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Evaluation of the Total Aflatoxin Level in Certain Cereals and Legumes Highly Consumed in Mali

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Abstract. The World Summit on Sustainable Development (WSSD) reaffirmed lofty and ambitious goals on seven main issues. Similarly, the Consolidated Action Plan for Africa (CAAP) has set priority science and technology projects. The achievement of an essential objective; common to both, namely: "Agriculture and food security", will strongly depend on the good knowledge and sustainable use of biological diversity, particularly microbial. Mali depends largely on agriculture and needs an annual growth of 6% or more in this sector to achieve sustainable development in general. However, the contamination of cereals and legumes essential to reduce food insecurity in Africa, aggravated by the proliferation of toxigenic fungi, constitutes a main constraint to the supply of healthy foods. However, in order to optimally target and implement these options, it is imperative that mycotoxin contamination is quantified and mapped. Three hundred and eighty-four (384) representative samples (1-2 kg per sample) were collected from different markets in Koutiala, Ségou, Bamako and Koulikoro in 2018 and 2020 to determine their fungal flora, the natural incidence of mycotoxins. All samples analyzed were contaminated with fungi, in the range of $1.2.10^5$ to $33.5.10^5$ CFU/g. SAS software was used for all analyzes and differences were considered significant when $p<0.05$. All cereals analyzed contain aflatoxins. Because of the high consumption and high levels of aflatoxins in beans and maize in the samples analyzed, we suggest periodic monitoring and the application of Hazard Analysis and Control (HACCP) prevention and control of mycotoxins in the production and industry of human food in Mali.

Keywords: Food, Contamination, Aflatoxins, Bean, Maize, Peanut, Mali

Introduction

The World Summit on Sustainable Development (WSSD) reaffirmed lofty and ambitious goals on seven main issues. Similarly, the Consolidated Action Plan for Africa (CAAP) has set priority science and technology projects. The achievement of an essential objective; common to both, namely: "Agriculture and food security", will strongly depend on the good knowledge and sustainable use of biological diversity, particularly microbial. Mali depends largely on agriculture and needs an annual growth of 6% or more in this sector to

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achieve sustainable development in general. However, the contamination of cereals and legumes essential to reduce food insecurity in Africa, aggravated by the proliferation of toxigenic fungi, constitutes a main constraint to the supply of healthy foods. In West Africa, only pathogenic microbial agents, pesticide residues and aflatoxins (Mensah et al., 2014; Koné, 2015; Diarra et al., 2017; Dembélé et al., 2020; Coulibaly et al., 2022) have been the subject of work or studies as part of an approach to protect the health quality of food intended for human and animal consumption. Food safety indicators will be difficult to achieve in Africa as long as the problems of contamination by molds and mycotoxins remain unresolved. In 2011, the 7th Global African Agriculture Development Partnership Platform highlighted the urgent need to mitigate mycotoxin contamination by recommending the establishment of consumption standards. It is in this context that this project proposes to quantify aflatoxin contamination in cereals. Rice, maize, groundnuts and beans have become highly strategic and priority products for food security in Africa. In Mali, the consumption of maize, beans and groundnuts is increasing faster than that of any other major component of the continent due to high population growth, rapid urbanization and changes in dietary habits (Seck et al., 2013). Unfortunately, these products face significant contamination with mycotoxins. Contamination by mycotoxins, highly toxic substances produced by fungi, starts in the fields and eventually travels through the value chain and affects farm families, traders, markets and ultimately consumers (WHO, 2008). Several mycotoxins exist, produced by a plethora of fungi, and toxin profiles differ between cultures in countries and regions (FAO, 1991). Maize, for example, among the most important staples in Mali, is one of the crops most susceptible to aflatoxins (Diarra et al., 2017). Mycotoxin contamination of beans, the most important smallholder crop in Mali, is poorly quantified, although visual observations of treated beans in households and markets show high contamination by several fungi. Mycotoxins and their presence in food and feed are linked to cancer, immune system suppression, growth retardation, liver disease and death (Dow, 1994). Mycotoxin contamination also limits the income of small-scale farmers, as food quality and safety issues resulting from contamination present trade restrictions. To make matters worse, produce is screened for mycotoxins prior to export, with no destruction of contaminated materials, concentrating contaminated crops in the local food chain. The fight against mycotoxin contamination is now considered a priority in sub-Saharan Africa. In many parts of the world, management options to mitigate mycotoxin contamination during the growth and storage of cereals and legumes are available. However, in order to optimally target and implement these options, it is imperative that mycotoxin contamination is quantified and mapped. In this project, we propose proper quantification of aflatoxin contamination as the basis for (i) risk assessment of key mycotoxins, which will help identify target areas for intervention; (ii) harmonization of mycotoxin standards to improve trade in the region, and (iii) boosting local mechanisms for monitoring/surveillance and enforcement of standards, thereby ensuring that commodities consumed locally are safe.

Material and Methods

Study Sites

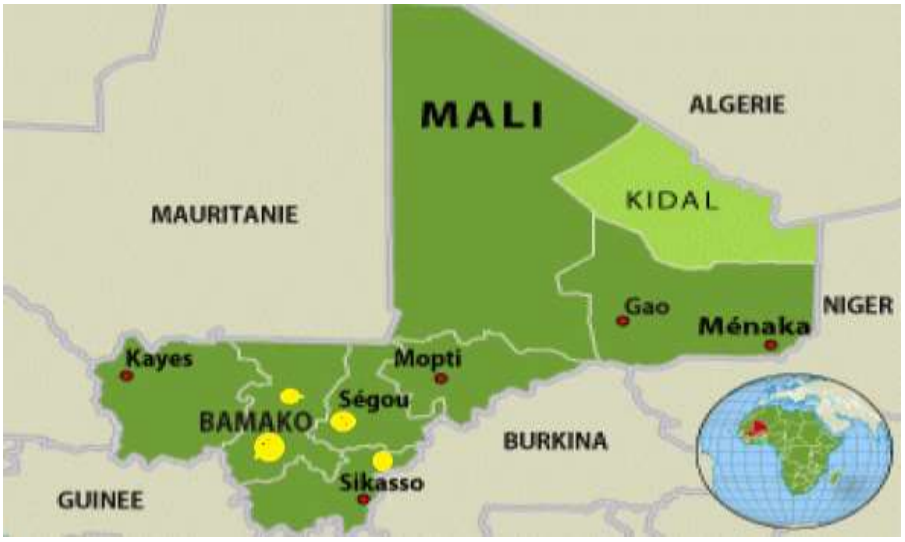

Figure 1. Map showing the different sampling sites (http://www.mapnall.com 05/24/2023 at 10:22 a.m.)

Biological Material

The samples of maize, bean and groundnut constituted the material of study during this thesis.

Sampling

The sampling method was reasoned for the choice of sampling sites. The selection criteria for localities and sales sites for beans, maize and groundnuts were based on the existence of storage warehouses and permanent sales markets and high consumption of the products studied. Samples were taken systematically for medium storage warehouses. The markets were chosen at random and in each market, on selected outlets, samples were taken in the same way.

Sample Size

The sample size was calculated from the Schwartz formula using the standard non-compliance rate of 50%, allowed in cases where a specific rate is not identified based on the results of the studies carried out.

Schwartz formula: N = Z2 pq/i2, with N= sample size; Z (constant)= 1.96 i = precision; here we took a precision i = 5% and q=1-p and in our case p= 0.50; so q=1-0.50 = 0.50

N = (1.96)2 x 0.50 x 0.50/ (0.05)2 = 384 samples

The study will then concern 384 samples of cereals sold on the local markets of the selected study areas.

Sampling

For analysis purposes, samples of maize, beans and groundnuts were collected in markets and storage areas at the various study sites selected. These samples provided information on the contamination of products stored in households and markets. Three hundred and eighty-four (384) representative samples (1-2 kg per sample) were collected from different markets in Koutiala, Ségou, Bamako and Koulikoro in 2018 and 2020. All samples were homogenized and divided to obtain a 1 kg working sample for analysis. Each sample was ground in a laboratory grinder. For mycological examination, cereal and legume samples were analyzed immediately upon arrival or they were stored for 2-3 days in paper bags at room temperature (about 25°C). Cereal and legume samples for mycotoxin analysis were stored at -4°C.

Enumeration and Isolation of Fungi

For the enumeration and isolation of fungi in cereal and legume samples, the dilute plate technique modified by O. Diarra et al in 2017 was used. For this, ten grams of each homogenized sample were mixed with 90 ml of 0.1% peptone and shaken on a horizontal shaker for 20 minutes. After this treatment, 0.1 ml of this dilution was inoculated onto each of the three different media: Dichloran Rose Bengal Chloramphenicol Agar (DRBC) to count the total cultivable fungi; Potato dextrose agar (PDA) and malt extract agar (MEA) were used, and the plates were incubated under a photoperiod of 12 hours light: 12 hours dark , at 25°C for 7 days. Plates containing 10 to 300 colonies were counted and results were expressed as colony forming units per gram of sample (CFU/g). Representative colonies of each type were purified by picking them onto plates with PDA.

Treatment and Analysis of Mycotoxins

All the corn, peanut and bean samples taken were processed at LaboREM-Biotech at the Faculty of Science and Technology, USTTB (University of Science, Techniques and Technologies of Bamako). Fifty (50) grams of each sample were taken and ground in a Stephan mill mixer at 2700 revolutions per minute for 3 minutes. The shredded material was sieved through a 2 mm mesh to obtain a powder that promotes the extraction of aflatoxins. All maize, peanut and bean samples were pre-screened for mycotoxins using the RIDASCREEN kit (R-Biopharm AG', Germany), available in the laboratory. Five (5) grams of powder from each ground material were taken for aflatoxin extraction in a 15 ml tube with cap. The extraction and identification of aflatoxins were done according to the manufacturer's instructions (R-Biopharm, 2003). Aflatoxins were extracted with a methanol:water (70:30) mixture. The final extracts were diluted with distilled water and used with the specific kit. The optical densities of the extracts were measured at 450 nm. All samples and standards were analyzed in triplicate. Data on the aflatoxins present in the samples were analyzed using Ridasofwin software from Biopharm (R-Biopharm AG', Germany).

Statistical Analysis

The analysis of variance test was used to analyze the number of fungi and the content of toxins. A multiple range test for variables was used to compare mean fungal counts and toxin content of samples from different villages. SAS software was used for all analyzes and differences were considered significant when $p<0.05$. The normal distribution of toxin contents, means, standard errors and variances were calculated using SAS 9.1 software (SAS, 1990).

Results

Fungal Flora and Aflatoxicogenic Strains Present in the Samples Analyzed

The quantities of total fungi and those capable of producing aflatoxins (aflatoxinogenic) obtained are presented in Figure 2 below:

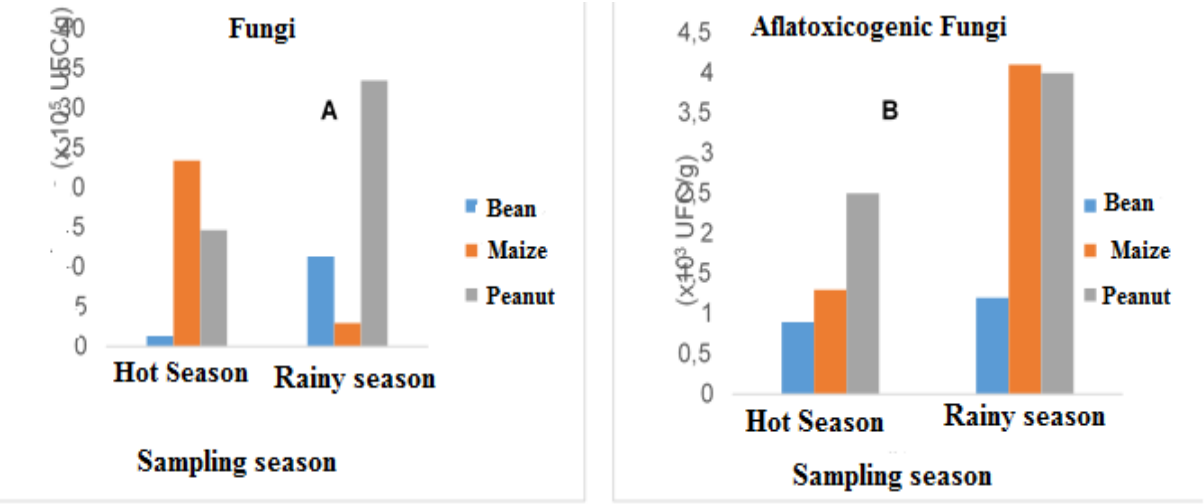

Figure 2. Quantities, depending on the season, of total (A) and toxigenic (B) fungi on the cereals analyzed

The analysis of Figure 2 shows that the contents of total and toxigenic fungi vary greatly depending on the cereals and the sampling seasons. The maize which presented the highest quantity of total fungi ($23.4.10^5$ CFU/g) in the hot season (Figure 2A), showed the lowest quantity of total fungi during the rainy season ($2.8.10^5$ CFU/ g). However, despite the low quantity of total fungi in maize in the rainy season, it is on the same maize that the highest quantity of toxigenic fungi ($4.1.10^3$ CFU/g) was found (Figure 2B). The highest amounts of total fungi, whatever the sampling site, were found on groundnut (Figure 2A), with the highest amount in Koutiala ($22.6.10^5$ CFU/g). On the contrary, the lowest quantities of total fungi were found on beans in Koutiala ($2.1.10^5$ CFU/g) and in Ségou ($0.4.10^5$ CFU/g) and on maize in Bamako ($1.03.10^5$ CFU/g) and Koulikoro ($0.9.10^5$ CFU/g).

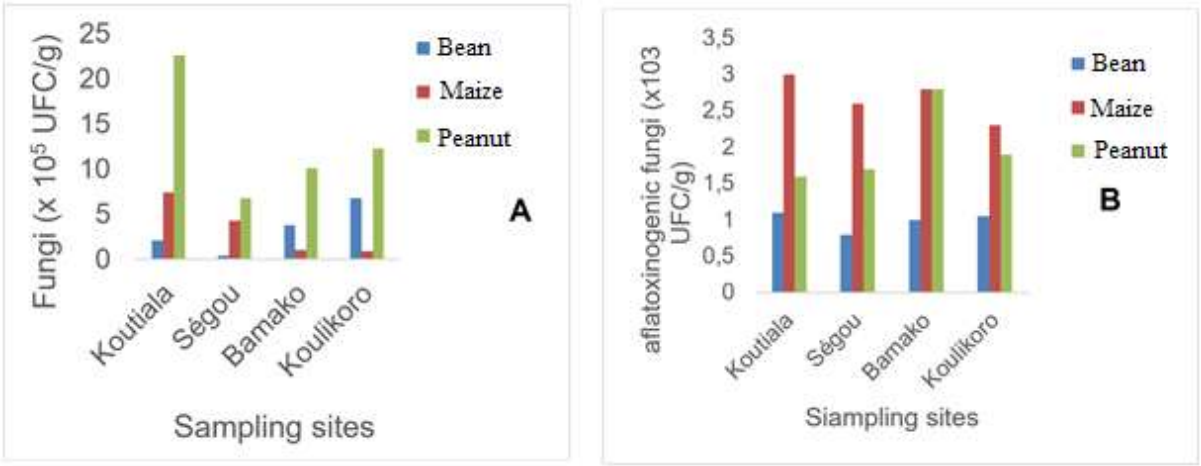

Figure 3. Quantities, depending on the sampling sites, of total (A) and toxigenic (B) fungi on the cereals analyzed

Maize and groundnut showed high amounts of toxigenic fungi at all sampling sites (Figure 3B). However, maize samples from Koutiala showed the highest amounts of toxigenic fungi ($1.6 .10^3$ CFU/g). Similarly, peanut samples from Bamako showed the highest amounts of toxigenic fungi ($2.8.10^3$ CFU/g) (Figure 2B).

Aflatoxin Quantities of Analyzed Cereals

Three hundred and eighty-four (384) samples of corn, peanuts and beans (128 for each) were analyzed at the LaboREM-Biotech (Research Laboratory in Microbiology and Biotechnology) of the F.S.T. (Faculty of Science and Technology) of the U.S.T.T.B. (University of Sciences, Techniques and Technologies of Bamako). These samples were analyzed using the RIDA QUICK standard aflatoxin kit method. Regarding the samples: 96 presented from Koutiala, 96 from Ségou, 96 from Bamako and 96 from Koulikoro. Aflatoxins were detected in 100% of the samples analyzed.

ANOVA results for aflatoxin concentrations measured in bean, maize, and peanut samples are reported in Table 1:

Table 1. Analysis of variance (ANOVA) for aflatoxin levels in cereals analyzed

Sources of variation	Degree of freedom	F
Samples	384	***
Repeat	2	NS

Analysis of the data presented in Table 1 shows that the aflatoxin content of the samples analyzed varies significantly from one cereal to another. However, no significant variation was observed between the different repetitions demonstrating the repetitiveness of the results obtained. The results of the comparisons of the average aflatoxin concentrations of the cereals analyzed according to the sampling season are presented in Table 2 below:

Table 2. Effect of cereal type and sampling period on aflatoxin concentration in bean, maize, and groundnut samples analyzed by sampling season

Cereals	Quantity of aflatoxins (µg/kg)	
	Hot season	Cold season
Bean	$22,4^{c}$	$66,5^{c}$
Maize	$86,5^{a}$	$162,1^{a}$
Peanut	$66,25^{b}$	$122,5^{b}$

Analysis of the data presented in Table 2 shows that regardless of the sampling season, maize and groundnut presented the highest concentrations of aflatoxins with the highest minima and maxima of aflatoxins (Table 2). The highest concentrations were found in maize with 86.5 µg/kg in the hot season and 162.1 µg/kg in the cold season (Table 2). Beans gave the lowest aflatoxin concentration with the lowest minima and maxima whatever the season (Table 3). Aflatoxin concentrations were higher either during the cold season regardless of the cereal analyzed (Table 3).

Table 3. Comparison of aflatoxin levels in cereals and legumes analyzed

Cereals	Hot season		Cold season	
	Minimum	Maximum	Minimum	Maximum
Bean	0,6	178	13	857
Maize	0,9	453	8,9	1244
Peanut	2,1	321,5	22	1059

Aflatoxin concentration in maize was significantly higher ($p<0.05$) in maize (detected: in all samples; mean 162.1 µg/kg; max: 1244 µg/kg) than in peanut (122.5 µg/kg with a maximum of 1059 µg/kg) and beans (average 66.5 µg/kg; max: 857 µg/kg) (Figure 4; Table 3).

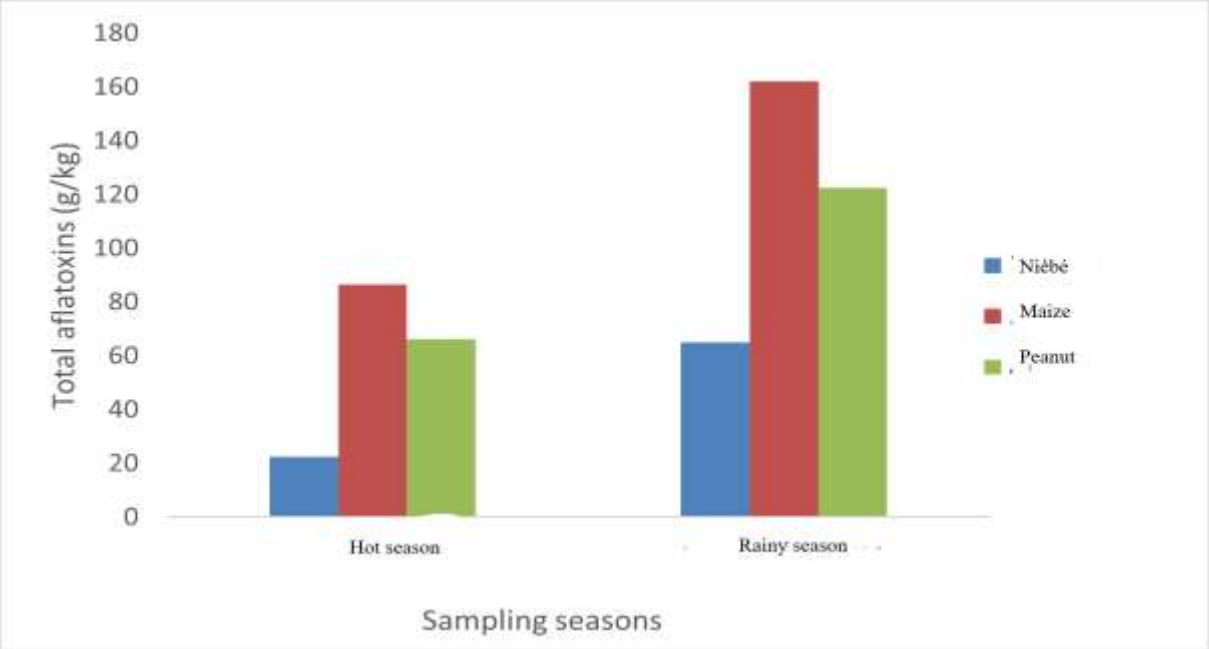

Figure 4. Aflatoxin concentration in beans, maize and peanuts in hot and cold season

As shown in Table 3 and Figure 4, the second highest concentration of total aflatoxin in this study was quantified in groundnut. Aflatoxins were detected in 100% of peanut composites (rainy season: 100%; average concentration 122.5 µg/kg).

Quality of Cereals Analyzed

Aflatoxin levels in ready-to-eat maize samples meet EU standards (4 µg/kg) and Codex (15 µg/kg) for beans. Unprocessed beans must be sorted or physically treated before human consumption or use as an ingredient (Table 4).

Table 4. Total aflatoxin content and European Union (EU) and Codex standards for trade and consumption

Cereals	Quantity of aflatoxins (µg/kg)		Standards	
	Hot season	Cold season	European Union	Codex
Bean	22,4	66,5	4 µg / kg	15 µg / kg
Maize	86,5	162,1		
Peanut	66,25	122,5		

It was noted that while 10% of the samples contained aflatoxin concentrations below the EU standard (4 µg/kg) and 21% below the codex standard, 90% and 79% of the samples respectively had a concentration in aflatoxins higher than EU and Codex standards. The fact that: (i) all maize samples collected during the rainy season contained on average a high concentration of aflatoxins (162.1 µg/kg) which is 40.5 times higher than the EU standard and 10.6 times higher to the Codex standard and that groundnuts and beans also contain very high levels of aflatoxins, and that (ii) maize, groundnuts and beans are heavily used as food and feed; we demand that contamination of cereals be reported as a potential risk for the population.

Discussion

All analyzed samples were contaminated with fungi, in the range of 1.2. 10^5 to 33.5.10^5 CFU/g. According to Pitt and Hocking (2009), xerophilic fungi, which are capable of rapid growth above about 0.77 water activity, can damage food as shown by our results.

Maize and groundnut showed high amounts of toxigenic fungi at all sampling sites. However, maize samples from Koutiala showed the highest amounts of toxigenic fungi. Aspergillus capable of producing aflatoxins were the most detected genera during this study. The level of toxigenic fungi varies greatly depending on the area and the sampling period with the highest concentrations during the rainy season. These results are adapted to those of many researchers who have shown that toxigenic fungi, particularly those of the genus Aspergillus, are mainly concerned in tropical countries (Kana et al., 2013; Greco et al., 2014; Kehinde et al., 2014; Diarra et al., 2017).

Variations in the concentration of total and toxigenic fungi are linked, on the one hand, to the composition of the cereals, and, on the other, to the climatic conditions prevailing in each season.

All cereals analyzed contain aflatoxins. However, the fact that aflatoxins have been detected in foods heavily consumed in Mali does not mean that all pose a threat to human health because aflatoxins do not have the same harmfulness. Indeed, aflatoxin B1 has been recognized as the most toxic for humans and animals (Vargas et al., 2001). Currently, in the case of many analyses, the lack of knowledge about the combined (and potentially synergistic) effect with other substances limits our interpretation. It does, however, represent a contribution to knowledge that could be used when new toxicological data on other mycotoxins becomes available.

In the remainder of this discussion, we have found it relevant to focus on public health aflatoxins and the importance of international trade. However, although the prevalences of aflatoxins in groundnut and maize samples (Keita et al., 2013) are often singled out, the prevalence of aflatoxin in beans has not often been pointed out. in previous surveys. Worldwide, several total dietary studies from various countries have included aflatoxins, including France (Leblanc et al., 2005; Sirot et al., 2013; Fleury et al., 2017), Canada (Tam et al., 2011), Lebanon (Raad et al., 2014), Vietnam (Huong et al., 2016) and China (Qiu et al., 2017). In Africa, studies on urinary biomarkers (Abia et al., 2013; Ediage et al., 2013; Ezekiel et al., 2014), food product surveys (Misihairabgwi et al., 2017; Ssepuuya et al., 2018) and l analysis of prepared foods as eaten (Abia et al., 2017) helped bring the public health community's attention to the threat of mycotoxins. Not surprisingly, the elevated concentration of the sum of AFB1, AFB2, AFG1, and AFG2 in foods (beans, corn, and peanuts) as consumed is likely the most significant public health and commercial finding of this analysis compared to other regions of the world (Marin et al., 2013). The fact that maize, groundnut and bean heavily consumed in Mali could contain high concentrations of total aflatoxin has been rarely reported (Shephard, 2018). Beans, groundnuts and maize are therefore likely to contribute significantly to aflatoxin exposure. The Joint Expert Committee on Food Additives and Contaminants (JECFA) noted at the 83rd session (World Health Organization, 2016) that the current estimate of global exposure was established with occurrence data belonging to the countries of the WHO European region and that no information was available on the levels of fumonisins in maize in the African, Eastern Mediterranean and South-East Asian regions. JECFA has also noted (World Health Organization) that the interaction between AFB1, a compound with known genotoxic properties, and fumonisins, which have the potential to induce regenerative cell proliferation, is of concern. We will highlight the lack of Codex standards for mycotoxins in edible oils and, in light of the occurrence data reported in this document, the need to monitor aflatoxin contamination levels in beans. Mitigation measures for the risk of exposure to aflatoxins

include preventing the growth of toxin-producing fungi via biological control (Ji et al., 2016) in the field, good post-harvest practices (Suassuna et al., 2018) and the biodegradation of aflatoxins (Liu et al., 2022; Diarra et al., 2020). Since human co-exposure to natural toxins through typical African foods is currently unavoidable, national food safety authorities should ensure that risk assessments are properly carried out to protect human health and maintain food safety. international trade.

As this study demonstrates, aflatoxin contaminants common in many foods (such as corn and beans) threaten human health. Populations in southern Mali may be subject to strong and repeated exposure to natural toxins (aflatoxins). In a recent study (Wangia et al., 2019), the combined effects of various toxins at realistic concentrations were further investigated and revealed antagonistic additives.

Conclusion and Perspectives

This work is a contribution to the current debate on the risks associated with food contamination by mycotoxins. The objective was to characterize the degree of aflatoxin contamination of food (beans, maize and peanuts) sold in Mali and intended for humans and animals. High levels of aflatoxins demonstrate that there are food quality issues for consumers. In this work, all food samples analyzed were contaminated with aflatoxins with the highest concentration of aflatoxin in beans during the rainy season. It was in Koutiala that we obtained the highest contamination during the rainy season.

Because of the high consumption and high levels of aflatoxins in beans and maize in the samples analyzed, we suggest periodic monitoring and the application of Hazard Analysis and Control (HACCP) prevention and control of mycotoxins in the production and industry of human food in Mali.

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