Original Research Article

MICROBIOLOGICAL SAFETY AND QUALITY ASSESSMENT OF MAIZE (*Zea Mays* L.) PRODUCED AND STOCKED FROM RURAL CONDITIONS IN CÔTE D'IVOIRE

ABSTRACT

Aims: Fungal pathogens are one of the main biological agents causing maize post-harvest loss and affect food security in the country. Thus, this study was conducted to assess fungal pathogens associated to post-harvest maize (*Zea mays* L.) with especial focus to mycotoxin-producing fungi at producer's storage condition in different regions of Côte d'Ivoire.
Study design: A total of 1 500 samples of maize as grains, cobs and husks were collected at rate of 500 samples by region (Gbêkê, Poro, Hambol, Indénié-Djuablin and Gontougo) and sent to the laboratory in order to analyse their sanitary quality.
Place and Duration of Study: This study was carried out during March 2016 to January 2017. Then, the analyses of the collected sample took place at the Biotechnology, Agriculture and Valorisation of Biological Ressources Laboratory of the Félix Houphouët-Boigny's University, Abidjan.
Methodology: Mold counts, toxic fungal contamination and mycotoxin (aflatoxin and ochratoxin A) concentrations were determined using standard methods.
Results: The microbiological analysis showed an absence of pathogenic microorganisms in the maize samples regardless of the different regions. However, significant contamination levels of microorganism's hygiene and spoilage indicator were detected in almost maize stored samples. Total aerobic mesophilic bacteria ranged from 10^4 and 10^{11} cfu/g while yeast and moulds were estimated between 10^4 to 10^7 cfu/g. Five fungal genera were isolated. *Aspergillus*, *Fusarium*, *Penicillium* and *Mucor* were predominantly identified in all samples with decreasing order. More importantly, maize sample stored as grain, cobs and husks were affected by aflatoxins (B1, B2, G1 and G2) and ochratoxin A. Sixty percent (60%) of the maize samples, mostly husks, showed aflatoxin B1 (from 12.73 to 130.31 µg/kg) and OTA (from 16.75 to 134.21 µg/kg) concentrations above the Maximum Authorized Limit of 5 µg/kg.
Conclusion: A significant variability from one region to another can be noticed at level of maize quality regardless the type of maize. The sanitary quality of maize seems to be tied to postharvest treatments (drying), type of storage (grains, cobs and husks) and structure of storage.

Keywords: fungal contamination; aflatoxin, ochratoxin A, maize grains; cobs; husks; production region; Côte d'Ivoire.

1. INTRODUCTION

Maize is one of the most widely cultivated grasses in the world [1]. World maize production was around 875 million tonnes in 2012 [2]. The demand continues to increase and cannot be

satisfied without strong technological interventions [3]. In the Ivory Coast cereal cultivation is dominated by rice, maize, millet and sorghum. Like rice, several improved or traditional local varieties of maize are grown there. It is the second cereal produced and consumed after rice [1]. It is used for human and animal food (poultry, pigs, cattle) and serves as a raw material in certain industries (brewing, soap and oil mill) [4]. Long considered a simple subsistence product, maize is now the subject of agricultural speculation which is intensifying in Côte d'Ivoire, due to the economic stakes of this crop which has become increasingly important. Its national production estimated at 1,025,000 tonnes in 2018, for a total sown area of 523,538 ha [5] is still low to meet the needs of the populations. Nearly 50% of this production is located in the Savannah region located in the north of the country. Despite the growth in its production and its socio-economic importance, post-harvest losses during storage remain a real challenge for farmers [6]. Storage practices and traditional storage structures can make maize susceptible to different types of damages including storage pests and disease [7]. Study conducted in Côte d'Ivoire to monitor the merchant quality of maize grains stored for 9 months in polypropylene bags and traditional granaries revealed respectively 47.40% and 60.42% mean grain damage caused both by weevil and mould [8]. In another studies, the characterization of the fungal flora of maize grains (*Zea mays*) intended for the preparation of compound feeding stuffs of poultry have been reported [9]. Furthermore, so far there is little information available on mycobiota population dynamics in traditional storage structures. Moreover, earlier studies highlighted the need for much attention for bio-deterioration which is caused by fungal pathogen particularly mycotoxin-producing fungal pathogens that leads to the loss of physical, nutritional qualities and health impact (grain unsuitable for human consumption) [10, 11]. However, there is limited study conducted in major maize producing areas on fungal pathogens associated with stored maize in Côte d'Ivoire. Therefore, the objective of the current study is to investigate incidence of mould infection and mycotoxin (aflatoxin and ochratoxin A) concentrations in five regions of Côte d'Ivoire.

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2. MATERIAL AND METHODS

2.1 Materials

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2.1.1 Biological material

The biological material is composed of dry maize in the form of grains, cobs and husks deriving the major region production of this resource in Côte d'Ivoire.

2.1.2 Study site

The samples were collected from the regions of Gbêkê (Center), Poro (North), Hambol (North - Center), Indénié-Djuablin (Northeast) and Gontougo (East). Each of these regions has a geographical specificity and climatic characteristics which influence the seasons of maize production. Indeed, the regions of Gbêkê (7º50'nord 5º18'west), Hambol (8º10'nord 5º40'west), Indénié-Djuablin (7º02'nord 3º12'west) and Gontougo (8º30'N 3º20'West) are characterized by a humid tropical climate (Baouléen climate). It has four seasons including two rainy seasons favouring maize production twice a year and two dry seasons. Except the other four regions, the climate of region of Poro (9º27' Nord 5º38' west) is of Sudanese type characterized by a rainy season favourable to maize production and a dry season [12, 13]. Maize (*Zea mays* L.) is the main food crop in these regions taken into account in the study.

2.2 Methods

2.2.1 Sampling of stored maize

The strategy adopted consisted of two phases. The first phase consisted in identifying the regions where maize cultivation constitutes the main subsistence activity. In each region, meetings were organized with the traditional chiefdom to present the study. Then, samples of 1 kg of maize as husks, cobs and grains were taken from the stocks of producers constituting the second phase. A total of 1500 samples were collected for each form of maize from March 2016 to January 2017 (500 grains, 500 cobs, and 500 husks, Table 1). Maize samples were then conveyed to laboratory in sterile plastic bags and kept at 4°C for the microbiological and mycotoxin analysis.

Table 1. Number of samples collected according to maize variety and department

Regions	Grains	Epis	Spathes	Total
Gbêkê	100	100	100	300
Poro	100	100	100	300
Hambol	100	100	100	300
Indénié-Djuablin	100	100	100	300
Gontougo	100	100	100	300
Total	500	500	500	1500

2.2.2 Microbiological analysis of maize stored

Enumeration of microorganisms. The culture dependent approach was performed as follow: 2100 mL of peptone water (Oxoid, Basingstoke, United Kingdom) was added to 100 g of maize grains in a sterile Stomacher bag that was vigorously shaken for 5 min in a Stomacher 400 (Seward, Worthington, United Kingdom) to obtain a uniform homogenate. Samples (1 mL) of the homogenate were serially diluted *10*-fold in peptone water, from which aliquots (0.1 mL) were spread-plated onto different selective agar media and incubated at different temperatures for 1 to 4 days for isolation and enumeration (by recording the number of CFU) by using a colony counter (JP Selecta, Spain) of specific groups of microorganisms [14] : plate count agar (PCA; Oxoid) for the total aerobic bacterial count (30 °C), yeast glucose chloramphenicol (YGC; Oxoid) agar for yeast and moulds (30 °C); Baird Parker (BioRad) agar for S. aureus (37 °C); Violet Red Bile Lactose (VRBL, AES Laboratoire) agar for coliforms (30 °C for total coliforms and 44 °C for thermotolerant coliforms); Hektoen (BioRad) for Salmonella and Trypton Sulfite Neomycin (TSN, BioRad) agar for anaerobes (46 °C).

Fungal isolation. To know the frequency and a relative percentage of fungi, maize grains were plated on potato dextrose agar (PDA, BioRad) medium by agar plating method [15]. Ten (10) maize grains from each sample were surface sterilized with 3% sodium-hypochlorite solution for 3 min and rinsed twice with sterile distilled water. Samples were then plated on PDA plates at the rate of five (5) seeds per plate (9 cm in diameter). The plates were incubated for 5 to 7 days at 100°C. Fungal isolates were sub-cultured on Malt Extract and Czapek Yeast medium agars (Oxoid, UK) and incubated for 5 to 7 days at 100°C for purification. Fungi were identified by using taxonomic schemes based on microscopic observation and culture appearance including colonies colours, texture, reverse colour, hyphae arrangement, conidia shape and nature of spores [15]. The frequency of fungi and relative percentage of particular species with in a genus of fungi was calculated using the formula of [16]:

Frequency (%) = number of samples infected with fungi x 100 / total number of samples analysed
Relative percentage (%) = number of fungal species isolated x 100 / total number of fungi isolated

2.2.3 Analysis of aflatoxins and ochratoxin A

Extraction and purification of aflatoxins. Aflatoxins (AFs) were extracted and purified from maize using the official guidelines of AOAC [17]. To 100 g of maize put in an erlenmeyer flask, 100 mL of 80% methanol aqueous solution were added. The mixture was homogenized, put in darkness at room temperature for 12 h, and then filtered with a Whatman paper (Wathman N°4). Thereafter, 50 mL of the filtrate were added with 40 mL of a mixture deriving from phosphotungstic acid-zinc sulfate-water (5/15/980, w/w/v), and kept at ambient temperature for 15 min before filtration upon Whatman paper. Aflatoxins were extracted from the out coming filtrate with 3 volumes of 10 mL of chloroform. The extract was collected into a 50 mL flask and processed with rotative evaporator (Buchi Rotavapor R-215) at 40 °C for evaporation of the chloroform reagent. Finally, 0.4 mL of hydrochloric acid and 4.6 mL of bidistillated water were added to the dry extract, and the solution was filtered through filter Rezist in a chromatographic tube then passed through an immunoaffinity column (column RiDA aflatoxin, Biopharm, Germany).
Extraction and purification of ochratoxin A. 100 g of the sample of maize was crushed in a hammer mill to obtain a homogeneous fine grind. In a Nalgene jar containing 15 g of grind, 150 mL of aqueous methanol-bicarbonate 1% (m / v, 50:50) were added. The mixture was homogenized by Ultra-Turax for 3 minutes and the homogenate was centrifuged at 5000 rpm for 5 min at 4°C. The supernatant was filtered through a Whatman paper (Wathman N°4) into tubes of 100 mL. 11 mL of filtrate were added 11 ml of saline phosphate buffered (PBS) at pH 7.3. Immunoaffinity columns brand Ochraprep and RBiopharm were conditioned with 10 mL of PBS. Purification of 20 ml of the mixture was made on immunoaffinity columns and OTA extraction was performed with two volumes of 1.5 mL of PBS at a flow rate of 5 mL/minute. The resulting sample was packed in a chromatographic tube and the analysis of OTA was made by HPLC using the European community regulation [18].
Quantification of aflatoxins and ochratoxin A. Determination of AFs and OTA contents was achieved with high performance liquid chromatography column, using a Shimadzu liquid chromatograph (Kyoto, Japan) fitted with fluorescence detector (Table 2).

2.2.4 Statistical analysis

All the analyses were carried out in three-fold test and data processed with software Statistical Product and Service Solutions, SPSS version 20.0, an IBM product since 2009. For each characteristic, the results were expressed in form of averages followed by their standard deviations as parameters of data spread. A two-way analysis of variance (ANOVA 2) was also made in order to test the impact of region and the ways of preserving maize on assessed characteristics to 5% significant threshold statistical. For the statistically different averages, the Tukey's test served for the classification. Furthermore, the correlation between data and samples was estimated on basis of main components analysis (MCA), thanks to STATISTICA version 7.1 software.

Table 2. Conditions of aflatoxins and ochratoxin A analysis by HPLC

ITEM	Aflatoxins (AFB1, AFB2, AFG1, AFG2)	Ochratoxin A (OTA)

Pre-column	Shim-pack GVP-ODS 10 x 4,6 mm	
Column	Shim-pack GVP-ODS, 250 mm x 4,6 mm	
Detector fluorescence	λ excitation: 365 nm	λ excitation: 330 nm
	λ emission: 435 nm	λ emission: 460 nm
Mobile Phase	Acetonitrile/Water/ Methanol (20/20/60)	Acetonitrile/Water/ Acetic acid (49/49/2)
Inject volume	20 µl	100µl
Flow rate	1 mL/min	
Column Temperature	40°C	
Rising solvent	Methanol	Acetonitrile

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Loads of spoilage microorganisms and hygiene indicator microorganisms

The results of microbiological analysis make it possible to assess the hygienic and sanitary qualities of maize samples taken from producers' stocks. The microbial load found in the maize samples is shown in Table 3. Maize samples (grains, cobs and husks) show a varying load between 5.5 x 10^4 and 9.8 x 10^{11} cfu/g of total mesophilic aerobic bacteria count. Except the Hambol maize grains which has a load lower than the standard criteria which is 10^5 cfu/g, all the other samples have loads higher than the standard. However, the regions of Gontougo and Indénié-Djuablin recorded the highest loads for values between 8.4 x 10^6 and 9.8 x 10^{11} cfu/g. For the total and thermotolerant coliforms, the counting varies between 1.8 x 10^3 - 9.9 x 10^4 cfu/g and 10 - 3.3 x 10^3 cfu/g, respectively. Samples of maize grains and maize cobs from the regions of Gbêkê, Poro and Hambol are free from thermotolerant bacteria, while the other samples show loads greater than the standard criteria which is 10 cfu/g. Yeast and moulds were present on all the maize samples regardless of the form and the region and represents the predominant flora of total microorganisms at 30 °C. The most significant loads were enumerated on maize husks samples from the regions of Gontougo and Indénié-Djuablin. These values are estimated between 1.5 x 10^4 and 9 x 10^4 cfu/g for yeasts and between 5 x 10^6 and 3.9 x 10^7 cfu/g for moulds. All of the maize samples (grains, cobs and husks) from the various regions showed no contamination due to pathogenic microorganisms such as Salmonella, *Staphylococcus aureus* and sulfite-reducing anaerobic microorganisms.

Table 3. Hygienic and microbiological quality of samples of maize grains, cobs and husks from the five collection regions

Regions	Maize forms	MAG	TC	FC	Yeasts	Mold	ASR	*Salmonella*	*S. aureus*
Gbêkê	**Grains**	5.0×10^5	1.8×10^3	<10	3.4×10^2	4.5×10^3	$<10^2$	<10	<10
	Cobs	8.1×10^8	5.6×10^3	<10	4.2×10^3	7.2×10^3	$<10^2$	<10	<10
	Husks	6.0×10^9	8.6×10^3	1.3×10^2	7.1×10^4	2.2×10^5	$<10^2$	<10	<10
Poro	**Grains**	2.6×10^5	2.1×10^3	<10	2.2×10^2	2.7×10^3	$<10^2$	<10	<10
	Cobs	6.7×10^8	4.7×10^3	<10	7.0×10^2	6.6×10^3	$<10^2$	<10	<10
	Husks	6.5×10^9	8.0×10^3	1.3×10^2	8.8×10^2	1.5×10^4	$<10^2$	<10	<10
Hambol	**Grains**	5.5×10^4	2.9×10^3	<10	2.7×10^2	3.6×10^3	$<10^2$	<10	<10
	Cobs	7.7×10^7	7.5×10^3	<10	5.6×10^2	8.4×10^3	$<10^2$	<10	<10
	Husks	4.5×10^{10}	7.6×10^3	1.9×10^2	7.7×10^3	1.8×10^4	$<10^2$	<10	<10
Indénié-Djuablin	**Grains**	8.4×10^6	6.3×10^3	1.1×10^2	5.0×10^2	7.5×10^4	$<10^2$	<10	<10
	Cobs	6.5×10^9	6.8×10^3	1.8×10^2	3.2×10^3	5.3×10^4	$<10^2$	<10	<10
	Husks	9.8×10^{11}	7.9×10^4	3.3×10^3	9.0×10^4	3.9×10^7	$<10^2$	<10	<10
Gontougo	**Grains**	9.2×10^6	7.5×10^3	1.5×10^2	7.2×10^2	5.5×10^4	$<10^2$	<10	<10
	Cobs	8.1×10^9	8.1×10^3	2.9×10^2	9.2×10^3	8.8×10^4	$<10^2$	<10	<10
	Husks	1.2×10^{11}	9.9×10^4	2.9×10^3	1.5×10^4	5.0×10^6	$<10^2$	<10	<10
Microbiological criteria		10^5 CFU/g	10^3 cfu/g	10 cfu /g	10^3 cfu /g	10^3 cfu /g	10^2 cfu /g	Not present	Not present

Each value is the average of the analysis of three tests; MAG: Mesophilic Aerobic Germs; TC: Total Coliforms; FC: Fecal Coliforms; ASR: Anaerobic Sulphito-Reducers.

3.1.2 Fungal flora isolated

The fungal count of maize sample, collected from the five regions, is given in Table 3. In the present investigation, mycological examination of maize samples revealed the occurrence of five genera: *Aspergillus*, *Fusarium*, *Penicillium*, *Mucor* and *Alternia* (Table 4). Aspergillus genus shows a greater number of species isolated from samples of maize stored. The percentage of contamination of *this genus* varies between 41 and 70.2 %. The regions of Indénié-Djuablin and Gontougo record the highest percentages for the various maize forms with values ranging from 55.3 to 70.2 %. The low percentages are recorded on maize cobs and grains in the region of Hambol (41 and 42.3 %, respectively) and on maize grains from the region of Poro (42 %). Occurrence due to *Fusarium* species is higher on grains, cobs and husks samples in the regions of Gontougo and Indénié-Djuablin with percentages ranging from 20 to 36 %. The lowest percentages are recorded in the regions of Gbêkê for maize grains sample (10 %) and Poro respectively for maize cobs (12 %) and husks (11 %) samples. The contamination rate of *Penicillium* species is higher on maize husks samples in the region of Gbêkê, on maize grains and cobs samples in the region of Hambol with values of 15.9%, 16% and 18% respectively. Samples of maize grains, on the cobs and on the husks the highest *Mucor* contamination (12%) with values between 12% and 31%. Samples of maize on the cobs and husks from the region of Indénié-Djuablin recorded the lowest values of 4%. With regard to the *Alternia* genus, the lowest contamination rates were recorded in the regions of Indénié-Djuablin and Gontougo regardless of the forms of maize sampled with percentages between 2% and 6%. The highest rates were determined in samples of maize in grains (10% to 13%) and on the cobs (14%) respectively for the regions of Gbêkê, Hambol and Poro.

Comment [P.3]:
Please discuss and explain the effect of a high or low percentage of isolated fungal flora on maize crops?

3.1.3 Relative density of Aspergillus species

The relative density of Aspergillus species is shown in Figure 1. Six (6) species were identified. The regions of Gontougo and Indénié-Djuablin have a high percentage of *A. flavus* regardless of the shape of the sample compared to other areas. The highest percentages were recorded on husks (17%), followed by cobs with values ranging from 12 ± 0.50 to 14.6 ± 0.65%. In addition, high percentages were also determined on the husks of Gbêkê, Poro and Hambol with respective values of 8.5 ± 0.45%, 9 ± 0.30% and 12 ± 1.50%. A predominance of *A. niger* is observed on Gbêkê husks (16 ± 0.15%), followed by samples of Gontougo husk and cobs maize with respective rates of 11 ± 0.50% and 12 ± 0.65%. The region of Hambol has the lowest occurrence rates of A. niger with values between 2 ± 0.17% and 4.8 ± 0.100%. As regards the *A. fumigatus* species, a high occurrence is observed on all the maize samples regardless of the region with percentages varying between 9.6 ± 0.65% and 15 ± 0.50%. However, low percentages were recorded on samples of grains maize from the regions of Poro and Gontougo with respective rates of 5.5 ± 0.40% and 5.8 ± 0.17%. The regions of Gbêkê and Poro have the highest levels of contamination of *A. terrus* on samples of maize on the grains and on the cobs with percentages between 10.5 ± 0.50% and 14.40 ± 0.35%. Likewise, strong contaminations were recorded on samples of maize husks from Poro (12%), grains from Gontougo and Indénié-Djuablin and husks from Gontougo. The region of Hambol presented the lowest percentages with rates ranging between 6.7 ± 0.100% and 9.5 ± 0.60%. Of all the samples analysed, the regions of Poro, Indénié-Djuablin and Gontougo recorded the highest percentages of contamination in *A. versicolor*, with contents between 8 ± 0.50% and 10.5 ± 0.56%. In addition, the samples of Hambol husks showed high proportions reaching the value of 9 ± 0.55%. On the contrary, the regions of Gbêkê and Hambol have low percentages with an average proportion of 4.32%. The species of *A. glaucus* isolated from maize samples showed the strongest contamination in the regions of Indénié-Djuablin and Gontougo with percentages ranging from 11 ± 0.00% to 14 ±

0.20%. The lowest levels were recorded in the regions of Gbêkê, Hambol and Poro with values ranging from 1.4 ± 0.15% to 6 ± 0.10%.

Comment [P.4]: Please explain and discuss, what is the effect of the relative density of Aspergillus species on maize?

Table 4: Contamination levels of stored maize samples according to isolated fungi

Regions	Form of maize	*Aspergillus*	*Fusarium*	*Penicillium*	*Mucor*	*Alternia*
Gbêkê	**Grains**	50.5 ± 2.40^{d}	6 ± 0.100^{d}	10 ± 0.90^{b}	21 ± 0.45^{a}	12.5 ± 1.72^{bc}
	Cobs	48.8 ± 2.65^{d}	8.2 ± 0.84^{c}	11 ± 1.20^{b}	18 ± 1.70^{b}	14 ± 0.60^{b}
	Husks	58.1 ± 2.32^{c}	4 ± 0.80^{d}	15.9 ± 1.50^{a}	20 ± 1.94^{ab}	2 ± 0.10^{e}
Hambol	**Grains**	42.3 ± 2.15^{e}	5.2 ± 0.60^{d}	16 ± 1.10^{a}	23.5 ± 0.30^{a}	13 ± 0.50^{b}
	Cobs	41 ± 3.17^{e}	5 ± 0.33^{d}	18 ± 1.73^{a}	17 ± 2.100^{b}	19 ± 0.78^{a}
	Husks	48 ± 3.24^{d}	14 ± 0.90^{a}	4 ± 0.60^{d}	20 ± 1.60^{ab}	14 ± 1.15^{b}
Poro	**Grains**	42 ± 2.10^{e}	7 ± 0.50^{c}	14 ± 1.00^{b}	20 ± 1.50^{ab}	17 ± 0.50^{a}
	Cobs	58 ± 3.57^{c}	4.1 ± 0.76^{d}	6 ± 1.20^{c}	18 ± 1.80^{b}	14 ± 0.95^{b}
	Husks	59 ± 2.63^{c}	8 ± 0.20^{c}	11 ± 0.100^{b}	10 ± 1.50^{c}	12 ± 0.78^{bc}
Indénié-Djuablin	**Grains**	60 ± 3.75^{b}	16 ± 0.82^{a}	10 ± 0.56^{b}	10 ± 1.35^{c}	4 ± 0.03^{d}
	Cobs	62 ± 9.65^{b}	10.2 ± 0.10^{b}	11 ± 0.20^{b}	11 ± 0.56^{c}	6 ± 0.20^{d}
	Husks	67.5 ± 2.96^{a}	15.5 ± 0.56^{a}	8 ± 0.50^{c}	4 ± 0.05^{d}	5 ± 0.57^{d}
Gontougo	**Grains**	55.3 ± 2.41^{c}	12.4 ± 1.65^{b}	7.3 ± 0.15^{c}	13 ± 1.50^{c}	12 ± 0,75^{bc}
	Cobs	60 ± 3.10^{b}	10.3 ± 1.15^{b}	9 ± 0.65^{bc}	10 ± 0.45^{c}	10,9± 0.56^{c}
	Husks	70.2 ± 2.76^{a}	12.8 ± 0.95^{b}	7 ± 0.50^{c}	10 ± 0.56^{c}	0 ± 0.00

On the same column, the numbers followed by the same letter are statistically identical to the 5% threshold (Tukey's test mean ± standard deviation).

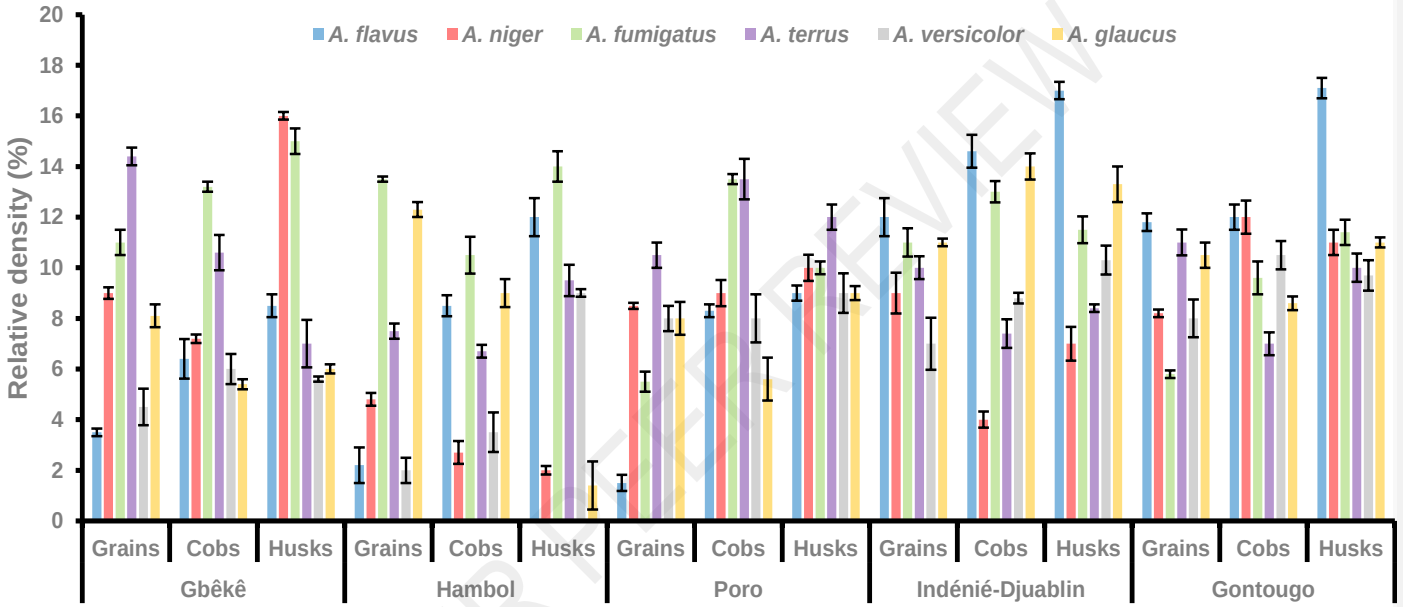


Figure 1. Relative density of *Aspergillus* species isolated from stored maize samples

3.1.4 Aflatoxin concentrations in stored maize samples

The aflatoxin concentrations of the samples of maize stored in the different regions are recorded in Table 5. Aflatoxin B1 contents vary from 0.80 ± 0.75 to 20.92 ± 27.63 µg/kg for maize grains, from 2.40 ± 2.67 to 32.22 ± 50.40 µg/kg for maize cobs and from 12.73 ± 26.26 to 130.31 ± 92.56 µg/kg for maize on husks. Samples of maize in grains and on cobs from the regions of Gbêkê, Hambol and Poro recorded the lowest concentrations below 5 µg/kg, the maximum limit reference value. The highest concentrations exceeding the standard were recorded on all samples of maize husks which differ significantly from other forms of maize regardless of the region. In addition, samples of maize from Indénié-Djuablin and Gontougo show concentrations ranging from 9.30 ± 9.76 to 32.22 ± 50.40 µg / kg, above the normative value. In total 40% of the maize samples analysed have a content below the reference standard of the European Union. For aflatoxin B2 concentrations, the values vary between 0.10 ± 0.12 and 1.43 ± 2.04 µg/kg for grains, between 0.28 ± 0.28 and 3.21 ± 4,84 µg/kg for maize as cobs and between 0.33 ± 0.35 and 3.26 ± 4.89 µg / kg for husks. The region of Indénié-Djuablin stands out significantly from other regions by the highest levels on maize samples as cobs and husks. Regarding aflatoxin G1, the various maize samples recorded values ranging from 3.36 ± 4.52 to 3.90 ± 4.00 µg/kg for the region of Gbêkê, from 1.65 ± 1, 52 to 4.12 ± 4.57 µg/kg for Poro, from 2.35 ± 2.33 to 8.71 ± 19.16 µg/kg for Hambol, from 16.07 ± 17.45 to 32.31 ± 47.48 µg/kg and from 27.56 ± 51.44 to 37.11 ± 48.85 µg/kg for Gontougo. With values varying from 0.10 ± 0.10 to 0.63 ± 1.21 µg/kg, maize samples as grains, cobs and husks from the regions of Gbêkê, Hambol and Poro show the lowest concentrations of aflatoxin G2 unlike the other samples with concentrations ranging from 1.33 ± 1.89 to 3.35 ± 5.10 µg/kg. The total aflatoxin concentrations resulting from the sum of different aflatoxins differed significantly (P <0.05) from maize form and region for maize samples as cobs and husks. The contents vary from 2.63 ± 2.36 to 60.79 ± 80.24 µg/kg for maize grains, from 7.04 ± 7.04 to 71.04 ± 91.59 µg/kg for maize cobs and from 17.66 ± 31.30 to 169.19 ± 150.13 µg/kg for maize husks (Table 4).

Comment [P.5]: Please explain and discuss, what is the impact of high or low aflatoxin content on maize?

3.1.5 Concentrations of ochratoxin A in stored maize samples

Table 6 shows ochratoxin A (OTA) levels determined in the maize samples. All maize samples are contaminated regardless of the different regions. However, maize samples as grains and cobs from the regions of Gbêkê, Poro and Hambol show concentrations between 0.84 ± 0.78 and 2.61 ± 2.24 µg/kg, below the normative value set at 5 µg/kg. These samples represent 40% of the total samples conforming to the standard. With values varying from 16.75 ± 32.42 to 134.21 ± 77.24 µg/kg, husks differ significantly from other samples by contents greater value than to those recommended European Union. In addition, maize grains and cobs from the regions of Indénié-Djuablin and Gontougo have concentrations ranging from 5.58 ± 5.43 to 18.60 ± 18.16 higher than the normative value set by the European Union.

Comment [P.6]: please state the standard value recommended by the European Union. Then, explain why are these results different with standard value?

Comment [P.7]: Please discuss why is the result higher than the normative value?

Table 5. Aflatoxin concentrations in stored maize samples

Aflatoxins	Regions	Grains	Cobs	Husks
AFB1 (µg/kg)	Gbêkê	1.97 ± 2.70^{cB}	2.27 ± 2.40^{bB}	12.73 ± 26.26^{cA}
	Poro	0.80 ± 0.75^{cB}	2.53 ± 2.93^{bB}	18.28 ± 30.97^{cA}
	Hambol	1.31 ± 1.37^{cB}	2.40 ± 2.67^{bB}	55.41 ± 65.00^{bA}
	Indénié-Djuablin	9.30 ± 9.76^{bB}	32.22 ± 50.40^{aB}	130.31 ± 92.56^{aA}
	Gontougo	20.92 ± 27.63^{aA}	13.78 ± 24.46^{aA}	19.92 ± 32.50^{cA}
AFB2 (µg/kg)	Gbêkê	0.9 ± 0.28^{aA}	0.39 ± 0.37^{bA}	0.44 ± 0.42^{bA}
	Poro	0.10 ± 0.12^{aA}	0.28 ± 0.28^{bA}	0.33 ± 0.35^{bA}
	Hambol	0.19 ± 0.23^{aA}	0.34 ± 0.33^{bA}	0.56 ± 0.10^{bA}
	Indénié-Djuablin	0.59 ± 0.77^{aB}	3.21 ± 4.84^{aA}	3.26 ± 4.89^{aA}
	Gontougo	1.43 ± 2.04^{aA}	1.51 ± 2.57^{aA}	1.56 ± 2.62^{aA}
AFG1 (µg/kg)	Gbêkê	3.36 ± 4.52^{bA}	3.85 ± 3.95^{bA}	3.90 ± 4.00^{bA}
	Poro	1.65 ± 1.52^{bA}	4.07 ± 4.52^{bA}	4.12 ± 4.57^{bA}
	Hambol	2.35 ± 2.33^{bA}	3.96 ± 4.24^{bA}	8.71 ± 19.16^{bA}
	Indénié-Djuablin	16.07 ± 17.45^{aA}	32.31 ± 47.48^{aA}	31.37 ± 47.58^{aA}
	Gontougo	37.11 ± 48.85^{aA}	27.56 ± 51.44^{aA}	27.61 ± 51.49^{aA}
AFG2 (µg/kg)	Gbêkê	0.18 ± 0.26^{aA}	0.54 ± 0.57^{bA}	0.59 ± 0.62^{bA}
	Poro	0.10 ± 0.10^{aA}	0.50 ± 0.31^{bA}	0.55 ± 0.36^{bA}
	Hambol	0.16 ± 0.21^{aA}	0.52 ± 0.44^{bA}	0.63 ± 1.21^{bA}
	Indénié-Djuablin	0.51 ± 0.64^{aB}	3.30 ± 5.05^{aA}	3.35 ± 5.10^{aA}
	Gontougo	1.33 ± 1.89^{aA}	1.68 ± 2.68^{aA}	1.73 ± 2.73^{aA}
AFT (µg/kg)	Gbêkê	5.70 ± 7.68^{cB}	7.04 ± 7.04^{bB}	17.66 ± 31.30^{cA}
	Poro	2.63 ± 2.6^{cB}	7.39 ± 7.79^{bB}	23.28 ± 36.100^{cA}
	Hambol	4.01 ± 4.02^{cB}	7.22 ± 7.42^{bB}	65.31 ± 85.47^{bA}
	Indénié-Djuablin	26.46 ± 28.100^{bC}	71.04 ± 91.59^{aB}	169.19 ± 150.13^{aA}
	Gontougo	60.9 ± 80.24^{aA}	44.53 ± 80.88^{aA}	50.82 ± 89.34^{bA}

AFB1: Aflatoxin B1; AFB2: Aflatoxin B2; AFG1: Aflatoxin G1; AFG2: Aflatoxin G2; AFT: Total Aflatoxins; By columns and rows the averages with the same letters are statistically identical. The upper-case letters are representative of the lines and the lower-case letters are representative of the columns.

Table 6. Ochratoxin A concentrations in stored maize samples

Regions	Grains	Cobs	Husks
Gbêkê	1.46 ± 2.14^{cB}	1.47 ± 1.89^{bB}	16.75 ± 32.42^{cA}
Poro	0.84 ± 0.78^{cC}	2.28 ± 2.86^{bB}	47.80 ± 88.13^{cA}
Hambol	1.69 ± 1.83^{cB}	2.61 ± 2.24^{bB}	68.40 ± 55.06^{bA}
Indénié-Djuablin	5.58 ± 5.43^{bC}	18.60 ± 18.16^{aB}	134.21 ± 77.24^{Aa}
Gontougo	11.35 ± 11.13^{aB}	13.11 ± 18.63^{aB}	45.51 ± 44.38^{Ca}

By columns and rows, the averages with the same letters are statistically identical. The upper-case letters are representative of the lines and the lower-case letters are representative of the columns.

3.1.6 Correlations between the parameters of the sanitary quality of the maize samples

Table 7 shows the existence of several significant positive correlations between mold loads and the mycotoxins levels. Indeed, an increase load of *Aspergillus flavus* strongly coincides with an increase in mycotoxin concentrations, r varying from 0.58 to 0.79. Likewise, the load of *Aspergillus versicolor* depends very significantly on the contents of mycotoxins (r ranging from 0.52 to 0.62). The level of aflatoxin B1 significantly influences the other mycotoxins (r varying from 0.71 to 0.95). Also, ochratoxin A concentration is proportional to those of the mold and the aflatoxin levels (r between 0.56 and 0.88).

Comment [P.8]: what is the standard value of R to be used as a benchmark that the results have a significant effect?

3.1.7 Differentiation of maize samples in relation to the sanitary quality parameters studied

The principal components analysis was carried out using the F1 and F2 components which records an eigenvalue higher than 1, according to the Kaiser rule (Table 8). The projection of analysed variables in factorial design F1-F2 shows strong negative correlation between all the parameters studied (mold load, relative density of *Aspergillus* species, aflatoxin and ochratoxin A concentrations) with F1 factor (Fig. 1, A). Based on the projection of samples in the same design, they are organised in two groups. Group 1 is composed of two individuals presenting high levels of mold load, relative density of *Aspergillus* and mycotoxins contents. It deals with husks maize coming from Gontougo and Indénié-Djuablin. Group 2 includes individuals having low mold load, relative density of *Aspergillus* and mycotoxins contents. It deals with all maize samples from Gbêkê, Poro and Hambol on the one hand and maize grains and cobs from Indénié-Djuablin and Gontougo on the other (see Fig. 1, B).

Table 7. Correlation matrix between sanitary parameters of maize samples

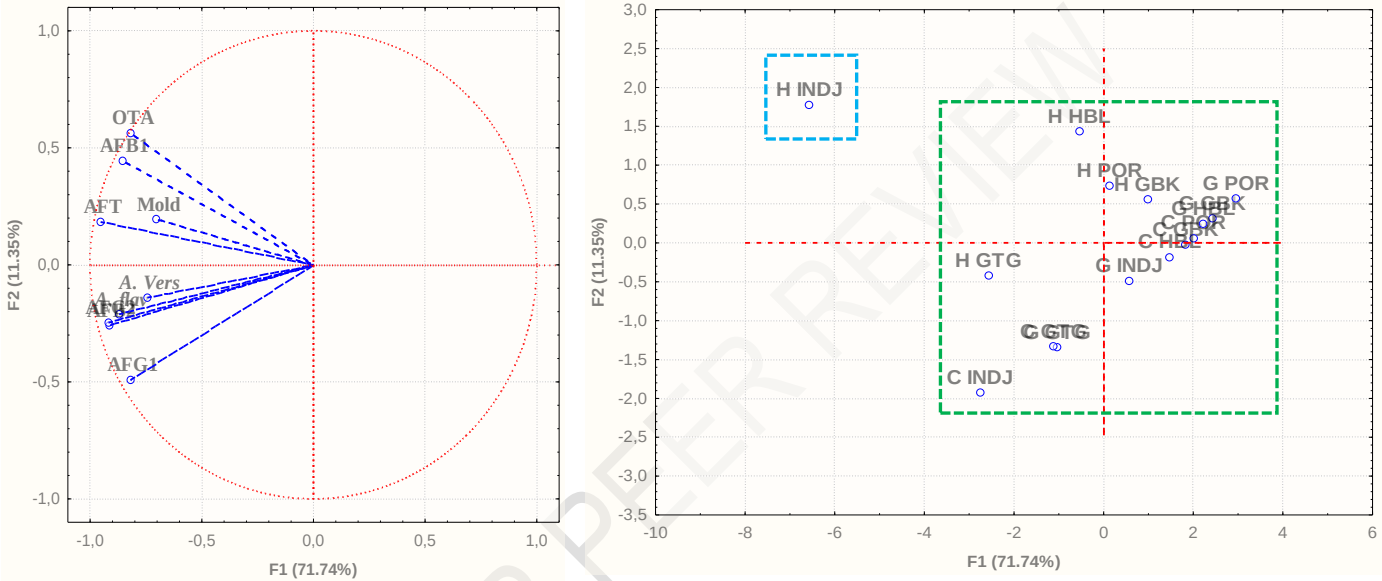
	Mold	*A. fla*	*A. Ver*	AFB1	AFB2	AFG1	AFG2	AFT	OTA
Mold	**1.00**								
A. fla	**0.71**	**1.00**							
A. Ver	0.47	**0.69**	**1.00**						
AFB1	**0.53**	**0.58**	**0.52**	**1.00**					
AFB2	0.50	**0.76**	**0.60**	**0.71**	**1.00**				
AFG1	0.47	**0.78**	**0.62**	0.50	**0.86**	**1.00**			
AFG2	**0.53**	**0.79**	**0.61**	**0.71**	**0.99**	**0.83**	**1.00**		
AFT	**0.58**	**0.72**	**0.62**	**0.96**	**0.86**	**0.73**	**0.85**	**1.00**	
OTA	**0.65**	**0.60**	**0.56**	**0.95**	**0.59**	0.39	**0.60**	**0.88**	**1.00**

In bold, significant correlation values. Mold: mold loads; A. fla: relative density of A. flavus; A. ver: relative density of A. versicolor; AFB1, aflatoxin B1 content; AFB2, aflatoxin B2 content; AFG1, aflatoxin G1 content; AFG2, aflatoxin G2 content; AFT, total aflatoxin content; OTA, ochratoxin A content

Table 8. Matrix of the eigenvalues of the factors resulting from the principal components analysis and correlation with the parameters of the sanitary quality of maize samples according to the regions.

	Axis 1	Axis 2
Contribution of the axis		
Proper value	6.46	1.02
Variability (%)	71.74	11.35
% cumulated	71.74	83.09
Definition of axis and factor weights		
Molds	**-0.70**	0.19
A. flavus	**-0.87**	-0.21
A. versicolor	**-0.74**	-0.14
AFB1	**-0.86**	**0.45**
AFB2	**-0.91**	-0.26
AFG1	**-0.82**	**-0.49**
AFG2	**-0.92**	-0.25
AFT	**-0.95**	0.18
OTA	**-0.82**	**0.56**

In bold, significant correlation values.



(a) Projection of variables

(b) Projection of individuals

Figure 2. Projection of the sanitary parameters (a) and individuals (b) of maize grains, cobs and husks in the factorial design 1-2 of the principal component analysis.

mould, mold loads; ***A. fla*,** relative density of *A. flavus*; ***A. vers*,** relative density of *A. versicolor*; **AFB1,** aflatoxin B1 content; **AFB2,** aflatoxin B2 content; **AFG1,** aflatoxin G1 content; **AFG2,** aflatoxin G2 content; **AFT,** total aflatoxin content; **OTA,** ochratoxin A content; **GBK**, Gbêkê; **POR**, Poro; HBL, Hambol; **INDJ**, Indénié-Djuablin; **GTG**, Gontougo; **G**, Grains; **C**, Cobs; **H**, Husks.

3.2 Discussion

The results of the microbiological analyses indicate a significant level of contamination of the various forms of stored maize sample (grains, cobs and husks) in the five regions visited during this study. The flora of maize sample is composed of saprophytic germs which proliferate in parallel with an increase in the humidity level causing alterations. This high-water content could be a limiting factor for traditional maize storage. Indeed, it promotes the proliferation of microorganisms, capable, using their amylases, of hydrolysing starch and producing mycotoxins that are harmful to humans and animals. Similar remarks were noted by [19] implying a high proportion of germs of hygienic interest in samples of maize flour marketed in nine communes of Abidjan. Microbiological analyses of maize samples show that all maize forms are predominantly contaminated by aerobic mesophilic germs regardless the region (of the order of 10^5 to 10^{11}). Maize samples as cobs and husks are well above the standard which is 10^5 CFU/g recommended by the Codex Alimentarius. These microorganisms consist of pathogens and non-pathogenic germs for the most part not very demanding at the level nutritional [20]. Indeed, for this type of germs, temperature and humidity remain important criteria for their growth. These results are similar to those obtained by [21] which showed the large predominance of the aerobic mesophilic flora in the contamination of wheat flour from storage condition in Morocco. The count of thermo-tolerant coliforms indicates results well above the standard for almost all samples. The strong presence of these germs is justified by the ubiquitous nature of the latter. These bacteria, which are widespread in the environment and saprophytic in humans and warm-blooded animals, are found in maize after harvest and throughout the drying and storage period. Indeed, these germs are considered as hygiene indicators in the food manufacturing process [22]. Results of this study are in agreement with those of [19] and [20] who enumerated these germs in the samples of maize flour collected in different markets of Abidjan with charges between 10^3 and 10^6 CFU / g. However, it should be noted that maize grain and cobs from Gbêkê, Poro and Hambol are free from faecal coliforms probably reflecting good post-harvest maize hygienic conditions practiced by producers in these regions. All maize samples analysed were free from pathogenic bacteria such as *Salmonella*, *S. aureus* and Sulfite-Reducing Anaerobes. This absence of pathogenic germs in maize stored could reflect the respect of good hygiene practices during storage by producers. Conservation of crops remains one of the key factors in a country's food security. Indeed, agricultural production is generally seasonal as consumer needs extend throughout the year. It is an art that requires the establishment of an adequate sanitary policy to spare populations from the risk of food shortages during the agricultural off-season. In this perspective, particular emphasis should be placed on the control of crop pests in stocks such as molds. Indeed, the damage caused by the latter can lead to financial losses, famines and risks of intoxication linked to the consumption of spoiled products [23, 24]. Analysis of the results showed that all samples were contaminated with yeasts and molds. The highest loads were recorded on maize husks. This contamination could be due to a poor storage condition of the maize. According to [25], fungal contamination probably takes place before harvest, in the field, during drying and storage. In fact, molds have the property, under unfavorable conditions, of becoming spore-forming and of multiplying by germination when conditions become favorable. The most common spoilage fungi, and the most destructive of foods, belong to the genera *Aspergillus*, *Penicillium* and *Fusarium*. Contamination of cereal grains by a multitude of molds, particularly in maize, has been documented in other studies [9, 26, 27, 28], and results of this study also confirm this state of affairs. Five (5) genera of molds (*Aspergillus*, *Fusarium*, *Penicillium*, *Mucor* and *Alternia*) were isolated and identified at varying percentages on all maize samples (grains, cobs, husks). The presence of fungal flora in maize samples can have serious consequences on the health of consumers. Indeed, these molds can produce mycotoxins which are toxic to humans and animals [29]. The frequency of isolation shows that the genus *Aspergillus* shows a greater number of species isolated on

samples of stored maize with a percentage varying between 40% and 70% with a predominance on maize husks. The dominance of the genus *Aspergillus* in the contaminating flora of cereals has been reported in several studies [9, 10, 30, 31]. Furthermore, the results of the incidence of *Aspergillus* show that *Aspergillus flavus* and *Aspergillus niger* are the fungi most present in maize samples. *Aspergillus* strains of the flavi and nigri section can be isolated from different farming systems, be it maize, rice or peanuts [32]. These are fungi that proliferate at high temperatures and support relatively low water activity. They are considered “storage fungi” although contamination frequently begins in fields [33]. Under optimal growth conditions, *Aspergillus* is able to produce a biologically significant amount of toxins within days. This same observation was made by [9] on maize grains intended for the preparation of compound feed for poultry in Côte d'Ivoire. The frequencies of occurrence of these filamentous fungi, according to these authors, were 35.47% and 17.78% respectively for *A. flavus* and *A. niger*. In addition, the work of [26] showed that these two species *A. flavus* and *A. niger* were the most present among the fungal flora isolated on maize grains stored in traditional structures such as Gombisa and polypropylene bags in Ethiopia with a frequency of 90% and 51%. According to [34], *Aspergillus flavus* is an opportunistic pathogen of crops and has a cosmopolitan distribution. The presence of *Aspergillus flavus* in maize stored, which is either intended for human and animal consumption, will pose health concerns given that this fungus is likely to produce a dreaded toxin of aflatoxin in maize before and after harvest, in almost all stored foods and the latter is a potent carcinogen that is highly regulated in most countries [24]. *Aspergillus fumigatus*, *A. terrus*, *A. glaucus* and *A. versicolor*, also present on maize sample, are common contaminants on various substrates and frequently isolated in nuts and sun-dried products [35] as is the case with the maize samples analysed in our study. Some strains of *A. niger*, *A. versicolor* and *Penicillium* are producers of ochratoxin A (OTA), a carcinogen classified by the International Agency for Research on Cancer [10].

All analyzed maize samples in our study were also contaminated with *Fusarium* sp, Mucor sp and *Alternia* sp. The presence of these fungi may lead also to the formation of mycotoxins, which are secondary fungal toxic metabolites to humans and animals, causing disorders like cancer, immune suppression or endocrine disruption [36]. Mycotoxins such as aflatoxins B1, B2, G1, G2 and OTA were detected in the different forms of maize (grains, cobs, husks) from the five regions. The presence of these toxins in our samples could be explained by the fact that the storage conditions of the maize favored the growth of the molds responsible for the production of these toxins. In addition, the detection of these mycotoxins in maize is a public health concern, in places where this cereal is consumed as a staple food and is also used as an ingredient in animal feed. In fact, in Côte d'Ivoire, as in other developing countries, maize is one of the staple foods of the population. It is eaten in the form of fresh boiled (kaba-belégué) or simply braised, porridge or baked pancakes made from maize flours, “kabatoh” or “akassa Boulle” [20]. Sixty percent (60%) of maize samples, mostly husks, showed aflatoxin B1 and OTA concentrations above the maximum authorized limit of 5 µg/kg [37]. These results are in agreement with the work carried out by [38] who reported a high level of aflatoxin B1 and ochratoxin A contamination on maize from the Abidjan markets with concentrations ranging from <1.5 µg/kg to 20 µg/kg. Substantially equal results were also reported by [39]. These authors detected concentrations between 6.3 - 150 µg/kg and 2 - 186.5 µg/kg for total aflatoxins and ochratoxin A in maize samples stored in five producing regions of Ethiopia. This study is similar to that reported by [40] in Brazil who reported a consumption of foods contaminated with aflatoxins. The determination of aflatoxins in maize showed 42% positive samples (ranging from 0.05 to 8.3 µg/kg), with a greater incidence in maize flour. Similar results on the presence of OTA in breakfast cereals have been obtained in Canada. To this end, 30% of the samples analysed were contaminated, with low contamination levels ranging from 0.01 to 0.38 µg/kg [41].

However, low concentrations of aflatoxin B1 (0.80 to 2.53 µg / kg) and OTA (0.84 to 2.61 µg / kg) were detected in maize in grains and cobs from the regions of Gbêkê, Poro and

Hambol, representing 40% compliance of maize samples with respect to the normative value. These results are similar to those obtained by [24]. These authors found average of aflatoxin B1 concentrations of 0.92 µg/kg in maize flours collected in the region of Séguéla in the North-West region of Côte d'Ivoire. These data can help encourage maize producers in these regions to better promote good production and storage methods. This same observation is also made for total aflatoxins representing the total sum of aflatoxins (B1, B2, G1 and G2). The lowest concentrations of total aflatoxins, below the reference value which is 10 µg/kg [37], were recorded on all maize grains and cobs samples of the regions of Gbêkê, Poro and Hambol with levels varying from 1.75 to 4.06 µg/kg.

4. CONCLUSION

The evaluation of the microbiological parameters revealed an absence of pathogenic microorganisms in the maize samples regardless to the region. However, significant contamination levels of maize hygiene and spoilage indicator germs were detected in almost all of the maize samples. With regard to the quality criteria specified, the husks are not of good microbiological quality. Regarding the isolation and identification of molds, the genus *Aspergillus* was the most prominent with an occurrence of three species which are *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus versicolor* potential producers of mycotoxins. Thus, analysis for mycotoxin contaminants revealed the presence of aflatoxins B1, B2, G1, G2 and ochratoxin A in all forms of maize. With the exception of maize grains and cobs from the regions of Gbêkê, Poro and Hambol, all other samples showed concentrations above the Maximum Allowable Limit for aflatoxins B1, total aflatoxins and ochratoxin A as regulated by the European Union. This study highlighted poor agricultural practices used in Côte d'Ivoire. Current practices should be revised for the production of maize that is safe for consumption by the population in this region.

REFERENCES

1. Siene LA, Conde M, Bayala R, N'guettia TV, Kouadio AF. Response of two local varieties of maize (Zea mays L.) to two types of fertilization in post-floral water deficit conditions in sudanian zone of Côte d'Ivoire. Int. J. Innovat. Applied Stud. 2020 ; 29(3) : 443-455.
2. Planetoscope-Statistique. Statistiques mondiales en temps réel sur les céréales www.planetoscope.com/cereales/193-production-mondiale-de maïs. 2013 ; Consulté le 19/01/2021 à 16h30mn
3. Semassa AJ, Padonou SW, Anihouvi VB, Akissoé NH, Aly D, Adjanohoun A, Baba-Moussa L. Diversité variétale, qualité et utilisation du maïs (Zea mays L.) en Afrique de l'Ouest : Revue Critique. Euro. J. Scien. Res. 2016; 12 (18): 197-217.
4. Bamba S, Biego H, Coulibaly A, Nyamien Y, Konan Y, Sidibe D. Determination of merchantability of maize (Zea mays L.) as epis, spathes and grains stocked in the production regions of Côte d'Ivoire. J. Agricul. Ecol. Res. Inter. 2019; 20(4): 1-10
5. FAOSTAT. Statistical databases on African countries "food commodities" trade, production, consumption, and utilization. FAO, Rome, Italy, disponible sur : http://www.uneca.org/sites/default/files/uploads/ice17-report-secu-aliment-industrie-fr-ecawa. 2020-09-final.pdf.
6. Die GR, Chatigre KO, Fofana I, Biego GHM. Nutritive parameters evolution of maize seeds conserved by triple bagging system and biopesticides (Lippia multiflora and

Hyptis suaveolens) leaves in Côte d'Ivoire. Inter. J. Biochem. Res. Review. 2019; 28 (3): 1-15
7. Yao VG, Konan KC, Niamketchi GL, Aka BA, Coulibaly A, Biego HG. Evolution of merchantability during storage of maize triple bagged containing biopesticides (Lippia multiflora and Hyptis suaveolens). Euro. J. Nutrit. Food Safet. 2019; 11 (4): 274-283.
8. Ezoua P, Coulibaly A, Konan Y, Sidibé D, Chatigre KO, Biego GHM. Efficacy of Lippia multiflora (Verbenaceae) and Hyptis suaveolens (Lamiaceae) leaves on merchant quality of stored maize grain (Zea mays L.) in Côte d'Ivoire. J. Agri. Eco. Res. Inter. 2017; 11(3):1-10.
9. Dedi K, Diomande Y. Caractérisation de la mycoflore de grains de maïs (Zea mays) destinés à la préparation d'aliments composés pour volaille. Inter. J. Biolog. Chim. Sci. 2017 ; 11 (6) : 10094-2603.
10. Toffa D. Etude de la contamination de certains aliments d'origine végétale de la république du Niger, les moisissures toxinogènes. Thèse de Doctorat, Faculté des sciences Rabat, Université Mohammed V, Maroc. 2015 ; 246 p.
11. Yao VG, Biego GH, Konan KC, Niamketchi GL, Coulibaly A. Evolution of mycotoxins during maize grains storage in triple bags containing plants biopesticides (Lippia multiflora and Hyptis suaveolens). AFSJ. 2020; 17(3): 22-33.
12. Amani MK, Koffi FK, Yao BK, Kouakou BD, Jean EP, Sekouba O. Analysis of climate variability and its influences on seasonal rainfall patterns in West Africa: Case of the watershed N'zi (Bandama) in Ivory Coast. Euro. J. Geogr. 2010 ;12: 200-222.
13. Kouassi AM, Kouamé KF, Goula BTA, Lasm T, Paturel JE, Biémi J. Influence of climatic variability and modification of land use on the rain-flow relationship from a global modeling of the N'zi (Bandama) watershed in Ivory Coast. Ivoi. Sci. Techno. Review. 2008; 11:207-229.
14. AFNOR. Analyse microbiologique, contrôle de la qualité des produits alimentaires. AFNOR Ed, Paris; 1996.
15. Pitt JI and Hocking AD. Food and Fungi Spoilage, 2nd edition, Blackie Academic & Professional, London, UK. 1997.
16. Larone DH. Medically Important Fungi: A Guide to Identification, 5th ed. ASM Press: Washington, DC. 2011.
17. AOAC. Aflatoxins in corn, raw peanuts and peanut butter: immunoaffinity column (aflatest) method. AOAC International; 2005.
18. CE. Commission Européenne : Règlement (CE) No 401/2006 de la commission du 23 février 2006 portant fixation des modes de prélèvement d'échantillons et des méthodes d'analyse pour le contrôle officiel des teneurs en mycotoxines des denrées alimentaires. Journal Officiel de l'Union Eupéenne L70/12; 2006b.
19. N'goran-Aw ZB, Coulibaly KJ, Assidjo NE, N'gatta. C. Qualité microbiologique des farines de maïs commercialisées sur les marchés de la ville d'Abidjan. Rev. Mar. Sci. Agron. Vét. 2018 ; 6 (4) : 476-482.
20. N'guessan YD, Bedikou ME, Zoue LT, Goualie BG, Niamke SL. Physicochemical, nutritive and safety evaluation of local cereal flours sold in areas of the District of Abidjan-Côte d'Ivoire. J. Applied Biosc. 2014 ; 83 : 7579-7594.
21. Ennadir J, Cromwell GL, Calvert CC, Cline TR, Crenshaw JD, Crenshaw TD et al. Qualité microbiologique des farines de blé consommées au Maroc. Rev. Can. Microbiol. 2012 ; 58 : 145-150.

22. Birollo GA, Reinheimer JA, Vinderola CG. Enterococci vs. nonlactic acid microflora as hygiene indicators for sweetened yoghurt. Food Microbiol. 2001; 18: 597-604.
23. Udoh JM, Cardwell KF, Ikotun T. Storage structures and aflatoxin content of maize in five agroecological zones of Nigeria. J. Stored Prod. Res. 2000; 36: 187-201
24. Fofana-Diomandé A, Kouakou JM, Aka-Diéméléou C, Traoré KS, Dembélé A. Exposition alimentaire aux mycotoxines cancérogènes dans le département de Séguéla (Nord-Ouest de la Cote d'Ivoire) : cas de l'aflatoxine B1. Inter.J. Biolog. Chem. Sci. 2019 ; 13 (2) : 937-949. French
25. Tabuc C. Flore fongique de différents substrats et conditions optimales de production des mycotoxines. Institut national polytechnique de Toulouse (France). 2007. French
26. Dubale B, Solomon A, Geremew B, Sethumadhava R, Waktole S. Mycoflora of grain maize (Zea mays L.) stored in traditional storage containers (gombisa and sacks) in selected woredas of Ji mma zone, Ethiopia. Afri. J. Food Agri. Nutri. Development. 2014; 14 (2): 8676-8694.
27. Baoua IB, Amadou L, Bakoye ON, Abdoulaye O, Baributsa D, Murdock LL. Maize quality in markets in four West African countries. J. Stored Prod. Res. 2016; 69: 26-30
28. Birgen JK, Cheruiyot RC, Akwa TE. Mycotoxin Contamination of stored maize in Kenya and the associated fungi. J. Plant. Pathol. Res. 2020; 2(1):7-13
29. Manizan AL, Akaki D, Piro-Metayer I, Montet D, Brabet C, Koffi-Nevry R. Évaluation des pratiques culturales de l'arachide favorisant la contamination par les aflatoxines dans trois régions de Côte d'Ivoire. Inter. J. Biolog. Chem. Sci. 2018; 12 (4): 1590-1600. DOI: https://dx.doi.org/10.4314/ijbcs.v12i4.7.
30. El Sayed TI, El Desouky TA, Abd EAM. Investigation of fungus associated within co-occurrence of aflatoxins and ochratoxin a in cereals from Egypt. MOJ Toxicol. 2019; 5 (3) :92–99. DOI: 10.15406/mojt.2019.05.00161.
31. García-Díaz M, Serna JG, Vázquez C, Botia MN, Patiño B. A Comprehensive Study on the occurrence of mycotoxins and their producing fungi during the maize production cycle in Spain. Microorganisms. 2020; 8 (141) 1-15; doi :10.3390/microorganisms8010141.
32. Wicklow DT, Mcalpin CE, Platis CE. Characterization of the Aspergillus flavus population within an Illinois maize field. Mycological Res. 1998; 102, 263–268.
33. Whitlow W, Hagler WM. Mycotoxin contamination of feedstuffs - An additional stress factor for dairy cattle, in 100^{e} symposium sur les bovins laitiers, October. Quebec; 2001.
34. Klich MA. Aspergillus flavus : le principal producteur d'aflatoxine. Mol. Plant. Pathol. 2007. 8 (6) : 713-722. DOI : 10.1111/j.1364-3703.2007.00436
35. Esteban A, Abarca ML, Bragulat MR, Cabanes FJ. Effect of water activity on ochratoxin A production by Aspergillus niger aggregate species. Int. J. Food Microbiol. 2006; 108 (2): 188-195.
36. CAST (Council for Agricultural Science and Technology). Ames, Iowa, USA Mycotoxins: Risks in plant, animal and human systems, Task force report. N^{o}. 139. 2003.
37. CE (Commission Européenne) : Règlement N° 165/2010, du 26 Février 2010 modifiant le règlement N° 1881/2006 portant fixation de teneurs maximales pour certains contaminants dans les denrées alimentaires en ce qui concerne les aflatoxines. J. officiel UE, L50, 11-12.
38. Sangaré-Tigori B, Moukha S, Kouadio J, Betbeder A-M, Dano S, Creppy E. Co-occurrence of aflatoxin B1, fuminosin B1, ochratoxin A and zearalenone in cereals and peanuts in Côte d'Ivoire. Food Additi. Contam. 2006; 23: 1000-1007.
39. Worku AF, Abera M, Kalsa KK, Subramanyam B, Habtu NG. Occurrence of mycotoxins in stored maize in Ethiopia. Ethiop. J. Agric. Sci. 2019; 29 (2) 31-43

40. Jager AV, Tedesco MP, Souto PC, Oliveira CA. Assessment of aflatoxin intake in São Paulo, Brazil. Food Control. 2013; 33: 87-92.
41. Roscoe V, Lombaert GA, Huzel V, Neumann G, Mmelietio J, Kitchen D, et al. Mycotoxins in breakfast cereals from the Canadian retail market: a 3-year survey, Food Additi. Contam. 2008: 100 (3), 347-355.