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Occurrence and risk assessment of mycotoxins in subsistence farmed maize from Zimbabwe

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ABSTRACT

Maize is the staple food of Zimbabweans and is consumed daily in the majority of households, particularly subsistence farming households. The objectives of this study were first, to determine the occurrence of mycotoxins in maize produced and consumed by subsistence farmers in Zimbabwe and second, to determine mycotoxin exposure through maize consumption and subsequently the human health risk. A total of 95 maize meal samples were collected from the household stores of randomly selected subsistence farming households. Maize intake data and agronomic practices of these households were investigated A multi-mycotoxin LC-MS/MS method was used to analyze and quantify mycotoxin contamination in the maize samples. Mycotoxin contamination was compared across agro-ecological zones in order to determine differences in mycotoxin contamination levels and presented. Of the toxicologically relevant mycotoxins, aflatoxin B1 (AFB1), fumonisin B1 (FB1), FB2, deoxynivalenol (DON) and zearalenone (ZEN) were detected in 1, 95, 31, 24 and 15 % of the samples at mean levels of 11, 242, 120, 217 and 110 μg/kg respectively. Other mycotoxins detected in the maize were 15-acetyl-deoxynivalenol (15-ADON), nivalenol (NIV), FB3, alternariol-methylether (AME), AFB2, AFG1 and diacetoxyscirpenol (DAS) and the percentage contamination ranged between 1 and 4 % in the maize samples. Contamination of the maize by the mycotoxins was observed at minimum levels below limit of detection for each mycotoxin and maximum levels of 105, 530, 67, 108, 3, 4 and 14 μg/kg for 15-ADON, NIV, FB3, AME, AFB2, AFG1 and DAS respectively. The median levels of each mycotoxin were reported below the limit of detection, with the exception of FB1 (median, 146 μg/kg), which was further considered in the exposure and risk assessment. Dietary exposure was derived from combining mean maize intake data and median FB1 contamination. Mean maize intake was estimated to be 26.8, 37.2, 30.1, 15.8 and 15.0 g/kg bodyweight (bw)/day for under 5s, children, adolescents, adults and the elderly respectively. Subsequently FB1 exposure from maize was calculated, to be 3.91, 5.40, 4.40, 2.30 and 2.20 μg/kg bw/day for these populations. Exposure to FB1 through maize intake was observed to equate to 196, 272, 220, 115 and 110 % of the provisional maximum tolerable daily intake (2 μg/kg bw/day) for under 5s, children, adolescents, adults and the elderly respectively. Subsistence farming communities in Zimbabwe are at risk of high exposure to FB1 and the risk was highest for under 5s, children and adolescents respectively.

1. Introduction

Mycotoxins are small molecular weight chemical substances (Steyn, 1995, Pitt, 2000) classified as secondary metabolites that are toxic in nature and produced by a variety of fungi in a number of plants, crops, fruits and seeds (Richard, 2007). They are often naturally occurring in either free or modified forms (De Boevre et al., 2012). As such they can find their way into the food chain resulting in human exposure either by direct consumption (humans eating contaminated plants, crops, fruits and seeds) or indirectly through ingestion of exposed animals (chicken, pigs or cows, among others). This process triggers acute or chronic effects

Abbreviations: **LSD**, least significant difference; **PMTDI**, provisional maximum tolerable daily intake.

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depending on severity and duration of exposure (Steyn, 1995, Pitt, 2000). Some mycotoxins have been further classified as mutagenic, carcinogenic or teratogenic based on evidence of such effects in various experimental animals. These include alternariol, aflatoxin B1 (AFB1), T-2 toxin and ochratoxin A (OTA) respectively (Fleck et al., 2016, IARC, 2011, Wangikar et al., 2010). The nature and quantities of these ubiquitous hazards in food materials determine whether or not they have the potential to harm human beings if ingested in sufficient amounts (Battilani et al., 2009). In most cases, they are frequently occurring as a mixed cocktail of sub-members of different mycotoxin groups such as aflatoxins, fumonisins, trichothecenes and ergot alkaloids among others in grain (Binder et al., 2007). In sub-Saharan Africa, the most notorious mycotoxins in terms of occurrence are produced mainly by *Aspergillus* spp. (aflatoxins, citrinin and ochratoxins) and *Fusarium* spp. (fumonisins, deoxynivalenol and zearalenone) (Munkvold, 2003). In maize, *Fusarium* spp. toxins are more commonly found and co-occurrence with *Aspergillus* spp. toxins has been identified and documented in literature (Hove et al., 2016). The occurrence of mycotoxins in maize is largely dependent on environmental and agronomic factors. As such, maize is typically contaminated by both field fungi (those occurring at any stage throughout the growth cycle of the crop prior to harvest) and storage fungi (those occurring at any stage throughout the post-harvest phase of the crop). These fungi may produce mycotoxins in the maize given an enabling environment. Pest infestation also plays a significant role in contamination of raw maize by mycotoxins either in the field or during storage, for example, fumonisins (IARC, 2011).

Maize, a major cash crop in Southern Africa, is the staple food of Zimbabweans and is consumed daily in the majority of households. Evidence of contamination of maize from Southern Africa by mycotoxins has been documented in South Africa, Zambia, Mozambique and Zimbabwe from various surveys conducted in the last 20 years (Shephard et al., 2013, Mukanga et al., 2010, Warth et al., 2012, Gamanya and Sibanda, 2001). The co-occurrence of multiple mycotoxins, for example aflatoxins and fumonisins, in maize is of particular concern given the evidence indicating towards modulatory effects on each mycotoxin's individual toxicity (Hove et al., 2016). Furthermore, very limited knowledge exists on levels of combined exposure to various mycotoxins particularly through the consumption of staple foods such as maize. This scenario is undesirable as it pre-disposes affected communities and individuals to both food and health insecurity. Food security is affected when crops are spoiled due to fungal infestation. As a consequence human health is at risk due to consecutive mycotoxin contamination and the result is often the development of chronic illness. Although epidemiological links are clear regarding chronic aflatoxin exposure in humans (IARC, 2011), substantive assumptions have been proposed regarding links to oesophageal cancer in humans as a result of dietary exposure to fumonisins through maize consumption (Yoshizawa et al., 1994, Sydenham et al., 1990). Furthermore, positive correlations have been derived for maize consumption and HIV (Human Immuno-deficiency Virus) infection, hepatocellular and oesophageal cancers in sub-Saharan Africa (Williams et al., 2010) and impaired childhood growth (Kimanya et al., 2010). It is therefore necessary to curtail health risks associated with mycotoxin exposure using a variety of preventive approaches such as good agricultural practice, monitoring of contamination and enforcement of legislation among other risk management measures. Formulation of risk management measures for mycotoxins requires, at first instance, evidence and data on their occurrence in foods and exposure levels. Accurate exposure data on mycotoxins is an important input in risk assessment and management efforts as well as in establishment of appropriate legislation for the monitoring and control of mycotoxin exposure in food (van Egmond, Schothorst, & Jonker, 2007). To date, studies on mycotoxin occurrence in food consumed in Zimbabwe are not sufficient to draw meaningful conclusions on mycotoxin contamination in maize and human exposure. Thus, the aim of this work was, first, to determine the occurrence of mycotoxins in maize produced and consumed by subsistence farmers in Zimbabwe, and second, to identify correlations between mycotoxin occurrence and factors such as agronomic and post-harvest practices, and geographical location.

2. Materials and Methods

2.1. Sampling

Zimbabwe is divided into 6 agro-ecological zones according to rainfall characteristics (Table 1).

Manicaland and Mashonaland West provinces contain all the agro-ecological zones, with the exception of Region I which is only found in Manicaland province. In addition to these natural boundaries, administrative boundaries exist and these are classified into districts, wards and villages in descending order of magnitude. In each province wards were selected in different agro-ecological zones (Figure 1) according to the probability-proportionate-to-size (PPS) method (Magnani, 1997) in the year 2014. From the selected wards, only wards with subsistence farming activities were further selected and wards without subsistence farming activities were excluded in the first knock-out step. Proximity to agrometeorology stations was also a key consideration in the sampling procedure therefore regions III and IV were omitted because of the large radius between the nearest agrometeorology station and the wards containing subsistence farming areas. For the present study, a total of 95 maize meal samples were collected in December 2014 from 95 randomly selected households residing in subsistence farming communities from the selected villages. As a second knock-out criteria, each household had to express willingness to participate in the study and those that were unwilling to participate were excluded from the study. Each participating household provided a 500 g sample of raw maize meal. The samples were collected from the current cooking stock of each household regardless of the source of the maize. The sources of maize were either from the household's harvest (84.2 %), obtained from neighbours (9.5 %) or commercial maize bought from local retailers (6.3 %). The sampling protocol used to select households is illustrated in Figure 2.

2.2. Determination of mycotoxins in maize

A multi-mycotoxin LC-MS/MS method for the detection and quantification of 23 mycotoxins (Ediage et al., 2015), validated according to the European Commission decision 2002/657/EC, was used to analyze and quantify mycotoxin contamination in the maize samples. The method consisted of extraction of 3 g sample with 20 ml methanol/ethyl acetate/water (70/20/10 v/v/v) followed by defatting in 10 ml hexane. Subsequently liquid-liquid extraction of 2.5 ml defatted extract with 10 ml dichloromethane/formic acid (95/5 v/v) and solid phase extraction (SPE) of the remaining defatted extract on Grace® amino SPE cartridges (1000 mg packing) was performed. The product of liquid-liquid extraction was centrifuged and the supernatant was combined with the product from the SPE. This was followed by evaporation to dryness under a gentle nitrogen flow. The samples were re-constituted in injection solvent (48/51/1 v/v/v water/methanol/acetic acid + 5mM ammonium acetate) and analyzed on a Waters Micromass™ Quattro Micro LC-MS/MS system. The limits of detection and quantitation (LOD and LOQ respectively) are given in Table 2. The organic