

Determination of Aflatoxin Level in Stored Maize Flour and Risk of Exposure from Consumption of Aflatoxin Contaminated Meal in Boarding School

Mfinanga Mariam Abdu¹, Suleiman Rashid², Kilima Beatrice^{2,*}

¹Department of Food Science and Agro-Processing, School Engineering and Technology, Sokoine University of Agriculture, Morogoro, Tanzania

²Tanzania Bureau of Standards, Dar es Salaam, Tanzania

Email address:

mrsissamnasha@gmail.com (Mfinanga Mariam Abdu), Rashid@sua.ac.tz (Suleiman Rashid), bkilima@sua.ac.tz (Kilima Beatrice)

*Corresponding author

To cite this article:

Mfinanga Mariam Abdu, Suleiman Rashid, Kilima Beatrice. (2024). *Determination of Aflatoxin Level in Stored Maize Flour and Risk of Exposure from Consumption of Aflatoxin Contaminated Meal in Boarding School*. *International Journal of Microbiology and Biotechnology*, 9(1), 7-14. <https://doi.org/10.11648/ijmb.20240901.12>

Received: December 31, 2023; Accepted: January 15, 2024; Published: February 1, 2024

Abstract: Maize (*Zea mays*) is an important staple food crops for the majority of Africans population including Tanzanians. However, maize is mostly contaminated by mycotoxins which produced by fungal species like *asperigillus*. This study aimed to determine the aflatoxin contamination level in stored maize flour and assess the risk of consumption of aflatoxin-contaminated meal in boarding schools in Dar es Salaam and Coastal regions. A total of 33 samples of maize flour (dehulled and un-dehulled) were collected. High-Performance Liquid Chromatography connected to a fluorescence detector (HPLC-FLD) was used to analyze the levels of aflatoxin contamination (AFB1 and total aflatoxin) in maize flour. In addition, cancer risk was assessed using models prescribed by the Joint FAO/WHO Expert Committee on Additives (JECFA). The result shows aflatoxin contamination (AFB1 and total aflatoxin) in maize flour samples collected from different boarding secondary schools ranged from (0 – 12.98 ppb) for total aflatoxin and (0 – 8.83 ppb) for AFB1. The estimated daily exposure to aflatoxins for girls and boys ranged from (0.11 – 0.26 ppb /kg/day) and (0.10 – 0.26 ppb /kg/day) respectively. For the cancer risk assessments, the result shows range from (0.01 – 0.02 per 100,000 population) for girl and (0 – 0.02 per 100,000 population) for boys. The result surveyed area is at lower risk of developing aflatoxin health related effects such as liver cancer.

Keywords: Maize Flour, Aflatoxins, Boarding School Meals, Cancer Risk, Daily Intake, Estimated Daily Exposure to Aflatoxin, Tanzania

1. Introduction

Maize (*Zea mays*) is an important staple food for more than 1.2 billion people in sub-Saharan Africa (SSA) and Latin America [25]. Maize and its products have long been part of African culture, and they form part of everyday meals in most homes and schools [2, 23]. According to the World Food Programme, maize is a major staple ingredient in school meals in African countries such as Malawi, and approximately 368 million children in developed and developing countries are fed through school meals every day and in most developing countries like Tanzania, they use mostly maize products to prepare meals for the students at school [8, 28]. There are

different causes of maize and its products being contaminated with aflatoxin and among them is improper storage of the product. Storage of maize and maize flour for a long period of time can cause mold growth and may result in contamination.

According to a study, aflatoxin levels increased with storage time, especially in dry and moist areas [9]. This occurs when maize and maize flour are stored for more than six months. Environmental factors like light, moisture, temperature, relative humidity, and atmospheric gases are responsible for aflatoxin contamination. *Aspergillus flavus* growth and aflatoxin production are also affected by the presence of light. Darkness increases aflatoxin production while sunlight inhibits it [13]. The availability of oxygen and carbon dioxide also influences

aflatoxin production. Aflatoxin production and fungal growth are inhibited at a higher level of carbon dioxide and a lower level of oxygen [18]. Consumption of maize and maize-based foods has been reported to have food safety concerns due to the presence of mycotoxins [14]. The toxicants are a global safety concern as they cause foodborne illnesses [5, 12]. Children are most at risk of dietary mycotoxin exposure compared to older people, due to low immune systems, increased food demand, and an uncontrolled diet [10]. The most commonly occurring mycotoxins found in maize and maize-based foods are aflatoxins and fumonisins, which are caused by *Aspergillus* and *Fusarium* species, respectively. The most common types of aflatoxins in foodstuffs include aflatoxin B1, B2, G1, and G2, while fumonisins include fumonisins B1 and B2. Aflatoxins B1 and fumonisins B1 are the most toxic and carcinogenic to humans and animal [26].

Moreover, consumption of aflatoxin-contaminated maize-based food can cause adverse health effects on humans [17]. Aflatoxin toxicity depends on dosage and duration of exposure, nutritional status, immunity, and health. Regular intake of aflatoxin-contaminated meals can lead to high exposure to aflatoxins which can cause health effects in humans (aflatoxicosis). Acute exposure may cause nausea, vomiting, and abdominal pain, which may warrant symptomatic treatment only. Acute high-dose intoxication can be fatal in children. Chronic exposure to low-dose aflatoxin can lead to cirrhosis and hepatocellular carcinoma, which are irreversible disease conditions that can cause death [11, 16]. Many studies have been done to determine aflatoxin levels in maize and maize flour and assess the risk of consumption of aflatoxin-contaminated meal in different areas but few studies have been done on the determination of aflatoxin level in stored maize flour and the risk assessment of consumption of aflatoxin-contaminated meal in boarding schools. This study will determine the level of aflatoxin in maize flour used to prepare meals for boarding school students and assess the risk of exposure to the consumption of aflatoxin-contaminated food. The information obtained from this

study will be useful to government agencies to strengthen school kitchen inspections and provide training to school personnel on the issues of food safety and quality.

2. Materials and Methods

Sample Collection

Seven districts were randomly selected from two regions (Dar es Salaam and Coast region found in Tanzania) based on the criteria of having a large number of boarding secondary schools. The sample size was estimated using the Kothari equation (Kothari and Garg, 2014): -

$$n = \frac{z^2 p(1-p)}{e^2} \quad (1)$$

where, n= sample size, Z= standard variate at a given confidence level, for this study a 95% confidence level = 1.96 and e = acceptable error (the precision/ estimation error) set at 8% (0.08) for this study.

$$n = \frac{(1.96)^2 (0.05)(1-0.05)}{(0.08)^2} \approx 30$$

A systematic random sampling technique was used to select boarding secondary schools that were involved in the study. A total of 33 samples of maize flour (dehulled and unde-hulled) were collected in between April and May 2023. Thirty boardings secondary schools which are found in seven districts of Dar es Salaam (Kinondoni (Kn), Kigamboni (Kg), Ubungu (Ub)) and Coastal region (Kibaha town (KBTc), Kibaha Rural (KBR), Mkuranga (MK) and Bagamoyo (Bg)), Tanzania. Collected samples were packed in clean polythene bags from 30 selected boarding secondary schools. The samples were coded, transported to the Tanzania Bureau of Standards (TBS) food laboratory, and stored at a temperature of 4°C prior to aflatoxin analysis. Figure 1 below shows some districts where this study was conducted.

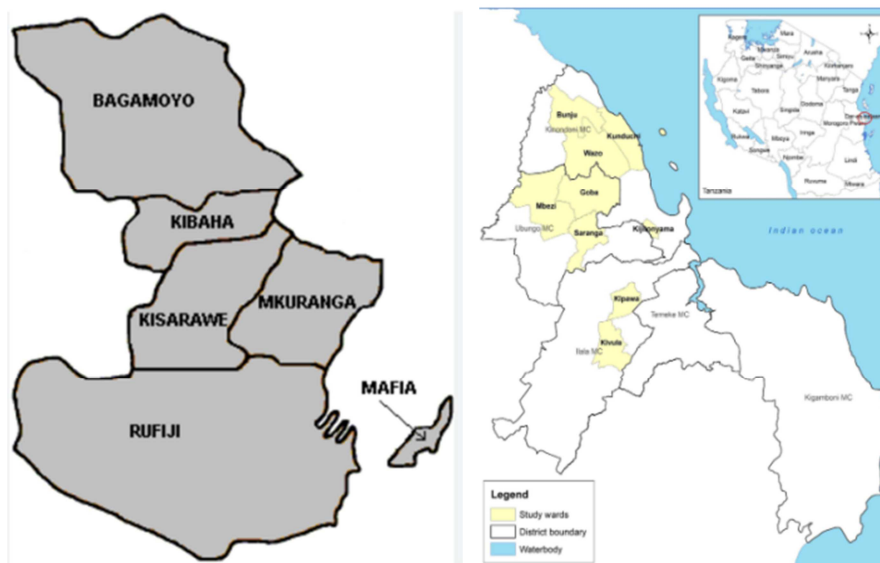


Figure 1. Shows the district of Dar es Salaam and the Coastal region in Tanzania Aflatoxin analysis.

The analysis of aflatoxin (AFB1 and total aflatoxin) maize flour in this study was analyzed/detected by using High-Performance Liquid Chromatography with Fluorescence Detection (HPLC- FLD). The method used for the analysis of aflatoxin B1, B2, G1, G2, and total aflatoxin content in cereals and nuts by HPLC-FLD (FCL/SOP-TM/13-02) and has five steps as follows:

Chemicals and standards

The reagents and chemicals utilized in the various sample preparation techniques were of chromatographic grade. These included distilled water (HPLC quality water type one 1 water) from the lab Honeywell Lot from Germany, acetonitrile Lot No L192A (LC-MS quality), and methanol Lot No M003M (LC-MS Grade). Aflacolumn (immunoaffinity column) and Aflatoxins Standards (AFB1, AFB2, AFG1, and AFG2) (AflaTest from Romer Labs GmbH, Technopark 1, 3430 Tulin, Austria).

Extraction of samples

Each sample of maize flour was put into an Erlenmeyer flask and weighed to a precision of 25 0.1g using an analytical balance that had been calibrated. The sample was placed in a 250 ml Erlenmeyer flask along with 100 ml of methanol: water (70:30 methanol/water) as the extraction solvent. The flask was wrapped with aluminum foil and set on a gyratory shaker (Stuart® Orbital Shaker SSL1, Cole-Parmer LLC, USA) at 250 rpm for 30 minutes. The extract was then filtered into a 250 ml Erlenmeyer flask using Whatman No. 1 filter paper according to the procedure described by [7].

Dilution stage

4 ml of the sample extract was added with 8 ml of distilled water into a Teflon tube, and vortex for 30 seconds to obtain a homogenous mixture (Talboys® Hvy Dty Vortex, Troemner LLC, USA).

Clean-up and elution stage of aflatoxins

Solid Phase Extraction (SPE) immunoaffinity columns were loaded with the diluted extract and allowed to pass through them by gravity. The sample-loaded columns were then washed twice with 10 ml of HPLC-grade water. 1 ml of HPLC-grade methanol was used to elute the adsorbed aflatoxins, and the eluates were then collected in vials. To remove any leftover liquid, a small amount of pressure was applied to the column's top. In accordance with ISO 16050, 0.3 ml of the eluate was combined with 0.6 ml of water and 0.1 ml of acetonitrile before being vortexed for 30 seconds.

HPLC system

Following extraction, dilution, cleaning, elution, and post-column derivatization, the extracts were analyzed using HPLC with a fluorescence detector (FtLD) (Model Agilent ChemStation technology, series 1200, 5301 Stevens Creek Blvd, Santa Clara, CA 95051, USA). Water, methanol, and acetonitrile made up the mobile phase (60:30:10, v/v). AFB1, AFB2, AFG1, and AFG2 were separated on the C18 column at a flow rate of 1.2 ml/min at a temperature of 30 °C. The injection volume was 50 L for both the sample extracts and the standard solution. Following their separation, AFG1 and AFB1 underwent derivatization in order to enable

fluorescence detectors to detect them at 465 nm for emission and 360 nm for excitation.

Determination of the limit of detection and limit of quantitation of the HPLC method

The limit of detection (LOD) and limit of quantitation (LOQ) of the HPLC method for AFB1, AFB2, AFG1, and AFG2 were determined using equations the two equations.

$$\text{LOD} = \text{Mean of the lowest concentration} + 3\text{SD}$$

$$\text{LOQ} = \text{Mean of the lowest concentration} + 10\text{SD}$$

Where SD = Standard deviation of the lowest concentration (Armbruster et al. 2008). The LOD and LOQ values obtained for the four types of aflatoxins are shown in Table 1.

Table 1. The Limit of detection (LOD) and limit of quantitation (LOQ) for each analyzed aflatoxin.

| Compound | LOD | LOQ |
|----------|------|------|
| AFG2 | 0.12 | 0.17 |
| AFG1 | 0.12 | 0.18 |
| AFB2 | 0.12 | 0.17 |
| AFB1 | 0.10 | 0.13 |

Statistical Analysis

Data from the laboratory analysis of aflatoxins levels was analyzed using SPSS Version 27. A one-sample t-test as one of the analyses of variance tests (ANOVA) was used to compare the concentration levels of total aflatoxin and aflatoxin B1 in the maize flour samples against their respective maximum acceptable levels (10 ppb for total Aflatoxin and 5 ppb for Aflatoxin B1) among maize flour samples taken from different boarding secondary schools. The frequency, percentage of distribution, average mean, and overall mean of the assessed variable from the data set were obtained by using descriptive analysis. A p-value of less than 5% was considered significant throughout the conducted analyses.

Daily exposure or intake of students

Daily exposure/intake of students from schools found in each district obtained by using equation 2 below. The equation was inserted into SPSS software and the result is summarized in the Figure 2. The equation is expressed by the following mathematical equation 2 (Stroka, 2011).

$$\text{DI} = \frac{\text{aflatoxin concentration in food} * \text{amount of food consumed}}{\text{body weight}} \quad (2)$$

Estimated daily exposure risk to aflatoxin

The estimated daily exposure risk to aflatoxins was obtained mathematically by using the equation 3 [1] as follows

$$\text{EDI} = \sum_{i=1}^n \frac{D_i * M_i}{W} \quad (3)$$

where EDI = Estimated daily intake (ng kg⁻¹ body weight day⁻¹), Di= Daily consumption of food (person⁻¹ day⁻¹), Mi = Mean level of AFB_i, W = Body weight Kg and n = Number of population characterization of primary liver cancer risk. This equation was inserted in SPSS software version 27 and the results are shown in Figure 3.

Estimated cancer risk

The cancer risk was simulated in equation 3 below by multiplying the probable average potency of cancer [1]. The equation was inserted in SPSS software and provide cancer risk of girls and boys found in boarding secondary schools of all seven districts surveyed in Dar es Salaam and the Coastal region and the results are shown in the table 4.

$$\text{Cancer risk} = \text{Average Potency (P}_{\text{cancer}}) \times \text{Estimated daily intake (EDI)}$$

where

$$P_{\text{cancer}} = (\text{PHBsAg}^+ \text{ of pop} \times \text{HBsAg}^+) + (\text{PHBsAg}^- \text{ of pop} \times \text{HBsAg}^-)$$

Where by

PHBsAg⁺ - Preference rate to cancer of hepatitis B positive group population and PHBsAg⁻ - Preference rate to cancer of hepatitis B negative group population where individual's potency is estimated to be 0.3 cancers per year per 100,000 populations per ng AFB1/kg body weight per day for positive group and 0.01 cancers per year per 100,000 populations per ng AFB1/kg body weight per day for the negative group as estimated by JECFA. HBsAg⁺ - Hepatitis B positive group (assumed as 25% prevalence rate as per (WHO 1998). HBsAg⁻ - Hepatitis B negative group (assumed to be 75% prevalence rate WHO 1998).

3. Results and Discussion

Recovery, limit of detection, and limit of quantification of aflatoxin.

The recoveries of all aflatoxins were greater than 70% (97.8 to 109.7%) indicating the suitability and good performance of the approved aflatoxin extraction protocol and quantification as the acceptable recovery range from (70 to 120%) (Shah et al., 2000). LOD for AFB1, AFB2, AFG1, and AFG2 ranged from 0.10-0.12 (ppb) and LOQ from 0.13-0.18 (ppb).

Level of aflatoxin contamination in maize flour and their comparison by an acceptable level

Table 2. Shows the total aflatoxin and aflatoxin B1 concentration detected from the collected maize flour samples from boarding secondary school.

| Samples | Total Aflatoxin (ppb) | Aflatoxin B1 (ppb) |
|---------|-----------------------|--------------------|
| BG | 12.63 | 4.62 |
| KBR | 18.16 | 8.05 |
| KBT | 7.29 | 4.64 |
| KG | 16.01 | 4.81 |
| KN | 6.16 | 3.54 |
| MK | 12.98 | 8.83 |
| UB | 0.00 | 0.00 |
| Total | 10.41 | 4.72 |

Table 3. One sample p-value results to test the level of total Aflatoxin and Aflatoxin B1 concentration.

| Sample | Total Aflatoxin (Max Acceptable Level 10ppb) | | Aflatoxin B1 (Max Acceptable Level 5ppb) | |
|--------|--|-------------------------------|--|--------------------------------|
| | Sig. | Mean Difference (TAF- Sample) | Sig. | Mean Difference (AFB1 -Sample) |
| BG | 0.64 | 2.63 | 0.83 | -0.38 |
| KBR | 0.35 | 8.16 | 0.11 | 3.05 |
| KBT | 0.36 | -2.71 | 0.87 | -0.36 |
| KG | 0.30 | 6.01 | 0.86 | -0.19 |
| KN | 0.37 | -3.84 | 0.54 | -1.46 |
| MK | 0.71 | 2.98 | 0.46 | 3.83 |
| Total | 0.84 | 0.41 | 0.74 | -0.28 |

The aflatoxin concentration of the collected maize flour samples from boarding secondary schools was analyzed for detection of aflatoxin B1 which is the most harmful toxin to human and animal health [3]. Total aflatoxin which represent the sum of all four types of aflatoxin (AFG1, AFG2, AFB1, and AFB2) found in the analyzed sample. Table 2 above shows aflatoxin concentration (total aflatoxin and AFB1) from maize flour samples collected at different schools found in certain districts and Table 3 indicates aflatoxin concentrations compared to the acceptable level as set by the standards. According to the TBS standard (TZS 328-1:2020/EAS 44:2019 (E) for milled maize products), the average mean of total aflatoxin concentration was (10.41 ppb) across all samples exceeded the acceptable level as it is indicated by the non-significant p-values (0.05). The average mean of aflatoxin B1 across all samples was (4.7206 ppb) which had not exceeded the acceptable level as indicated by non-significant p-values (0.05). Boarding schools' maize flour samples coded as BG, KBR, KG, and MK (12.63ppb, 18.16ppb, 16.01ppb, and 12.98ppb respectively) had higher

concentrations of total aflatoxin compared to the acceptable values (10ppb) as indicated by non-significant p-values (0.05) while total aflatoxin for KBT and KN (7.29ppb and 6.16 ppb respectively) did not exceeding the acceptable level (10ppb) as indicated by non-significant p-values (0.05). This also includes the UB sample where total aflatoxin was not detected. For Aflatoxin B1, the p-values suggest that all samples except KBR, and MK had Aflatoxin B1 levels within the acceptable range. KBR and MK (8.05 ppb and 8.83 ppb respectively). The value is higher than the acceptable limit as indicated by non-significant p-values (0.05). Moreover, the samples had lower aflatoxin levels those detected on dehulled maize (35.88ppb) as reported by [24]. In addition, the study conducted by [22] show that a higher level of aflatoxin contamination (40.5 ppb) in maize flour almost five times higher than those detected in this study. Aflatoxin concentration levels in some of the analyzed maize flour samples are slightly higher than the acceptable level could be due batch procurement (monthly basis) of maize flour. The reasons may include the effect of maize flour production

procedures that can affect the aflatoxin concentration level in maize flour. For resistance efficiencies of dehullers used to prepare maize flour and the extent of fungal and toxin penetration in the maize grains may result in differences in aflatoxin concentration in the maize flour produced [21]. Another reason that could be due to storage conditions both at traders and school levels. If maize and maize flour stored for a long period of time may results mold growth especially in dry and moist areas [9]. Poor storage practices at school stores might also be the cause of the aflatoxin concentration of maize flour. Most of the storage rooms in schools don't allow enough ventilation, no pallets and some keep maize flour in contact with the walls which might cause maize flour to absorb the moisture from the wall to the maize flour. According to [13], environmental factors like light, moisture, temperature, and atmospheric gases are responsible for aflatoxin contamination.

A. flavus growth and aflatoxin production in maize flour is influenced by the presence of light where darkness increases aflatoxin production while sunlight inhibits it [13]. The availability of oxygen and carbon dioxide also affect aflatoxin production and fungal growth are inhibited at a higher level of carbon dioxide and a lower level of oxygen [18].

Daily Exposure/intake to Aflatoxin_{B1}

Daily intake refers to exposure to food chemicals that are inadvertently present in food, or added to food for a technological purpose. Daily exposure to (or intake of) food chemicals is estimated by combining food consumption data with food chemical concentration data divided by body weight which is presented by equation 1. Figure 2 indicates the result of daily exposure/intake of students from schools found in each district served district.

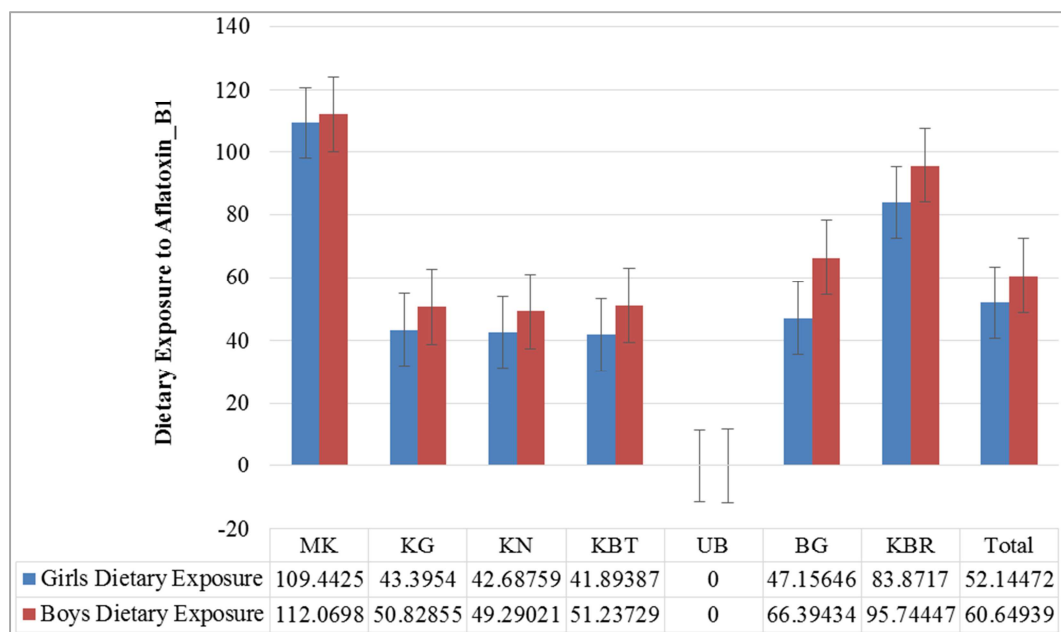


Figure 2. Summary of girls and boys' daily exposure/intake (ppb/kg body weight/day) to Aflatoxin in y axis and sample code from districts in x-axis.

Daily intake of aflatoxins found in maize meal for girls and boys across different boarding secondary schools was measured in g/person/day. The intake of meals prepared by dehulled maize flour for girls and boys 521g per person per day and 662 g per person per day respectively. The highest maize flour AFB₁ contamination range was (8.05-8.83ppb) while the lowest (0 – 4.81ppb). The highest total aflatoxin contamination ranged from (12.63 – 18.16ppb) while the lowest concentration ranged from (0 – 7.29ppb). The highest daily intake of total aflatoxins and AFB₁ due to consumption of maize flour for girls and boys 54.5 ppb/kg body weight/day, and (56.04 ppb/kg body weight/day) respectively. The result shows schools in district MK and KBR had higher daily exposure to aflatoxin compared to other schools (for girls 96.66 ppb/kg body weight (bw)/day) and for boys (103.91 ppb/kg body weight (bw)/day) respectively and aflatoxin BI and total aflatoxin contamination of 8.83ppb and 18.6ppb respectively. Students in these districts had high chance to be

exposed to aflatoxins contamination compared with other schools surveyed in this study. Regular intake of food contaminated with aflatoxins can cause acute aflatoxicosis whereas intake of low to moderate doses over a long period can result in chronic aflatoxicosis. Chronic primary aflatoxicosis results from the ingestion of low to moderate levels of aflatoxins (USAID, 2012). The effects are usually subclinical and difficult to recognize. Some of the common symptoms are impaired food conversion and slower rates of growth with or without the production of an overt aflatoxin syndrome. Chronic forms of aflatoxicosis include teratogenic effects associated with congenital malformations and mutagenic effects [4]. Overall average mean of daily exposure for girls and boys were 52.14 and 60.65 ppb/kg body weight (bw)/day respectively. These values are higher than those reported in Kilimanjaro region where daily exposure to aflatoxins AFB₁ was 0.0811ppb/kg bw/day [24]. The use of cereal based diet may increase the risk of aflatoxins exposure

in boarding schools.

Estimated daily exposure risk to aflatoxins

Dietary exposure assessment is one of the essential elements of the risk assessment process. It provides scientific evidence for the estimated dietary intake (EDI) of contaminants based on the amount of contaminant present in a particular food commodity and the consumption pattern for that food commodity. To determine EDI for AFs, accurate

information regarding their concentration and food consumption is essential [27]. The obtained EDI values can be used to characterize the risk which further helps to build strategies to reduce potential risks associated with aflatoxin exposure [6, 19]. The results of the estimated daily exposure risk to aflatoxins were obtained mathematically by using the equation 2 and the results were summarized on Figure 3 below.

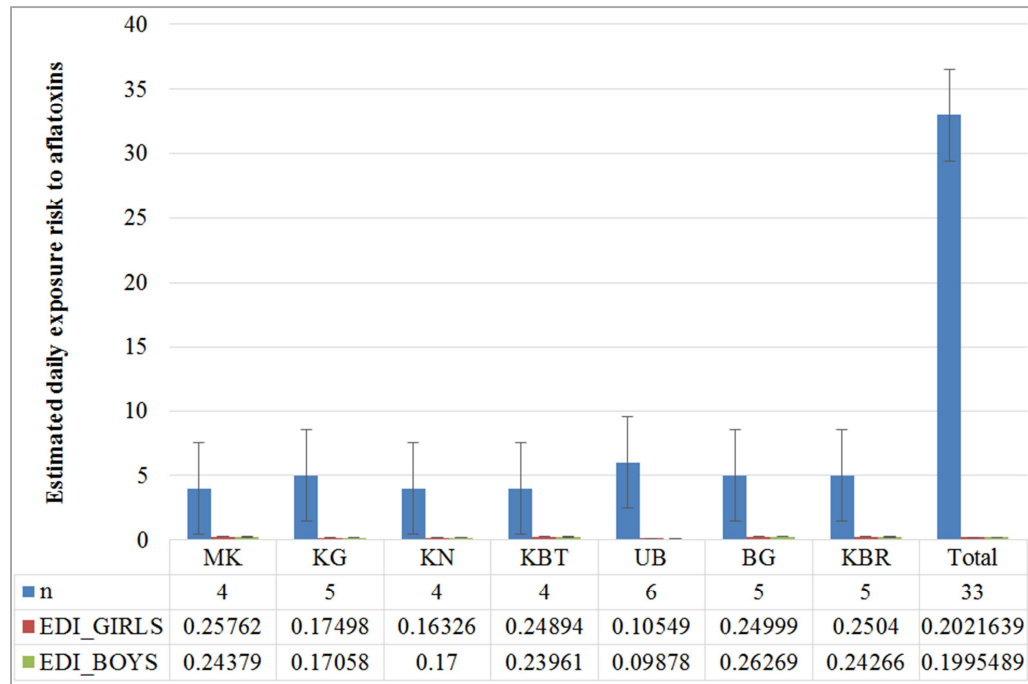


Figure 3. Summarizes the estimated daily exposure (ppb/kg. bw/day) risk to aflatoxin for girls and boys in y axis and sample code from district in x axis.

From Figure 3 above the estimated daily exposure to aflatoxins for girls and boys shows that estimated daily exposure risk to aflatoxin was (0.19 ppb /kg bw/day) and (0.18 ppb /kg bw/day) for all samples from different school found in all seven districts of the two regions (Dar es Salaam and Coastal region) respectively. The highest average of estimated dietary exposure for girls was observed in the MK (0.26 ppb /kg bw/day), BG (0.25 ppb /kg bw/day) and KBR (0.25 ppb /kg bw/day). On the other hand, the highest estimated daily exposure for boys was (0.26 ppb /kg bw/day), 0.24 ppb /kg bw/day in MK and, 0.24 ppb /kgbw/day in KBR samples. This suggests that students in those districts are at higher risk of aflatoxin exposure compared to other samples. The total average means daily exposure was 0.20 ppb/kgbw/day for girls and boys. The average mean of estimated daily exposure from this study is higher compared to those reported by [20]. In other studies, reported a lower average mean of estimated dietary exposures of (0.0035-0.133 $\mu\text{g/kg bw/day}$) in Kenya, and (0.039-0.180 $\mu\text{g /kg.bw/day}$) in Mozambique. The high dietary exposure of (0.070- 19.960 $\mu\text{g /kg bw/day}$) have been reported in Guangxi province (in China) [15]. AFB1 is genotoxic and can induce cancer at as low as <1 ng/kg bw/day [27]. Thus, students in these schools had higher risk of genotoxic exposure.

Estimated Cancer risk

The students' risk for aflatoxin which can cause liver cancer or HCC was estimated using the approach proposed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1998 and updated in November 2016 (WHO, 2017a). In brief, the cancer risk was simulated as shown in equation 3 and its results are summarized on table 4.

Table 4. Summary of cancer risk to aflatoxin B1 to girls and boys in the surveyed districts.

| District | Sample | n-number of collectd sample | Cancer risk girls | Cancer risk boys |
|-------------|--------|-----------------------------|-------------------|------------------|
| Mkuranga | MK | 4 | 0.02125 | 0.02011 |
| Kigamboni | KG | 5 | 0.01732 | 0.01407 |
| Kinondoni | KN | 4 | 0.01684 | 0.01402 |
| Kibaha town | KBT | 4 | 0.02054 | 0.01977 |
| Ubungo | UB | 6 | 0.01088 | 0.00815 |
| Bagamoyo | BG | 5 | 0.02062 | 0.02167 |
| KibahaRural | KBR | 5 | 0.02066 | 0.02002 |
| Total | | 33 | 0.01835 | 0.01646 |

From the results of cancer risk to aflatoxin for both girls and boys ranged from (0.01 – 0.02 per 100,000 population). The highest cancer risk (0.02 per 100,000 populations) was observed in Kibaha Rural, Bagamoyo, Kibaha Town, and Mkuranga districts. The lowest cancer risk (0.01 per 100,000 populations) was detected in Kigamboni, Kinondoni, and

Ubungo district. The overall average mean cancer risk for girls was 0.018 per 100,000 populations and (0.016 per 100, 000 populations) for boys. However, the results for current study are lower compared to cancer risk (i.e 1.86 per 100,000 populations) caused by the consumption of contaminated maize meal which was reported by [20].

4. Conclusions

The result obtained showed that maize flour is mostly used to prepare meals in the society including at boarding secondary school. Based on the results obtained in this study as compared with the standards set by the Tanzania Bureau of Standards (TBS), it can be construed that out of the 33 maize flour samples analyzed for total aflatoxin (total AFs) and AFB1 exceeded TBS limits by 36.36% and 40% respectively. Cancer risk for aflatoxin exposure via maize flour consumed in some of the surveyed boarding secondary schools by students exposes them to the risk of getting health effects through consumption of maize flour meal. This is because AFB1 is genotoxic and can induce cancer at as low as <1 ng/kg bw/day/0.001ug/kg bw/day according to (SCF 1994). Therefore, government institutions must take prompt action to provide guidelines that will provide them with accurate information about aflatoxin contaminations and their health impact. Training them on the important criteria that should be used during buying of maize flour from supplies, having suitable storage facilities and maintaining the cleanness of the storage room. Also, it is important to train them to use diverse diets for students in order lower the chance of consuming aflatoxins from only one type of diet. Regular monitoring of foods stored at schools and suppliers of maize flour is required.

Funding

The full financial support of the Tanzania Bureau of Standards (TBS).

Acknowledgments

The authors are highly grateful to the authority of the Tanzania Bureau of Standards (TBS) for funding this research and Sokoine University of Agriculture for their support in doing this research. Authors also express sincere thanks to people who helped in the execution of this study, particularly the Staff members of the Chemistry laboratory of Tanzania Bureau of Standards (TBS).

Conflicts of Interest

The authors declare no conflict of interests.

References

- [1] Adetunji, M. C., Atanda, O. O., & Ezekiel, C. N. (2017). Risk assessment of mycotoxins in stored maize grains consumed by infants and young children in Nigeria. *Children*, 4(7), 58.
- [2] Aklaku, E. K., Sowley, E. N. K., & Oforu, M. (2020). Incidence of fungi and aflatoxin contamination of maize in Tolon-Kumbungu district of Ghana. *African Crop Science Journal*, 28(2), 195-202.
- [3] Ali, N. (2019). Aflatoxins in rice: Worldwide occurrence and public health perspectives. *Toxicology reports*, 6, 1188-1197.
- [4] Fetaih, H. A., Dessouki, A. A., Hassanin, A. A., & Tahan, A. S. (2014). Toxopathological and cytogenetic effects of aflatoxin B1 (AFB1) on pregnant rats. *Pathology-Research and Practice*, 210(12), 1079-1089.
- [5] Fung, F., Wang, H. S., & Menon, S. (2018). Food safety in the 21st century. *Biomedical journal*, 41(2), 88-95.
- [6] Heshmati, A., Khorshidi, M., & Khaneghah, A. M. (2021). The prevalence and risk assessment of aflatoxin in sesame-based products. *Italian Journal of Food Science*, 33, 92-102. <https://doi.org/10.15586/ijfs.v33iSP1.2065>
- [7] Hussain, A., Suhail, M and Shafqatullah. (2012). Aflatoxin contamination of Spices Sold in Different Markets of Peshawar. *Journal of Chemical Society of Pakistan* 34: 1052-105.
- [8] Jere, G. M., Abong, G. O., Njue, L. G., Masamba, K., & Omayio, D. G. (2020). Exposure of school children to aflatoxins and fumonisins through maize-based diets in school meals programme in Salima District, Malawi. *African Journal of Food, Agriculture, Nutrition and Development*, 20(6), 16793-16809.
- [9] Kaaya, A. N., & Kyamuhangire, W. (2006). The effect of storage time and agro-ecological zone on mold incidence and aflatoxin contamination of maize from traders in Uganda. *International Journal of food microbiology*, 110(3), 217-223.
- [10] Kadan, G., & Aral, N. (2021). Effects of mycotoxins on child development. *Current Molecular Pharmacology*, 14(5), 770-781.
- [11] Kimanya, M. E., Routledge, M. N., Mpolya, E., Ezekiel, C. N., Shirima, C. P., & Gong, Y. Y. (2021). Estimating the risk of aflatoxin-induced liver cancer in Tanzania based on biomarker data. *PloS one*, 16(3), e0247281.
- [12] Kortei, N. K., Ayiku, P. O., Nsor-Atindana, J., Ansah, L. O., Wiafe-Kwagyan, M., Kyei-Baffour, V.,... & Odamtten, G. T. (2023). Toxicogenic fungal profile, Ochratoxin A exposure and cancer risk characterization through maize (*Zea mays*) consumed by different age populations in the Volta region of Ghana. *Toxicon*, 226, 107085.
- [13] Kumar, A., Pathak, H., Bhaduria, S., & Sudan, J. (2021). Aflatoxin contamination in food crops: causes, detection, and management: a review. *Food Production, Processing and Nutrition*, 3(1), 1-9.
- [14] Leite, M., Freitas, A., Silva, A. S., Barbosa, J., & Ramos, F. (2021). Maize food chain and mycotoxins: A review on occurrence studies. *Trends in Food Science & Technology*, 115, 307-331.
- [15] Liu, J., Sun, L., Zhang, N., Zhang, J., Guo, J., Li, C., Rajput, S. A., & Qi, D. (2016). Effects of nutrients in substrates of different grains on aflatoxin B1 production by *Aspergillus flavus*. *BioMed Research International*, Article 7232858. <https://doi.org/10.1155/2016/7232858>.
- [16] Liu, Y., & Wu, F. (2010). Global burden of aflatoxin-induced hepatocellular carcinoma: A risk assessment. *Environmental Health Perspectives*, 118(6), 818-824. <https://doi.org/10.1289/ehp.0901388>.

- [17] Mahato, D. K., Lee, K. E., Kamle, M., Devi, S., Dewangan, K. N., Kumar, P., & Kang, S. G. (2019). Aflatoxins in food and feed: An overview on prevalence, detection and control strategies. *Frontiers in microbiology*, 10, 2266.
- [18] Mahbobinejhad, Z., Aminian, H., Ebrahimi, L., & Vahdati, K. (2019). Reduction of aflatoxin production by exposing *Aspergillus flavus* to CO₂. *Journal of Crop Protection*, 8(4), 441-448.
- [19] Martinez-Miranda, M. M., Rosero-Moreano, M., & Taborda-Ocampo, G. (2019). Occurrence, dietary exposure, and risk assessment of aflatoxins in arepa, bread, and rice. *Food Control*, 98, 359–366. <https://doi.org/10.1016/j.foodcont.2018.11.046>.
- [20] Matumba, L., Kimanya, M., Chunga-Sambo, W., Munthali, M., & Ayalew, A. (2019). Probabilistic dietary-based estimation of the burden of aflatoxin-induced hepatocellular carcinoma among adult Malawians. *World Mycotoxin Journal*, 12, 409–419. <https://doi.org/10.3920/WMJ2018.2346>.
- [21] Matumba, L., Monjerezi, M., Chirwa, E., Lakudzala, D., & Mumba, P. (2009). Natural occurrence of AFB₁ in maize and effect of traditional maize flour production on AFB₁ reduction in Malawi. *African Journal of Food Science*, 3(12), 413-425.
- [22] Nabwire, W. R., Ombaka, J., Dick, C. P., Strickland, C., Tang, L., Xue, K. S., & Wang, J. S. (2020). Aflatoxin in household maize for human consumption in Kenya, East Africa. *Food Additives & Contaminants: Part B*, 13(1), 45-51.
- [23] Nduti, N. N., Njeru, P. N., Mwaniki, M., & Reid, G. (2017C). Probst, H. Njapau, and P. J. Cotty, “Outbreak of an acute aflatoxicosis in Kenya in 2004: Identification of the causal agent,” *Applied and Environmental Microbiology*, vol. 73, no. 8, pp. 2762–2764, 2007.
- [24] Nicholaus, C., Martin, H. D., Matemu, A., Kimiywe, J., & Kassim, N. (2021). Risks of aflatoxin exposure among adolescents in boarding schools in Kilimanjaro region, Tanzania. *World Mycotoxin Journal*, 14(2), 221-235.
- [25] Nuss T and SA Tanumihardjo Maize: a paramount staple crop in the context of global nutrition. *Comp. Rev. Food Sci. Food Safety*, 2010; 9(4): 417-436.
- [26] Paschoal, F. N., de Azevedo Silva, D., von Sperling de Souza, R., de Oliveira, M. S., Pereira, D. A. A., & de Souza, S. V. C. (2017). A rapid single-extraction method for the simultaneous determination of aflatoxins B₁, B₂, G₁, G₂, fumonisin B₁, and zearalenone in corn meal by ultra-performance liquid chromatography tandem mass spectrometry. *Food analytical methods*, 10, 1631-1644.
- [27] Udovicki, B., Tomic, N., Trifunovic, B. S., Despotovic, S., Jovanovic, J., Jacxsens, L., & Rajkovic, A. (2021). Risk assessment of dietary exposure to aflatoxin B₁ in Serbia. *Food and Chemical Toxicology*, 151, Article 112116. <https://doi.org/10.1016/j.fct.2021.112116>.
- [28] WFP. United Nations World Food Programme. School Meals Programme (SMP) in Malawi. 2018.