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MULTIPLE MYCOTOXINS IN CORN FLOUR SOLD IN BULK AT MARKETS IN CÔTE D'IVOIRE

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ABSTRACT

In Côte d'Ivoire, cereals, mainly maize, play a significant role in diets, especially for children. Maize and its products can be contaminated with mold in the field and during post-harvest treatments. This study assessed the sanitary quality of some maize flours used in human consumption, particularly for children in Côte d'Ivoire. Thus, 43 maize flour samples collected in the Abidjan, Bouaké, and Korhogo markets were subjected to multi-mycotoxin analyses. The results showed that more than 97 % of the samples contained mycotoxins such as aflatoxins, fumonisins, ochratoxin A, citrinin, trichothecenes, and beauvericin. Fumonisin B1 (FUMB1) was the mycotoxin with the highest contamination rate (95.3 %). Analysis of the contamination profile of the samples revealed that flour samples containing four mycotoxins simultaneously were the most common. The flours collected in Bouaké were the most contaminated in terms of both quality and quantity, with total aflatoxin and fumonisin levels ranging from 34.3 µg.kg-1 to 81.3 µg.kg-1. All recorded aflatoxin levels exceeded the limits set by the European Commission, that is, 0.1 µg.kg-1 for infants and 4 µg.kg-1 for adults. This location also had the highest levels of ochratoxin A. Positive correlations were observed between the mycotoxins. Consumption of this corn flour could expose young children to toxic infections.

Keywords: Côte d'Ivoire, Maize Flour, Children, Mycotoxins, Toxic Infection.

1 INTRODUCTION

Corn and corn-based products play a crucial role in the human diet, particularly in developing countries. These food products account for 32.6 % of the daily energy intake per capita (Robet et al., 2020). In Côte d'Ivoire, maize is the second most consumed cereal after rice, with an estimated annual production of 1,176,000 tons in 2020 [1]. However, this grain provides an ideal substrate for the growth of various strains of toxin-producing molds belonging to the genera *Aspergillus*, *Penicillium*, and *Fusarium*. These mold species grow on various food products, primarily of plant origin, under warm and humid conditions [2;3]. They are capable of producing several types of mycotoxins, such as ochratoxin A (OTA), aflatoxins (AF), deoxynivalenol (DON), fumonisins (FUM), and zearalenone (ZEA), which are among the thirty regulated mycotoxins [4;5]. These mycotoxins exert various toxic effects on human health. Most of these mycotoxins are known for their carcinogenic, mutagenic, reprotoxic, immunotoxic, and neurotoxic effects [6;7;8;9]. Several recent studies have revealed the presence of these toxins in corn flour, with levels sometimes exceeding current standards [10; 11; 12; 13; 14; 15]. In Côte d'Ivoire, corn flour is often introduced as a complementary food for children to diversify their diet after weaning. Furthermore, any food supplement intended for infants and children under three years of age must be free of contaminants in amounts that could pose a risk to their health [16]. Most of these mycotoxins are hardly degraded by processing methods such as grinding, extrusion, and heating [17; 18]. Thus, the presence of mycotoxins in corn flour constitutes a significant public health concern given its widespread use in staple foods for infants and young children. Mold growth and mycotoxin production in foodstuffs can be influenced by various environmental factors, including temperature, water activity (aw), and humidity [19; 12]. In Côte d'Ivoire, climatic and rainfall conditions vary from south to north [20] and can significantly influence the types of molds and mycotoxins produced in foodstuffs. The objective of this study was to assess the mycotoxin levels and the nature of toxin-producing molds in corn flour sold in the markets of three major cities in Côte d'Ivoire with different climatic and rainfall conditions. This study was conducted during two time periods (June and September) in the cities of Abidjan, Bouaké, and Korhogo, located in the south, center, and north of Côte d'Ivoire, respectively.

2 MATERIAL AND METHODS

2.1 Biological material

The biological material consisted of corn flour used in food products, sold in bulk at markets in three Ivorian cities: Korhogo (north), Bouaké (center), and Abidjan (south).

2.2 Description of the study areas

The three locations (Abidjan, Bouaké, and Korhogo) covered by this study are situated in different climatic zones of Côte d'Ivoire (Figure 1). The city of Abidjan, the economic capital (5° 19' 0.001"N 4° 1' 59.999" W), is located in southern Côte d'Ivoire and has a coastal Attié climate (transitional equatorial climate). This climate is characterized by high humidity, which is 82% in Abidjan, temperatures of approximately 26°C, and abundant rainfall reaching 1.675 mm. This climate consists of two dry seasons (December–April; August–September) and two wet seasons (May–July; October–November). The city of Bouaké (7° 40' 59.999"N, 5° 1' 0.001" W), the capital of the Gbêkê Region, is located in the center of the country and has a Baoulé climate (a mild equatorial transitional climate). Temperatures, with greater daily ranges, fluctuate between 14°C and 33°C, with humidity levels of 60% to 70% and annual precipitation of 1,200 mm in Bouaké. This climate region also has four seasons: two dry seasons (November–March; July–August) and two rainy seasons (June–October; March–May). The city of Korhogo (9.4669° N, 5.6143° W), the capital of the Poro Region, is located in the northern part of the country and has a Sudanese climate (tropical transitional climate). Annual temperatures also hover around 26°C, with daily temperature ranges that are relatively greater than those in the south. The humidity level, lower than that in the southern part of the country, is 63%. There are two seasons: a dry season (November–June) and a rainy season (July–October). The average annual rainfall recorded in Korhogo is 1,151 mm [21; 22].

Figure 1: Locations of the study areas

2.3 Sampling

Corn flour samples were collected in June and September 2023 from markets in Abidjan, Bouaké, and Korhogo. These sampling sites were selected, on the one hand, because of the high density of people in these areas who commonly use these flours for infant feeding. On the other hand, they were chosen based on the relative humidity in these areas. The samples from Abidjan were collected from two markets in the municipalities of Abobo and Adjamé. These include 16 samples collected in June and September at the Abobo Grand Market and the Adjamé Forum Market (Table 1). In Bouaké, 14 samples were collected at the Kôkô neighborhood market and the Grand Market during the same period in June and September. In Korhogo, 13 samples were also collected from sellers at the Grand Market and the Haoussadougou Market during the same periods of June and September. In total, 43 samples of corn flour, each weighing 500 g, were collected under aseptic conditions from all the markets, then packed in food-grade bags and placed in a cooler containing ice packs. These samples were transported to the laboratory, where each sample was divided into two parts. The first part was stored at -20 °C for mycotoxin analysis, while the second was used for phenotypic, microscopic, and molecular identification of the molds. All analyses were conducted at the Laboratory of Chemistry and Food Safety (LCSA) of the Centre for International Cooperation in Agricultural Research for Development (CIRAD) in Montpellier, France.

Table 1 : Sample collection sites

Study areas	Site of collection	Geographic coordinates		Number of samples		Total
		X.UTM	Y.UTM	June	September	
Abidjan	Abobo (main market)	5° 25'58,393	4° 2' 20,011	4	4	8
	Adjamé (market forum)	5° 21'22,036	4° 1' 12,796	4	4	8
Bouake	Kôkô Neighborhood Market	7,6899°	5,0332°	4	4	7
	Grand Market, the Commerce District	7,6899°	5,0242°	4	4	7
Korhogo	Grand Market	9,4597°	5,6311°	4	4	7
	Haoussadougou Market	9,4602°	5,63262°	3	3	6
Total				23	23	46

2.4 Detection and quantification of mycotoxins

Mycotoxins were extracted from the samples using liquid-liquid extraction and then quantified by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) according to methods previously used by [15].

2.4.1 Extraction

For each flour sample, 5 g was placed in a 50 mL Falcon tube. Next, 20 mL of extraction solvent (acetonitrile/water/acetic acid (75/24/1)) was added. The Falcon tube was then shaken using a rotary shaker for 15 minutes. QuEChERS salts were then added. The mixture was shaken again for 15 minutes. The Falcon tube was then centrifuged at 9,000 rpm for 10 minutes at room temperature. After centrifugation, 300 µL of supernatant was collected in another Falcon tube. To this sample, 900 µL of LC1 mobile phase (water/acetic acid (99.5/0.5)) was added. Finally, this mixture was filtered using a syringe fitted with a 0.45 µm PTFE filter.

2.4.2 Quantification of mycotoxins

Mycotoxin quantification was performed using high-performance liquid chromatography (HPLC)/LC-MS/MS. An injection volume of 50 µL was used. A Phenomenex Kinetex 2.6 µm C18 100 Å (50 x 2.1 mm ID) reverse-phase column, with a 2.1 mm ID C18 precolumn, was used at a temperature of 50 °C. The column was then pressurized to between 145 and 165 bar. In addition, the elution gradient had a flow rate of 0.4 mL/min. Mobile phase 1 was a 99.5/0.5 mixture of water and acetic acid, while mobile phase 2 was a mixture of isopropanol and acetic acid (99.5 / 0.5). The detector was a Shimadzu LC-MS 8040, with electrospray ionization (ESI). Finally, the nebulizing gas flow rate was 2.5 µL/min, and the drying gas flow rate was 15 µL/min.

2.5 Statistical analysis

An ANOVA was performed to compare mycotoxin levels using XLSTAT-2023.2.1414.1414 software. The ANOVA is based on the assumption that the total variability observed in the results is due to random fluctuation. Fisher's LSD test was used to analyze the differences between the treatments with a 95% confidence interval. The graphs were created using Excel 2016.

3 RESULTS

3.1 Contamination rate

Of the 43 corn flour samples analyzed, 42 samples, or 97.7 %, tested positive for mycotoxins (Figure 2). A total of ten (10) mycotoxins were identified. These include aflatoxins (AFB1, AFB2, AFG1), fumonisins (FUMB1, FUMB2), ochratoxin A (OTA), citrinin (CIT), trichothecenes [neosolaniol (NEO); 3-acetyl-doxynivalenol (3ADON)], and beauvericin (BEA). Fumonisin B1 (FUMB1) was the mycotoxin with the highest contamination rate (95.3 %) (Figure 4). It was followed by aflatoxin B1 (AFB1) and fumonisin B2 (FUMB2), which had contamination rates of 90.7% and 88.4 %, respectively. Aflatoxins B2 (AFB2) and G1 (AFG1) had contamination rates of 53.5% and 13.9 %, respectively. The contamination rates of ochratoxin A (OTA) and citrinin were 9.3%, while that of neosolaniol (NEO) was 18.6 %. 3-Acetyl-doxynivalenol (3ADON) was the least frequently detected mycotoxin, with a contamination rate of 4.6 % (Figure 3).

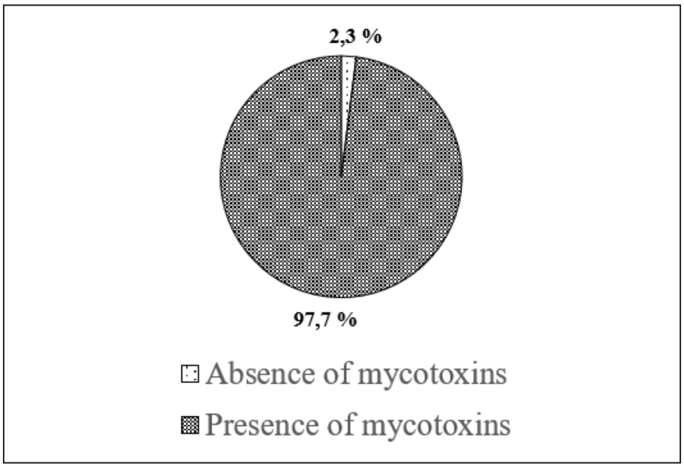
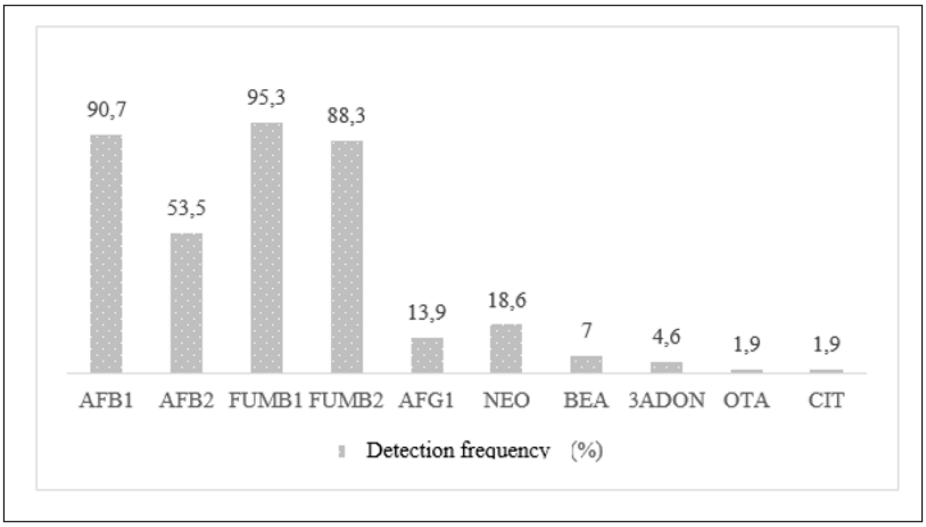


Figure 2: Percentage of samples contaminated with mycotoxins



AFB1: Aflatoxin B1; AFB2: Aflatoxin B2; FUMB1: Fumonisin B1; FUMB2: Fumonisin B2; AFG1: Aflatoxin G1; NEO: Néosolaniol; BEA: Beauvericin; 3ADON: 3-acétyldoxynivalénol; OTA: Ochratoxine A; CIT: Citrinin

Figure 3: Contamination profile and proportions of mycotoxins detected

3.2 Multiple contamination profiles

Figure 4 shows the multiple contamination profiles observed in the contaminated samples. It indicates that the number of mycotoxins detected in each sample ranged from one to six. Samples containing four mycotoxins simultaneously (C4) were the most common, with 39.5%. Next were those containing three mycotoxins (C3) and five mycotoxins, with respective prevalences of 21% and 18.6%. Contamination by six mycotoxins was observed in 9.3 % of cases. Contamination by one and two mycotoxins had the lowest proportions, at 2.3% and 7%, respectively.

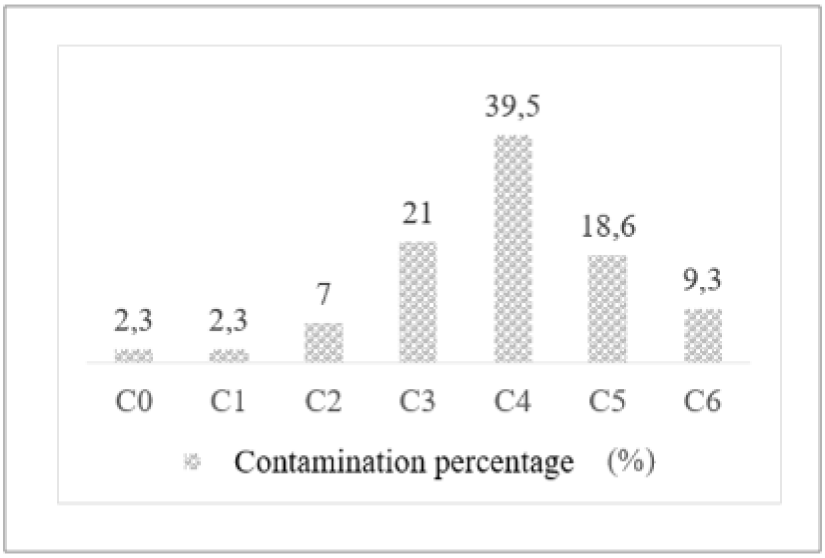


Figure 4: Multiple contamination profile (C1: sample containing one mycotoxin, C2: sample containing mycotoxins, C3: sample containing three mycotoxins, Cn: sample containing n mycotoxins.

3.3 Mycotoxin content

The contamination levels of the corn flour samples collected from the three locations are presented in Table 2. The samples from Bouaké were the most contaminated, both qualitatively and quantitatively. Total aflatoxin levels (AFB1+AFB2+AFG1) in this area were 34.3 $\mu g.kg^{-1}$ in June and 81.3 $\mu g.kg^{-1}$ in September. In Abidjan, total aflatoxin levels were 13.8 and 18.41 $\mu g.kg^{-1}$ in June and September, respectively. The lowest aflatoxin levels were found in the north, in Korhogo, with values of 9.9 $\mu g.kg^{-1}$ and 14.9 $\mu g.kg^{-1}$ in June and September, respectively. All aflatoxin levels detected across the three locations in Côte d'Ivoire exceeded the limits set by the European Commission: 0.1 $\mu g \cdot kg^{-1}$ for infants and 4 $\mu g \cdot kg^{-1}$ for adults. Fumonisin levels were also higher in Bouaké, with an average concentration of 69.34 $\mu g.kg^{-1}$ in June and 58.77 $\mu g.kg^{-1}$ in September. The samples collected in Korhogo had nearly identical fumonisin concentrations in June and September. They were 20.92 $\mu g.kg^{-1}$ and 20.29 $\mu g.kg^{-1}$, respectively. The lowest fumonisin levels were recorded in Abidjan, with concentrations of 17.02 $\mu g.kg^{-1}$ and 15.42 $\mu g.kg^{-1}$ in June and September, respectively. Unlike aflatoxins, total fumonisin contamination levels were below the maximum threshold recommended by the EU for infants and adults. Neosolaniol was detected in the towns of Bouaké and Korhogo. The highest levels were found in September in Korhogo, with concentrations of 568.21 µg.kg^{-1}. In Bouaké, concentrations of this emerging mycotoxin reached 280.16 in June and 110.41 in September. All detected neosolaniol levels exceeded the recommended standards. The mycotoxin 3ADON was found only in samples from Abidjan, while OTA, CIT, and BEA were found only in the Bouaké area. Among these mycotoxins, only the OTA levels found in Bouaké in June exceeded the recommended standards. No statistically significant difference was found in mycotoxin levels between samples collected in June and September from the same location ($p > 0.05$). However, a significant difference was noted between fumonisin levels in June in Bouaké compared to those in Abidjan and Korhogo ($p = 0.0112$).

Table 2: Mycotoxin levels in corn flour samples, expressed in µg.kg^{-1}

Mycotoxins	Abidjan		Bouaké		Korhogo		Maximum level (EU Regulation 2023/915)
	Jun	Sept.	Jun	Sept.	Jun	Sept.	
AFB1	6.7±14.1^{a}	3.9±6.4^{a}	16.7±22.6^{a}	42.1±9^{a}	4.3±6.8^{a}	14.9±13.6^{a}	(AFB1+AFB2+ AFG1+AFG2) 0.10* 4.0**
AFB2	7.1± 19.8^{b}	4.0± 9.8^{b}	17.6±30^{b}	39.2±103^{b}	5.6±9.3^{b}	Nd	
AFG1	Un	0.71± 0,8	Un	Un	Un	Un	
Total aflatoxins	13.8^{c}	18.41^{c}	34.3^{c}	81.3b^{c}	9.9^{c}	14.9^{c}	
FUMB1	11.00±23^{d}	8.63±16^{d}	49.33±40^{t}	33.87±64^{d}	12.63±14^{d}	11.31±10^{d}	(FUMB1+ FUMB2) 200 * 1000**
FUMB2	6.02±13.8^{e}	6.79±13^{e}	20.01±26^{v}	24.90±48^{v}	8.29±8.5^{e}	8.98±8.2^{e}	
Total fumonisins	17.02^{f}	15.42^{f}	69.34^{u}	58.77^{u}	20.92^{f}	20.29^{f}	
3ADON	1.67±4.2	Un	Un	Un	Un	Un	200 * 750**
NEO	Un	Un	280.16±372^{g}	110.41±261^{g}	Un	568.21±518^{g}	100**
OTA	Un	Un	5.5±9	Un	Un	Un	0.50* 3.0**
CIT	Un	Un	57.6±9	Un	Un	Un	Un
BEA	Un	Un	Un	29.6±65	Un	Un	Un

On the same line, mean values marked with the same letter are not statistically different at the $p < 0.05$ level.; *Maximum levels of mycotoxins in corn-based preparations intended for infants and young children; **Maximum mycotoxin levels in corn and corn products intended for human consumption; Un: undetected

3.4 Correlation matrix for mycotoxins

Figure 6 shows the correlation matrices between the various mycotoxins found in the locations of Abidjan, Bouaké, and Korhogo. Strong positive correlations were observed between AFB1 and AFB2 ($r =+ 0.651$), as well as between FUMB1 and FUMB2 ($r = +0.792$). A strong correlation was also noted between OTA and citrinin ($r = +0.757$) and between FUMB1 and OTA ($r = +0.778$). FUMB1 was also strongly positively correlated with citrinin ($r =+0.917$). Furthermore, moderate correlations were observed between aflatoxins (AFB1/AFB2) and fumonisins (FUMB1/FUMB2). Conversely, a significant negative correlation was noted between AFB1 and 3-ADON ($r = -0.733$).

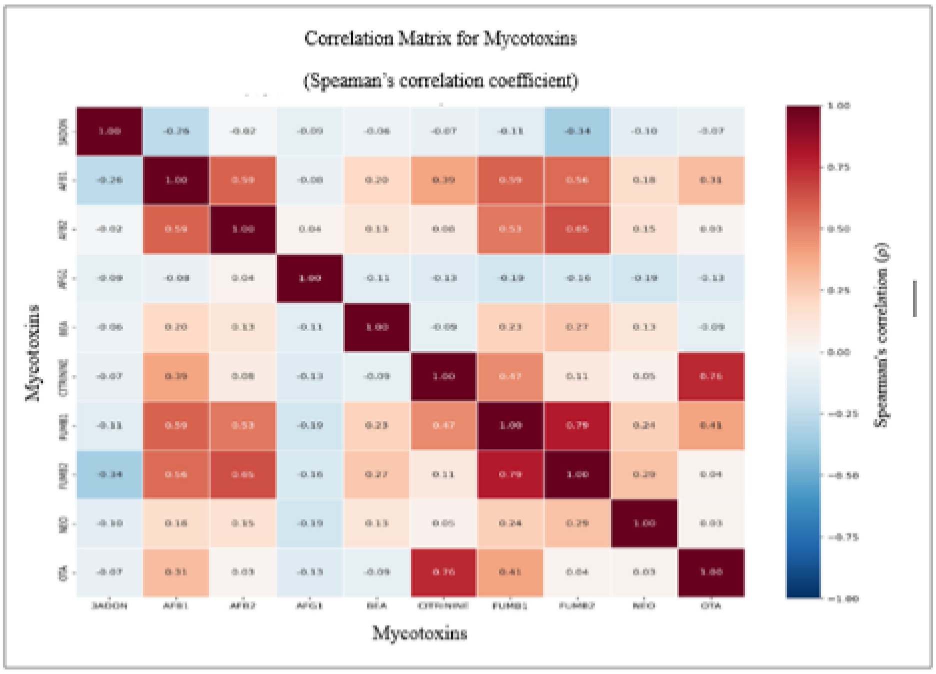


Figure 5: Correlation matrix for mycotoxins

4 DISCUSSION

The results of this study identified 10 different mycotoxins in corn flour samples collected from three locations in Côte d'Ivoire. These include three aflatoxins (AFB1, AFB2, AFG1), two fumonisins (FUMB1, FUMB2), two trichothecenes (NEO; 3ADON), ochratoxin A (OTA), citrinin (CIT), and beauvericin (BEA). The mycotoxins consistently found in the three locations—which have different climatic, rainfall, and temperature conditions-were aflatoxins (AFB1; AFB2) and fumonisins (FUMB1; FUMB2). The levels of fumonisins and 3-acetyl-deoxynivalenol were within the recommended threshold values. Furthermore, the concentrations of aflatoxins and ochratoxin A found in corn flour exceeded the EU recommendation [23]. This study also revealed the presence of emerging mycotoxins, not yet regulated, in the corn samples. These are beauvericin (BEA) and neosolaniol (NEO). These findings are corroborated by other studies conducted on corn flour in various locations across Côte d'Ivoire, which revealed contamination by aflatoxins, fumonisins, and ochratoxin A at levels exceeding the limits set by the EU. The average concentrations of mycotoxins in these samples ranged from 0.92 to 44.2 $µg.kg^{-1}$ for aflatoxin B1 and 297.2 $µg.kg^{-1}$ for fumonisin B1. Corn is the second most consumed cereal in Côte d'Ivoire after rice [24]. This cereal, in the form of flour, is used for human consumption, particularly among infants and young children. Consumption of this flour may pose a significant health risk to the population, particularly among children. Indeed, aflatoxins and ochratoxin A are classified as Group 1 and Group 2A carcinogens, respectively, by the International Agency for Research on Cancer (IARC) [25]. These mycotoxins are also known for their nephrotoxic, hepatotoxic, teratogenic, neurotoxic, genotoxic, immunotoxic, and reproductive toxic effects [26; 27]. Exposure to aflatoxins has also been linked to kwashiorkor [28; 29]. Cornmeal samples were collected during two different seasons: the dry season and the rainy season. The seasonal effect on mycotoxin levels varies considerably by location. In Abidjan and Korhogo, aflatoxin production was higher in June, whereas in Bouaké, levels of these mycotoxins were highest in September. Furthermore, the flour samples collected in Bouaké were the most contaminated in terms of both quality and quantity. This high production of mycotoxins in this location is linked to more favorable climatic conditions. Indeed, mycotoxins are produced by molds when the temperature, precipitation, and water activity are average [30; 31]. This region, characterized by a mild transitional equatorial climate with temperatures ranging from 14 °C to 33 °C and annual precipitation of 1,200 mm^3 [32], was more conducive to mycotoxin production.

5 CONCLUSION

This study, which involved multi-mycotoxin, microbiological, and molecular analyses of corn flour samples collected from market stalls in Korhogo, Bouaké, and Abidjan, showed that these flours could be contaminated with mycotoxins. Ten different mycotoxins were detected, with a contamination rate of 98 % across all analyzed samples. The most commonly found mycotoxins in the samples were AFB1, AFB2, DON, FUMB1, and FUMB2. In addition to these regulated mycotoxins, very high levels of emerging mycotoxins such as NEO, BEA, and 3-acetyl-deoxynivalenol (3ADON) were also detected. The levels of aflatoxins and ochratoxin A found exceeded regulatory thresholds. The samples collected in Bouaké were the most contaminated. The rainy and dry seasons had no impact on the mycotoxin content of the flour.

Consumption of these flours could pose a significant health risk to consumers given the highly toxic effects of the mycotoxins detected. Urgent food safety monitoring plans are essential to ensure a safe food supply for consumers.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

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