

Date	Submitted	Accepted	Published
	31 st July 2023	15 th August 2023	29 th February 2024

EXPOSURE OF KIANDUTU SLUM RESIDENTS TO AFLATOXIN THROUGH MAIZE-BASED PRODUCTS CONSUMPTION

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ABSTRACT

Kenyans have been highly affected by aflatoxicosis for a long time because of excessive exposure to high aflatoxin containing maize-based products. Maize has been the staple food in Kenya which is more highly consumed than any other cereal product. Many countries with strict aflatoxin threshold application, have consumers who still consume maize-based products, which has not gone through testing. The more remote and lower income areas are more affected by untested maize, ending up exposing themselves to Hepatocellular carcinoma which is a health hazard. The study was designed to determine the exposure of aflatoxin in Kiandutu slum which is a marginalized area in Thika, Kiambu County, Kenya. Ninety seven households were used in the study where number of maize-based products obtained from each household was sampled. Quantitative analysis was used to determine the aflatoxin levels by use of ELISA method. Questionnaires were used to collect the consumption data and sampling of maize-based products was done purposively. Aflatoxins were found in 30.93% of maize flour at between 0 to 34.19 $\mu\text{g}/\text{kg}$, 29.33% of composite flour at between 0 to 30.06 $\mu\text{g}/\text{kg}$, 18.67% of maize grain at between 0 and 20.92 $\mu\text{g}/\text{kg}$ and 6.97% of *Muthokoi* at between 0 to 7.14 $\mu\text{g}/\text{kg}$. Across all sampled households, daily consumption of maize-based products in kilograms per body weight was found to be highest in maize flour, followed by whole maize grains, composite flour and *muthokoi* in that order. Monte Carlo risk simulation was used to generate the quantitative exposure data. Daily maize flour consumption was higher than other maize-based products with a mean of 0.0038 kg/kg bw/day. It also had the highest daily aflatoxin exposure at a mean of 0.0301 $\mu\text{g}/\text{kg}/\text{bw}/\text{day}$. The percentage level of the health risk was found to be highest through maize flour consumption at 68.65. Results showed that the frequency of consumption of maize-based products is an important contributor to dietary exposure risk.

Key words: Aflatoxin, Exposure, Slum, Food safety, Maize-based products, Households, population

INTRODUCTION

In Kenya, maize grains are an important staple food in every household [1]. Their daily consumption is at an average of 400g per person while 98kg has been estimated as the annual consumption per capita [2]. The annual economic impact of aflatoxin contamination in developing countries has been estimated per year to be over \$ 100 million [3]. It is critical to acknowledge that, even in nations that have set strict aflatoxin regulations, many consumers are exposed to maize that has not undergone any regulatory inspection. The most affected population is the one that highly depend on subsistence farming, leading to adverse impacts on health and trade worldwide. [4]. Hepatocellular carcinoma (liver cancer) is the primary chronic disease associated with aflatoxins intake, being the third cause of cancer deaths globally WHO [5]. Food safety is a major challenge among the urban people who reside mainly in slums. These are part of marginalized and highly populated areas in developing countries including Kenya [6]. Such areas have a high prevalence of food insecurity and 85 % of the households are food insecure, with 50 % being severely insecure [7].

Factors associated with food insecurity include low level of income, scarce source of livelihood, small household size, high dependence ratio, illness, perceived insecurity, and slum residence. Residents in the slums generally eat for bare survival, with little concern for quality [8]. The households from developing countries earning a monthly income less than USD 65, were found to be affected by food insecurity [9]. In Kiambu County of Kenya, Kiandutu slum is one of the largest slums which has an approximate population of 50,000 people with an estimated 5,000 households [6]. No studies with published results have been conducted on the aflatoxin exposure to the dwellers of the slum where their main food is maize and its products including sifted maize flour, composite flour, whole grain and muthokoi. *Muthokoi* is a traditional Kenyan dish made through the removal of the maize kernels which are boiled until tender. Food commodities especially from developing countries are often reported to have aflatoxin levels beyond the maximum permissible limits of 10ppb [10]. Consumption of maize and maize-based foods has been reported to have food safety concerns due to the presence of mycotoxins and the toxicants are a global safety concern as they cause foodborne illnesses [11]. The most commonly occurring mycotoxins found in maize and maize-based foods are aflatoxins that are of concern at the various ranks of the food chain including harvesting, transportation, marketing, storage, processing, food preparation and end up in the final meal [12]. Mycotoxin proliferation is exacerbated by poor postharvest handling practices, particularly poor storage conditions, insect, and pest attacks.

The risk of exposure to aflatoxin contaminated foods includes acute and chronic toxicity. There are cases of stunting in children and adverse health conditions in the slums due to aflatoxin exposure from the maize-based products they consume [13]. The unemployment level and the low wages paid to the slum household members restricts them from having a diversity of food products.

This leads to high consumption of maize-based products that expose them to aflatoxin (14). The objective of this study was to determine the extent of exposure of the residents of Kiandutu slum to aflatoxins due to the consumption of maize-based products.

MATERIALS AND METHODS

Study area

The study was designed to assess the exposure to aflatoxin of the households in Kiandutu slum. It is an informal settlement located within Thika municipality. The settlement is about two kilometers south of Thika town Centre, off Garissa Road lying between latitudes 3°53' and 1° 45' south of Equator and longitudes 36° 35' and 37° 25' east (Google maps). Currently, it is the largest slum in Thika municipality.

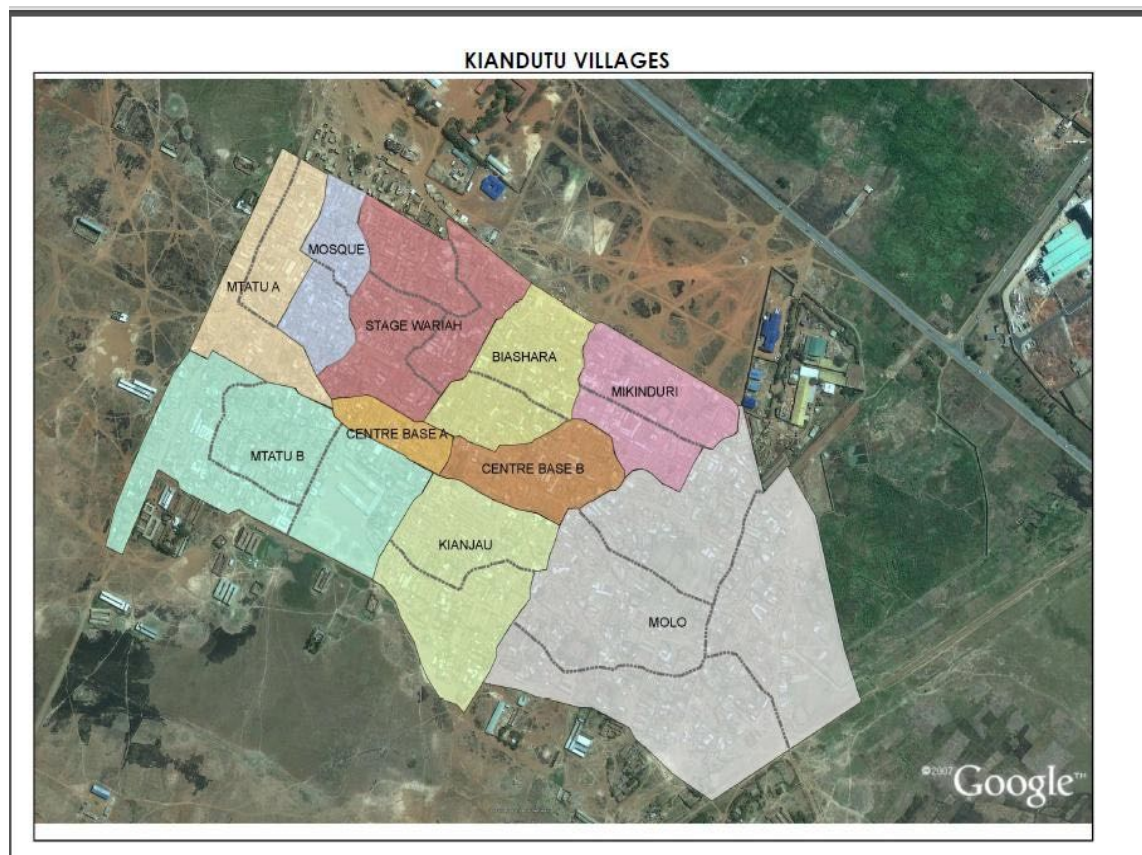


Figure 5: Kiandutu slum map showing all the villages

Source: Njeri KL [15]

Study Design

A cross-sectional study design was used and the study population was Kiandutu slum household dwellers.

Sample size determination

Ninety seven households were sampled from about 5,000 [16] and Yamane method [17] of sample size calculation was used ($n = N / (1 + Ne^2)$) by the use of line sampling method with a 95% confidence interval and error level of 0.1.

Household data collection

Semi-structured questionnaire was used to collect data on consumption of maize-based products from respondents aged 18 years and above. The survey for households was designed to capture the maize-based food products consumed, their aflatoxin levels, body weights (bw), and daily quantities of consumption. The quantities of meals and body weights (bw) were collected by weighing the respondents and the respective portions of the meals served by the use of certified digital weighing scales. The objectives of the study and the consent of participation were explained to the respondents before the commencement of the activity. The questionnaires were administered by trained individuals who translated the consumption questions into the language that all understood for easy communication.

Maize-based sample collection

Samples were collected from every household at a rate of 1 kg each of maize grains, maize flour, composite flour and Muthokoi. The non-bulky maize-based products found in the households were thoroughly mixed before sampling. The bulk samples were collected from the storage bags using the sampling probe from the top, middle and bottom parts and they were thoroughly mixed [18]. They were all packaged in an air tight plastic bag, all labelled and transported in cooler boxes to Kenya Bureau of Standards (KEBS) laboratories for aflatoxin analysis. All 97 households had maize flour products; 75 of them had maize grains, 70 composite flour and only 9 had the Muthokoi. The incentive presented to the members of the households who participated in the study was a packet of maize flour which was verified to meet the aflatoxin threshold before issuing.

Quantitative aflatoxin screening

Sample preparation

The maize grains and *Muthokoi* samples were ground uniformly to flour. Twenty grams of each were weighed and homogenized through shaking with 100 ml of 70 % methanol solution for 30 minutes. The mixture was filtered using Whatman filter paper 185 mm (Cat no. 1001 185) into a 250 ml conical flask.

ELISA method

The levels of aflatoxins in the maize-based samples were determined using the ELISA method of analysis [19] by the use of HELICA Technology. A sample filtrate of 100 µl was mixed with 200 ml of aflatoxin sample conjugate and homogenized in the micro wells. The mixture was transferred to the antibody-coated micro-wells and incubated for 15 minutes. After the incubation, the content was discarded into the basin and the wells were tapped upside down to discard the present buffer solution that was used to wash the content. The substrate solution was added at an amount of 100 µl and incubated for 5 minutes. Lastly, the stop solution was added and the optical density was led by the use of a leader machine at a wavelength of 450 nm and the aflatoxin levels were determined by the use of @Risk Top Rank Palisade (UK) software for Excel (Palisade, UK) V.8.0.

Kiandutu slum households' aflatoxins intake levels

The dietary exposure was modelled quantitatively in MS Excel spreadsheet (Microsoft, Redmond, WA, USA) by use of Monte Carlo simulation in @Risk software (version 4.0, Palisade Corp., Newfield, NY, USA). The mean and the 95th percentile (P95) exposure levels for the four products were estimated.

Margins of exposure (MoE) were calculated using Monte Carlo simulation after a 1,000,000 iteration runs for variability. Input parameters were varied according to the aflatoxin concentrations for each maize-based food product as determined by ELISA method of analysis. The Tolerable Daily Intake (TDI) of aflatoxins was estimated based on Margins of Exposure (MoE) of 10,000 as safety levels of public health, which is equivalent to 0.017 µg /kg bw/day [20]. Therefore, any exposure value of above 0.017 µg/kg bw/day was regarded as unsafe for consumption.

Data analysis

Maize-based products were the independent variables and the dependent variables were the risk estimate and the aflatoxin concentration in the products. Independent t-test was also used to compare the mean differences between continuous variables. The maize-based products consumption correlation with aflatoxin exposure levels was determined. The mean and 95th percentile for the estimated margins of exposure was determined by the Monte Carlo simulation in @Risk software (version 4.0, Palisade Corp., Newfield, NY, USA) as descriptive statistics. The results of tests carried out on the aflatoxin content was consolidated in an excel sheet and compared with the requirements of the Kenyan standards using analysis of variance (ANOVA). The mean intake was compared with WHO standard requirements [20] and analyzed for risk estimate.

Quantitative risk assessment for aflatoxin exposure

The consumption of maize-based products per kilogram body weight (bw/day) was estimated by dividing their intake (kg/ person) weekly according to JECFA guidelines [20].

$$\text{Consumption in (kg/kg bw/day)} = \frac{\text{Maize product consumed per day (kg)}}{\text{Body weight}} \quad (1)$$

Aflatoxins exposure was achieved through dividing the levels of the aflatoxins per kilogram of maize-based product.

$$\text{Aflatoxin levels (}\mu\text{g/kg)} = \frac{\text{Aflatoxins in Maize product (}\mu\text{g)}}{\text{Weight of maize samples (kg)}} \quad (2)$$

The levels of intake were obtained by multiplying the corresponding level of aflatoxins in the samples and the levels of consumption in maize-based products estimated as the levels of intake per kg bw/day for the respondents.

$$\text{Dietary exposure} = \sum \frac{(\text{Concentration of toxin in food} \times \text{food consumption})}{\text{body weight (kg)}} \quad (3)$$

RESULTS AND DISCUSSION

Maize-based meals prepared by Kiandutu slum households

Kiandutu slum respondents consume maize- based products as staple food. Maize- based products were consumed more highly than any other food product in an observation that was made in determination of slums households' dietary diversity in 7 days [21]. Maize grains, maize flour, composite flour and *muthokoi* were the maize-based products that were available in the households on which they depended for their daily consumption. Maize grains and *muthokoi* were used to make *Githeri* which is a mixture of boiled maize and beans and other cereals and pulses. Maize flour was used to make maize meal 'ugali' which is accompanied with vegetables, meat products, sea foods, milk products, other plant and animal sources of nutrients to improve the diet [22]. The composite flour was used to prepare porridge which mostly was used to be consumed during breakfast [23].

Levels of aflatoxin in the maize-based products

All the maize-based products were analyzed and found to contain detectable levels of aflatoxin ranging from 0 $\mu\text{g /kg}$ to 39 $\mu\text{g /kg}$, with their respective average levels as shown in Table 1. The order from the highest aflatoxin levels in each product was maize flour, maize grain, composite flour and *muthokoi* which included; 38.92 $\mu\text{g /kg}$, 24.96 $\mu\text{g /kg}$, 30.06 $\mu\text{g /kg}$ and 4.39 $\mu\text{g /kg}$, respectively. The lowest detected level was 0 $\mu\text{g /kg}$. The Kenyan aflatoxin threshold is set to be 10 $\mu\text{g /kg}$ by Kenya Bureau of Standards [10].

Maize flour had the highest levels of aflatoxin consisting of the highest mean value of all products and the least being Muthokoi. Maize flour sampled from Kampala Uganda households was found to contain the highest aflatoxin levels of 7 µg /kg compared to 38.92 µg /kg aflatoxin levels obtained in this study [24]. The levels of aflatoxin detected from the household's survey in some developing countries have been found to be as high as 411 µg /kg [25]. That report indicates that there are less efforts that are in place to ensure that the end products during harvesting and processing are free from aflatoxin invasion. Research has shown that maize based composite flour contain unsafe levels of aflatoxin [26]. *Muthokoi* was the least consumed product by Kiandutu slum dwellers, which was also found to contain the least levels of aflatoxins. Consistently, aflatoxin levels have been declared to be beyond thresholds of 10 µg /kg [27] mostly attracted from its method of its production from the whole grain which involves de- hulling process. The exposure of inner part of the maize grain leads to increase of the surface area of aflatoxin invasion [28]. Table 2 shows the percentage number of aflatoxin- contaminated maize-based products above the threshold: Maize flour; 28.9%, composite flour; 28.9% maize grains; 15.5%. All *Muthokoi* samples had complied with the set threshold.

Maize-Based Products consumption levels (kg/kgbw/day)

In all products, their consumption level was between 0.0038- 0.0012 kg/kgbw/day. Table 3 shows the order of the consumption, maize meal being highly consumed per day, maize grains, composite flour and *Muthokoi* in that order. Studies have shown that maize- based products consumption level can be up to 0.4 to 0.5 kg/kg bw/day [29]. Their consumption distribution fit with aflatoxin intake was high with maize meal (Ugali) than with all other products at R² of 0.858 as shown in Table 4. Their high level of correlation with aflatoxin exposure clearly displayed high aflatoxin risk to consumers [30].

Dietary exposure to toxins from the Maize-based Products (µg/kg/bw/day)

The exposure of Kiandutu slum household respondents to aflatoxin through consumption of maize-based products ranged from 0.0301- 0.0089 µg/kg/bw/day as described in Table 5. Maize flour displayed the highest exposure levels to aflatoxins compared to other consumed maize-based products from the slum' respondents. The 95th percentile and the mean for exposure to aflatoxin due to their consumption, were only calculable from the maize flour, maize grains and the porridge flour. This was due to the products available from the households.

The levels of aflatoxin exposure were 0.0305 µg/kg/bw/day, 0.025 µg/kg/bw/day and 0.0096 µg/kg/bw/day, respectively. The respondents' body weight was an average of 66.79kg. They were exposed to aflatoxin levels ranging from 0.0301 to 0.0089 µg/kg/bw/day. These results showed high consumption of the products led

by maize meal from the respondents. Table 6 showed the aflatoxin consumption from the maize meal, maize grains and *Muthokoi* led to aflatoxin exposure levels beyond the acceptable limits of 0.017 µg/kg/bw/day as recommended by European Food Safety Authority at the 95th Percentile [20].

Due to this report, there are chances that the residents may be experiencing various levels of aflatoxin intoxication from the products. The high levels of aflatoxin recorded in maize flour have emerged from the respective millers' failure to observe Good Manufacturing Practices (GMP) [31].

Previous studies and assessment, confirm that the consumption of maize-based products lead to aflatoxin exposure of about 0.39–0.56 and 0.47–0.66 (µg/kgbw/day) which was found to be higher than the results in this study [32]. Daily consumption of maize flour has been found to contain the highest aflatoxin levels compared to other maize-based products consumed which would lead to high mycotoxicosis effect. High level of aflatoxin exposure is related to high chances of Hepatocellular carcinoma (HCC) which is the highest risk of accumulative aflatoxin exposure effect [33]. The risk impact simulation for aflatoxin exposure was found to be highest through maize flour consumption and lowest through *Muthokoi* consumption; 68.65% and 56.57%, respectively as shown in Table 7. The increase in Biomarkers of HCC in Tanzania has been attributed to the increase of dietary aflatoxin exposure [34].

CONCLUSION, AND RECOMMENDATIONS FOR DEVELOPMENT

Kiandutu slum residents have been shown to be exposed to unacceptable aflatoxin levels through the consumption of the maize-based products. In this case, high aflatoxin intake may be attributed by the intake of aflatoxin contaminated maize-based products where maize is the main staple food. Daily and regular consumption of the products has shown to increase the chances of increase of health risk to the residents and, therefore, there is a need of raising the food safety concern mainly from the local millers "*Kisiagi*" who are not certified with Quality Management Systems. Promotion of good hygiene practices during the preparation of the maize-based products raw materials is required to reduce the levels of aflatoxin contamination.

Diversification of maize-based products with other cereal products that are less attacked by mycotoxins would reduce the residents' high consumption, reducing aflatoxin exposure. Awareness should be created countrywide to educate the consumers on mycotoxin intake health effects. Also, the researchers need to disclose often some of the food safety claims to the maize based product millers, an action that is rarely taken. As a result, the impact of such claims on consumer demand is not well understood. Good Agricultural Practices (GAP) need to be

enforced properly to avoid the invasion of aflatoxins into the maize grains during harvesting and storage. Government food safety agencies should enforce better monitoring of government laboratories' instrumentation, and strict monitoring of aflatoxin levels in each maize based-products in the market. It should be enforced for mandatory registration of mills selling sub-standard products under authorities which include KEBS and Public Health, for frequent premises inspection and monitoring.

Table 1: Aflatoxin concentration (µg /kg) in the Maize-based Products

Maize-based product		N	Minimum	Maximum	Mean	Std. Dev
Maize meal	Aflatoxin	97	0	39	8.32	7.197
Whole Grain	Aflatoxin	75	0	25	6.84	5.642
Porridge	Aflatoxin	70	1	7	4.21	1.463
<i>Muthokoi</i>	Aflatoxin	9	0	2	0.89	0.601

Table 2: Maize-based products aflatoxin safety thresholds

Aflatoxin Intake		Age Bracket (years)			No. of Samples	
	Statistic	18-29	30-50	51-77	> 10 ppb	
Maize Meal	P95	21.542	17.661	24.725	28/97	28.9%
	Mean	7.791	8.898	8.113		
	Min	1.330	1.160	1.340		
	Max	23.990	34.190	38.920		
Whole Grain	P95	18.458	16.499	8.290	15/97	15.5%
	Mean	5.432	5.753	3.133		
	Min	1.070	1.010	1.290		
	Max	20.920	24.960	8.350		
Composite Porridge	P95	21.640	17.671	26.015	28/97	28.9%
	Mean	7.791	8.775	8.535		
	Min	1.330	1.160	1.340		
	Max	23.990	34.190	38.920		

Table 3: Consumption of Maize-Based Products in (kg/kgbw/day)

Maize-based Product	N	Minimum	Maximum	Mean	Std. Dev
Maize Meal Consumption Per Day	97	0	0.010	0.0038	0.00143
Whole Grain Consumption Per Day	75	0	0.010	0.0031	0.00149
Porridge Consumption Per Day	70	0	0.010	0.0012	0.00102
<i>Muthokoi</i> Consumption Per Day	7	0	0	0.0027	0.00056



Table 4: Maize-based products consumption correlation with aflatoxin exposure levels

Model (Aflatoxin Intake)	R	R Square	Adjusted R Square	Std. Error of the Estimate
Maize Meal	.927a	0.858	0.852	0.01048
Whole Grain	.926a	0.857	0.849	0.00774
Porridge	.890a	0.792	0.779	0.00434
<i>Muthokoi</i>	.998a	0.997	0.991	0.00182

Table 5: Dietary exposure to toxins in the Maize-based Products ($\mu\text{g/kg/bw/day}$)

Dietary Exposure	Mean	Median	Std. Dev.	Minimum	Maximum	N
Maize meal	0.0301	0.0216	0.02727	0	0.14	97
Whole Grain	0.0212	0.0164	0.01992	0	0.09	75
Porridge	0.0089	0.0054	0.00924	0	0.05	70
<i>Muthokoi</i>	0.0198	0.0124	0.01901	0.01	0.06	7

Table 6: The total mean and the 95th percentile (P95) exposure levels to aflatoxins

Weight(kgs)	Aflatoxin Intake Maize Meal			Aflatoxin Intake Whole Grain			Aflatoxin Intake Porridge		
	Mean	Min	P95	Mean	Minimum	P95	Mean	Minimum	P95
40-49	0.075	0.030	0.075	0.0448	0.0450	0.0448	0.0071	0.0100	0.0071
50-59	0.031	0.027	0.026	0.0252	0.0250	0.0252	0.0082	0.0071	0.0082
60-69	0.021	0.007	0.012	0.0141	0.0167	0.0141	0.0014	0.0000	0.0014
70-79	0.046	0.045	0.053	0.0329	0.0367	0.0329	0.0227	0.0200	0.0227
80-89	0.013	0.015	0.013	0.0127	0.0150	0.0127	0.0110	0.0100	0.0110
90-99	0.006	0.000	0.004	0.0205	0.0200	0.0205	0.0069	0.0100	0.0069

Table 7: Monte Carlo Risk Simulation for Aflatoxin intake in Maize-based Products

Aflatoxin Intake	N	Min	Max	Mean	Std. Dev	Aflatoxin intake risk
Maize Meal	97	-0.06838	0.133752	0.029889	0.027088	68.65%
Whole Grain	75	-0.05244	0.098851	0.021389	0.019842	59.46%
Porridge	70	-0.03115	0.048173	0.008686	0.00927	18.51%
<i>Muthokoi</i>	7	-0.04689	0.105237	0.020076	0.018956	56.57%

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