_1	AFB_1	AFG_1	AFG_2	Total AFs
Maize	Kilosa	1.9921	0.6908	2.6564	0.0180	0.0067	0.0012	0.0069	0.0324
	Hanang	0.8957	0.2660	1.1559	0.0043	0.0006	0.0002	0.0008	0.0057
Sorghum	Kilosa	0.1139	0.0016	0.1107	0.0018	0.0003	0.0007	0.0003	0.0031
	Hanang	0.0586	0.0000	0.0486	0.0002	0.0001	0.0004	0.0003	0.0009

Table 18: Mean exposure to mycotoxins (µg/kg bw/day) by consumption of maize and sorghum in two districts in Tanzania in medium bound scenario

		FB_1	FB_2	Total FUMs	AFB_1	AFB_1	AFG_1	AFG_2	Total AFs
Maize	Kilosa	2.0237	0.7334	2.7301	0.0186	0.0069	0.0016	0.0069	0.0336
	Hanang	0.9387	0.3232	1.2454	0.0052	0.0009	0.0006	0.0008	0.0072
Sorghum	Kilosa	0.1248	0.0189	0.1341	0.0018	0.0004	0.0009	0.0004	0.0033
	Hanang	0.0744	0.0181	0.0844	0.0002	0.0001	0.0006	0.0004	0.0013

Table 19: Mean exposure to mycotoxins (µg/kg bw/day) by consumption of maize and sorghum in two districts in Tanzania in higher bound scenario

		FB_1	FB_2	Total FUMs	AFB_1	AFB_1	AFG_1	AFG_2	Total AFs
Maize	Kilosa	2.0336	0.7629	2.7588	0.0191	0.0071	0.0019	0.0069	0.0346
	Hanang	0.9766	0.3787	1.3275	0.0061	0.0011	0.0010	0.0009	0.0087
Sorghum	Kilosa	0.1357	0.0363	0.1575	0.0018	0.0004	0.0010	0.0005	0.0035
	Hanang	0.0901	0.0361	0.1202	0.0002	0.0002	0.0008	0.0005	0.0017

5. DISCUSSION

Overall aflatoxin and fumonisin analysis showed that all five crops are contaminated with aflatoxins and fumonisins. In the overall aflatoxin analysis, there is no trend in contamination observed, where one crop showed a complete significant different contamination for all four of the aflatoxins than another crop. There were, however, some differences in contamination for AFG_1 and AFG_2 between maize, sorghum and wheat. Rice shows higher contamination with AFB_1 and total AFs concentrations than wheat. However, mycotoxin contamination in rice is usually lower than in wheat (Tanaka et al., 2007). Wheat and finger millet have the lowest AFB_1 and total AFs contamination. Binder et al. (2007) detected no aflatoxins in wheat above the limit of quantification in surveys in Asia, Oceania, Europe and the Mediterranean region. In Ethiopia, also low concentrations of finger millet are found (Chala et al., 2014). Only maize and sorghum samples show aflatoxin concentrations above the regulatory limit of 5 µg/kg and 10 µg/kg for AFB_1. Maize shows the highest aflatoxin concentrations up to 219.45 µg/kg, which are higher than Kimanya et al. (2008b) found (158 µg/kg). Thus in the overall aflatoxin analysis, it seems that wheat and finger are the least aflatoxin contaminated crops, while maize, sorghum and rice show the highest contamination. Aflatoxins are typically formed during storage conditions when grains have low moisture content, as these toxins are able to grow in dry and hot conditions (Logrieco et al., 2003; Miller, 2008). Improper storage, transportation and processing facilities in poor hygienic conditions may stimulate fungal growth. The reason why no significant differences in aflatoxin contamination between the crops are found, could be due to the fact that aflatoxin contamination is mostly influenced by storage conditions and less by environmental, local conditions. Farmers might use the same storage facilities for the different crops (Darwish et al., 2014; Wagacha & Muthomi, 2008).

In the overall fumonisin analysis, it seems that maize and rice are higher contaminated than wheat, sorghum and finger millet. However, rice is usually lower contaminated than wheat (Tanaka et al., 2007). FB_2 contamination is very low in all the crops and absent in rice and finger millet. Maize and sorghum are the only crops with concentrations above the MTL of 1000 µg/kg, with more maize samples (16%) exceeding the MTL for FB_1+FB_2 than sorghum samples (3%). The maximum fumonisin concentrations found in maize (up to 6761 µg/kg) are lower than those found in previous studies (up to 11 048 µg/kg) (Kimanya et al., 2008). So, maize and rice show the highest fumonisin contamination, while wheat, sorghum and finger millet are the least contaminated. A great limitation of the analysis of fumonisins and aflatoxins was the fact that for each crop not the same amount of samples is analyzed and that a lot of samples can present

undetectable levels of mycotoxin contamination (table 9), while fewer samples can show high levels of contamination (Marin et al., 2013). Fumonisins are typically formed in the field and contamination is influenced by environmentel factors such as temperature, drought stress, insect infestation, rainfall during pre-harvest periods and improper agricultural practices, like delayed harvesting and intercropping. These factors are all very local and thus the levels of fumonisins vary between different growing areas and seasons (Darwish et al., 2014; Fandohan et al., 2005a, 2005b; Gnonlonfin et al., 2013; Miller, 2008; Placinta, D'Mello & Macdonald, 1999; Shephard, 2008 Wagacha & Muthomi, 2008).

In terms of aflatoxin contamination per AEZ, crops in Kilosa are more prone to contamination than crops in Hanang. Maize from Kilosa is higher contaminated with aflatoxins than maize from Hanang, with more maize samples exceeding the regulatory limit of 5 µg/kg for AFB_1 and 10 µg/kg for total AFs in Kilosa. Sorghum from Kilosa is also higher contaminated with AFB_1 than sorghum from Hanang. In Hanang, only one sorghum sample exceeded the regulatory limit for AFB_1 and total AFs, which is less than in Kilosa. Between the crops from Kilosa (maize, rice and sorghum), no differences in aflatoxin contamination are found, except for AFG_1 and AFG_2 levels. The same trend is observed between crops in Hanang (maize, sorghum, wheat and finger millet). However, a lot of samples can present undetectable levels aflatoxin contamination (table 9), while fewer samples can show high levels of contamination (Marin et al., 2013). For example, only 7% and 8% of the maize samples in Hanang have detectable AFG_1 and AFG_2 concentrations above the LOD, respectively. Thus, more aflatoxin contamination is found in crops in Kilosa. Farmers from different regions probably use different storage facilities, which could explain the variable aflatoxin contamination among the different AEZ. Farmers in Kilosa let their maize sun-dry for a shorter period than farmers in Hanang. Furthermore, in Hanang more farmers sort visually damaged grains out before storage. In Kilosa, more polythene bags are used for storage than in Hanang, where more traditional cribs are used. Polythene bags are not air-tight and this might facilitate fungal contamination and aflatoxin production. Drying and sorting out of damaged kernels, can greatly reduce post-harvest contamination in cereals (Degraeve, 2013; Hell & Mutegi, 2011).

The results of fumonisin contamination per AEZ, indicate that crops from Kilosa are more prone to fumonisin contamination than from Hanang. Maize from Kilosa is higher contaminated with FB_2 and total FUMs contamination than maize in Hanang, with more maize samples in Kilosa exceeding the MTL than in Hanang. Sorghum from Kilosa has no significant different fumonisin contamination than sorghum from Hanang. In Hanang however, wheat shows a higher fumonisin

contamination than sorghum, while sorghum from both regions has no different fumonisin contamination than wheat. So this could indicate that sorghum from Hanang has a lower fumonisin contamination than sorghum from Kilosa. In Hanang, maize is higher contaminated than sorghum, wheat and finger millet. Sorghum shows no FB_2 contamination and none of the sorghum samples exceed the MTL for fumonisins. In Kilosa, sorghum was the least contaminated crop, but the MTL is exceeded. So, crops from Kilosa are more prone to fumonisin contamination than crop in Hanang. Kilosa is located 300 km inland from the coast and has a warm and humid climate, with average rainfall between 800-1400 mm and average temperature of 25°C. Fumonisin contamination is more likely to occur when warm weather is followed by high rainfalls. The role of high humidity is very important, which is more favourable for field fungi to grow. Hanang, located in North-Eastern Tanzania with altitudes between 1000 m and 2000 m above sea level, has a more temperate, dry climate. Average rainfalls (700-900 mm) and temperature (20-25 °C) are lower than in Kilosa (Fandohan et al., 2005a, 2005b; Hanang district council, 2012; Kajembe et al., 2013).

In general, the risk of exposure to aflatoxins as well as to fumonisins is the highest by consumption of maize. The exposure to aflatoxins in wheat and finger millet is much lower than in rice and sorghum. Consumption of rice has the second highest risk to FB_1 and total FUMs exposure, followed by sorghum, wheat and finger millet. Consumption patterns show that wheat and finger millet are the least consumed cereals (0.000187 kg/kg bw/day and 0.000072 kg/kg bw/day, respectively). Rice and sorghum have equal consumption patterns (0.000957 kg/kg bw/day and 0,0008 kg/kg bw/day, respectively). The consumption of maize in Kilosa (0.0037 kg/kg bw/day) is almost twice as high as in rural Tanzania (0.0068 kg/kg bw/day) (Smith & Subandoro, 2007). The PMTDI of 2 µg/kg for fumonisins (total FUMs) is only exceeded by consumption of maize. The TDI of 1 ng/kg for aflatoxins is exceeded by consumption of maize (all aflatoxins), sorghum (AFB_1 and total AFs) and rice (total AFs). However, in this study only a deterministic exposure assessment could be performed. This point-estimate approach is normally used as a first step in assessing exposure. Inherent to the point-estimate approach are the assumptions that all individuals consume the specified food(s) at the same level, that the mycotocin is always present in the food(s) and that it is always present at an average or high level. It is therefore not as representative as a probabilistic approach (Lambe, 2002). Considering the exposure to mycotoxins per AEZ, the exposure risk to all of the mycotoxins is much higher in Kilosa than in Hanang, for both consumption of maize and sorghum. Exposure values by consumption of rice (Kilosa) are also much higher than exposures by consumption of wheat and finger millet (Hanang). In this study, only consumption data from Kilosa could be

used, therefore exposure values for Hanang are not very accurate and representative. So, it seems that mycotoxin exposure is the highest in maize, rice and sorghum. The exposure risk is also higher in Kilosa than in Hanang.

Since wheat and finger millet show the least overall mycotoxin contamination and risk exposures, these cereals would serve as the best partially replaceable cereals for maize as complementary foods. Millets, such as finger millet, are considered superior to cereals with respect to some nutrients especially protein, mineral and fat (Kaur et al., 2014). The 8-11 % total protein content, which is comparable with maize (12.1 %), is better balanced than that of other cereals, with higher amounts of lysine, threonine and valine. Finger millet is more palatable and the mineral content (2.7 %), especially calcium, is greater than that of rice (0.6 %) or wheat (1.5 %). It has potential health benefits, of which some are contributed to the polyphenol content (Alonso et al., 2013; Mamiro et al., 2005; Mbithi-Mwikya, 2002; Saleh et al., 2013). Unfortunately, millets contain substantial amounts of antinutritional factors such as tannins (up to 72 g/kg) (Gilani, Xiao & Cockell, 2012). Protein quality of wheat is of poor quality, because it has limiting amounts of two essential amino acids, namely lysine and threonine (Friedman, 1996). Wheat contains also some antinutritional components such as phytic acid (53 g/kg protein), but this is much lower than in maize (88 g/kg protein) (Gilani, Xiao & Cockell, 2012).

Rice has safe mycotoxin concentrations below the toxicological limits, but the risk of exposure to aflatoxins is higher than the toxicological limit. Rice has a protein content between 5 and 7%, which is lower than those found in most other cereals. However, rice is a better quality protein than wheat, because the lysine content of rice proteins (3-4%) is more than 50% greater than that of wheat and the amino acid balance is better (Friedman, 1996). So, on pure nutritional basis, rice could also serve as complementary food, better than wheat. Sorghum has toxicological mycotoxin concentrations and a dangerous risk of exposure to especially aflatoxins. It is however, one of the most important staple foods for many people in the semi-arid tropics of Africa, with a cultivated area of 24.5 million ha (Elbashir & Ali, 2013; Kaur et al., 2014). The essential amino acid concentrations are low, especially lysine and threonine. Sorghum contains some antinutritional components, like tannins (up to 79 g/kg) and phytic acid (101 g/kg protein), which are higher than finger millet and maize, respectively. Because of the very poor energy and digestibility, sorghum grain flour is not recommended for consumption by small children (Friedman, 1996; Gilani, Xiao & Cockell, 2012).

Another food source possible for complementary feeding is legumes. Legume seeds are important sources of food and feed proteins, which are a necessary supplement to other protein sources. Mixtures of cereals with locally available legumes that are high in protein and lysine, but low in sulphur amino acids, increases protein content of cereal-legume blends through complementation of their individual amino acids (Duranti & Gius, 1997; Osundahunsi & Aworh, 2003).

6. CONCLUSION

Maize serves as the main cereal used for complementary feeding in Tanzania, which is highly contaminated with fumonisins and aflatoxins. Improved farming practices and storage technologies for maize are not always possible to implement in African countries due to large capital investments. As population is rapidly increasing, more production of maize and other food sources are required. Strategies to reduce mycotoxin intake can be based on limiting the level of toxin in the complementary food, but contamination values can be extremely high. So limiting the consumption of contaminated food by partial replacement of cereals with lesser mycotoxin contamination is required.

In this study, maize, sorghum, rice, wheat and finger millet are analysed in two AEZ for aflatoxin and fumonisin contamination and exposure risks. Wheat and finger millet are generally the least contaminated with mycotoxins. Maize and sorghum are the only crops with toxicological mycotoxin contaminations and have the highest risk of exposure, exceeding the PMTDI for people consuming these cereals. Rice has also a high mycotoxin contamination and the exposure risk to mycotoxins by consumption is also alarming, but less than for maize and sorghum. Analysis per AEZ, showed that crops in Kilosa are more prone to mycotoxin contamination than in Hanang. This is probably due to better storage conditions, agricultural practices and longer drying periods in Hanang.

Wheat, finger millet and rice are recommended as alternative cereals for complementary foods, partially replacing maize. Finger millet and rice are nutritionally considered as the best options for additional use in complementary foods. Given the results, there is still a lot of research needed where the nutritional advantages and disadvantages of cereals have to be considered, together with the risk of mycotoxin contamination. The option of legumes of additionally complementary food has to be considered as well, with further mycotoxin research on these food sources. Mycotoxin problems in developing countries can only be handled when the overall food safety, health and agricultural issues are considered together. Therefore, training and awareness campaign are needed to inform farmers, traders and processors about the risk of toxin contamination.

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