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A Comprehensive Study To Explore Differences in Mycotoxin Patterns from Agro-ecological Regions through Maize, Peanut, and Cassava Products: A Case Study, Cameroon

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ABSTRACT: A total of 420 samples were collected from agrarian households. Whereas 51% (215/420) of the samples were contaminated with one or more toxins, the contamination rates for maize, peanut, and cassava products were 74, 62, and 24%, respectively. The fumonisins (20−5412 μg/kg), aflatoxin B_1 (6−645 μg/kg), roquefortine C (1−181 μg/kg), and deoxynivalenol (27−3842 μg/kg) were the most prevalent contaminants in maize. For peanut samples, aflatoxin B_1 (6−125 μg/kg) and ochratoxin A (0.3−12 μg/kg) were the main contaminants, whereas aflatoxin B_1 (6−194 μg/kg) and penicillic acid (25−184 μg/kg) were detected in the cassava products. Exposures calculated through maize intake for fumonisin B_1 and aflatoxin B_1 were several-fold higher (2−5 for fumonisin B_1 and 10^4−10^5 for aflatoxin B_1) than the health-based guidance values of 2 μg/kg bw/day and 0.15 ng/kg bw/day, respectively. The study design constitutes a good model that can be implemented in other sub-Saharan African countries.

KEYWORDS: *aflatoxin, fumonisin, ochratoxin A, mass spectrometry, feremented cassava, Africa*

INTRODUCTION

Mycotoxins are a heterogeneous group of toxic secondary metabolites produced by toxigenic fungi that contaminate a wide range of cereals, nuts, and their derived processed products with over 400 having been characterized. The aflatoxins (for example, B_1, B_2, G_1, and G_2), ochratoxin A (OTA), fumonisins (for example, B_1, B_2, and B_3), zearalenone (ZEA), and trichothecenes (deoxynivalenol, T2 toxin, HT2 toxin, and nivalenol) are considered to be significant.[1] This is due to their relatively acute and chronic effects observed in experimental animal studies and their frequent occurrence in food and feed worldwide.[2]

Formation of mycotoxins begins at preharvest plants and continues through postharvest in stored and processed products. Their occurrence in agricultural products and also in processed products significantly affects economies of developing countries and poses a serious risk for animal and human health. Preharvest and postharvest management strategies have therefore been recommended not only to reduce mycotoxin levels but also to control mycotoxin-related risks. Monitoring or surveillance of mycotoxin levels in crops and derived products is an important management tactic that is implemented along the food chain from farm to consumer.

Since the year 2000, mycotoxin monitoring programs have been carried out in several West African countries such as Benin Republic and Togo.[3,4] No surveys were done in Cameroon until 1994, when the fungal diversity in different maize species was characterized for the first time.[5−7] The presence of leaf, stem, and ear diseases in maize production areas in Cameroon was also reported.[8,6] The first data on multimycotoxin occurrence in dietary staples was reported in 2001.[6] Trace levels of aflatoxin B_1 (AFB_1) were detected in a few samples, whereas quantifiable levels of deoxynivalenol (DON; <100−1300 μg/kg), fumonisin B_1 (FB_1; 300−26000 μg/kg), and ZEA (50−110 μg/kg) were reported in maize.[6] Since 2007 there have been increasing numbers of reports on the occurrence of mycotoxins in dietary staples and their derived products from Cameroon.[9−12] Despite this upsurge in contamination data, the principal agricultural products (other than maize) such as cassava and peanuts have not yet been comprehensively investigated.

Cassava (*Manihot esculenta*) and cassava products constitute one of the main dietary staples consumed throughout West Africa, especially in Cameroon.[13] It accounts for 13% of the total energy intake of the Cameroonian population.[14] Despite its high consumption, very little is known about its mycotoxin profile. Recently, a large number of fungi (*Aspergillus* spp.) were isolated from stored cassava products, which raised serious concerns on the potential of this commodity as a natural substrate liable to mycotoxin contamination.[9] With respect to agricultural practices, cassava is often cultivated in a mixed-cropping system, which is a common practice among subsistence farmers in Cameroon. It is well documented that such practices increase the potential of mycological cross contamination.[9,15]

Cameroon is known as the bread basket of West and Central Africa with tens of thousands of tons of unprocessed agricultural food crops being exported daily to adjoining African and European countries. Traders often infiltrate the villages to purchase their desired products at low bargained prices. During trading, good-quality products are purchased for

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export while the poor-quality products are rejected and left behind for household consumption by the local farmers. The poor-quality products could consist of insect-infected and/or fungi-infested grains with possibly very high mycotoxin contamination. For the export products, usually minimal or no quality control is carried out at the points of entry of the importing African countries.

Although the European Union through the European Food Safety Authority (EFSA) and other advisory and legislative bodies have established maximum levels for certain mycotoxins in some foodstuffs,[16,17] these legislations are nonbinding in countries outside the European Union. On the African continent, by the year 2003, only 15 of the 54 African countries were known to have specific mycotoxin regulations,[18] mostly for controlling aflatoxin contamination. Meanwhile, for the majority of African countries, no specific mycotoxin regulations exist, including Cameroon. The absence of elaborate surveys and reports on economic consequences (losses) resulting from rejection of poor-quality products at the international market has hindered the establishment of mycotoxin legislations in most African countries.

All surveys on mycotoxin contamination carried out in Cameroon over the past decade so far have been based on random sampling of food commodities from street vendors.[10−12] Only one study[5] assessed the occurrence of mycotoxins (aflatoxins, DON, and ZEA) in 72 maize samples from farmers in three villages. An additional drawback was that this was not a household survey. Household surveys reflect direct exposure of individuals at the household level, but surveys of street vendors do not. In remote villages, the raw food used to prepare a family meal is usually cultivated at the household level in small plots, and for this, exposure is envisaged to be constant throughout the year with the exception of some perishable seasonal crops. The bottleneck with household surveys in developing countries is the difficulty in gaining access for sampling to these the remote villages, which are often the most affected with the mycotoxin problem, due to very poor nonexisting infrastructure. Studies were therefore limited to urban and/or semiurban areas.

This study was aimed to go beyond the boundaries of previous studies by providing a comprehensive insight into the differences in mycotoxin patterns from three agro-ecological regions in Cameroon using maize, peanut, and cassava products as model food staples. Sampling in very remote areas with a potentially higher risk of the mycotoxin problem tends to give more added value to the proposed study. Furthermore, this is so far the only report on the occurrence of mycotoxins in cassava and its products from Cameroon.

MATERIALS AND METHODS

Sampling. Cameroon has five agro-ecological zones, from which three were selected for this study: the western highland (WH), the humid forest with monomodal rainfall (HFM), and the humid forest with bimodal rainfall (HFB). The climatic conditions of the three regions sampled have been described by IRA-NCRE.[19] Figure 1 shows the map of Cameroon with the five agro-ecological zones.

The WH is characterized by an annual rainfall from 1300 to >3000 mm/year depending on the topography of the land with atmospheric temperature varying from <15 to 27 °C. In the HFM, the annual rainfall is about 10000 mm/year in Debuncha, which is the fourth wettest locality in the world, with an atmospheric temperature varying from 20 to 28 °C. Rainfall in the HFB ranges between 2000 and 4000 mm/year, and mean atmospheric temperature is 23 °C. The climate of this zone comprises four seasons (unlike the two other zones, which have just two seasons), a long dry season from December to May, a short wet season from May to June, a short dry season from July to October, and a long and heavy wet season from October to November.[19]

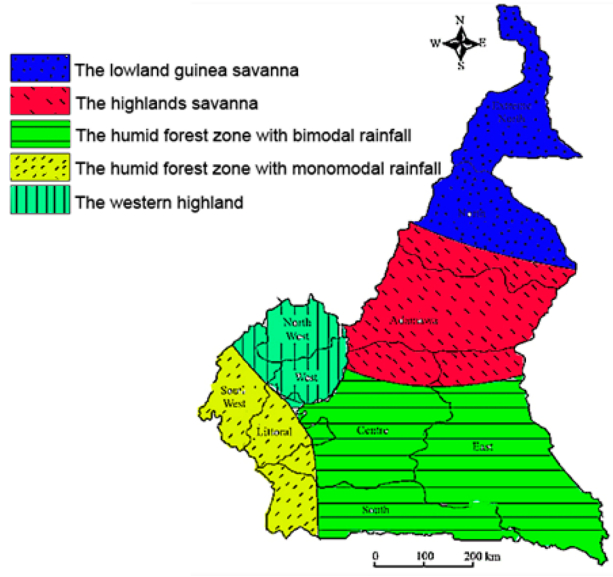

Figure 1. Map of Cameroon, showing the different agro-ecological regions.

The selection of these three zones was based on their agricultural contributions to the national food supply, which have always been greater than those of the two other nonselected regions. From these agro-ecological zones, a total of 10 villages were selected with 3 villages from each of the zones with the exception of the HFB zone, from which four villages were selected. A total of 165 maize grain (*Zea mays*), 90 peanut (*Arachis hypogaea*), and 165 cassava (*Manihot esculenta*; flakes (fermented cassava) and/or chips (nonfermented cassava)) samples intended for human consumption were collected from randomly selected households during two separate sampling sessions; July−August 2009 (rainy season) and December 2010−January 2011 (dry season). Sampling was performed by experienced and well-trained field facilitators. The villages included Malende, Kossala, and Ikiliwindi for the HFM; Bambui, Mbengwi, and Dschang for the WH; and Ndokayoka, Nkolbikong, Ngonai, and Nkolo for the HFB.

Sampling of maize and peanuts was carried out on the basis of the official guidelines described in Commission Regulation EC 401/2006[20] with some modifications. Briefly, the whole content of a traditional bag of stored maize and peanuts was considered as a lot. An aggregate sample size of 0.5 kg was composed of five incremental samples. Each incremental portion was about 100 g, and one incremental portion was taken for every 25 kg of product. In cases when the weight of the traditional bag exceeded 25 kg, the bag was subdivided into portions of 25 kg, and each section was sampled. Subsamples were taken at different depths or positions and then mixed together in a plastic container, and the 0.5 kg final sample was taken. During the first sampling session, peanuts were not collected due to time constraints. Maize and peanut samples were carefully labeled and sealed in paper bags. All samples were stored at −18 °C prior to their transportation to the Laboratory of Food Analysis, Ghent University, Belgium, by air shipment. Table 1 shows the distribution of collected samples across the three agro-ecological regions during the two sampling periods. Prior to analysis, 250 g of maize and peanut samples (unshelled) were milled to a sieve size of 0.5−1 mm using an IKA M20 universal mill (Sigma-Aldrich, Bornem, Belgium), from which a representative portion (250 g) was taken for analysis.

Materials and Reagents. Ammonium acetate was supplied by Grauwmeer (Leuven, Belgium). Acetic acid was purchased from Merck (Darmstadt, Germany). Dichloromethane, *N*,*N*-dimethylforma-

Table 1. Distribution of Samples Collected from Individual Households across the Different Agro-ecological Zones

	no. of samples						
	sampling period 1: July–August 2009			sampling period 2: December 2010–January 2011			
product	HFM[a]	HFB[a]	WH[a]	HFM	HFB	WH	total no. of samples collected
maize	26	23	26	30	30	30	165
peanut	–[a]	–	–	30	30	30	90
cassava flakes	20	15	18	20	22	21	115
cassava chips	6	8	8	10	8	9	50
subtotal	52	46	52	90	90	90	420

[a]HFM, humid forest with monomodal rainfall; HFB, humid forest with bimodal rainfall; WH, western highland; –, no samples were collected.

mide, and ethyl acetate were supplied by Acros Organics (Geel, Belgium). Methanol and *n*-hexane, both of HPLC grade, as well as Whatman glass microfiber filters (GFA, 125 mm) were purchased from VWR International (Zaventem, Belgium). Ultrafree centrifugal filter devices (0.22 μm) from Millipore (Billerica, MA, USA) were used. Bakerbond aminopropyl (NH_2) solid phase extraction (SPE) cartridges were obtained from Grace Discovery Sciences (Lokeren, Belgium). Water was purified on a Milli-Q Plus apparatus (Millipore, Brussels, Belgium).

Analytical standards, namely, 3-acetyldeoxynivalenol (3-AcDON), 15-acetyldeoxynivalenol (15-AcDON), alternariol (AOH), alternariol methyl ether (AME), altenuene (ALT), aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), aflatoxin G_2 (AFG_2), beauvericin (BEA), deoxynivalenol (DON), deepoxy-deoxynivalenol (DOM), fumonisin B_1 (FB_1), fumonisin B_2 (FB_2), fusarenone-X (FUS-X), nivalenol (NIV), HT2 toxin (HT2), neosolaniol (NEO), ochratoxin A (OTA), sterigmatocystin (STE), zearalenone (ZEA), and zearalanone (ZAN) were purchased from Sigma-Aldrich (Bornem, Belgium). Diacetoxyscirpenol (DAS) and T2 toxin (T2) were purchased from Biopure (Tulln, Austria). Fumonisin B_3 (FB_3) was obtained from Promec Unit (Tygerberg, South Africa). Penicillic acid (PA) was purchased from Fermentek (Jerusalem, Israel), whereas roquefortine C (ROQC) was obtained from Enzo Life Science (Lorrach, Germany). FB_2 and FB_3 standards (1 mg) were prepared in 1 mL of acetonitrile/water (50:50, v/v). Stock solutions of 3-AcDON, 15-AcDON, ALT, AFB_1, AFB_2, AFG_1, AFG_2, BEA, DON, DOM, FB_1, FUS-X, HT2, OTA, PA, ROQC, STE, T2, ZAN, and ZEA were prepared in methanol at a concentration of 1 mg/mL. AOH and AME stock solutions (1 mg/mL) were prepared in methanol/dimethylformamide (60:40, v/v). NIV, NEO, DOM, and DAS were obtained as solutions (100 μg/mL) in acetonitrile. All stock solutions were stored at −18 °C except FB_2 and FB_3, which were stored at 4 °C. New stock solutions were prepared every 6 months. From the individual stock standard solutions, a standard mixture was prepared at the following concentrations: AME, 20 ng/μL; AOH, FUS-X, HT2, NIV, and NEO, 10 ng/μL; ALT, 5 ng/μL; 15-AcDON, 2.5 ng/μL; AFB_2, 3-AcDON, DON, and ZEA, 2 ng/μL; T2, 1 ng/μL; DAS and STE, 0.5 ng/μL; FB_1, FB_2, and FB_3, 0.5 ng/μL; ROQC, AFB_1, AFB_2, AFG_1, and AFG_2, 0.2 ng/μL; BEA, 0.1 ng/μL; PA and OTA, 0.05 ng/μL. The standard mixtures were stored at −18 °C and renewed every 3 months.

Sample Preparation. The protocol used for sample preparation has been described.[21] One gram of homogenized sample was spiked with internal standards ZAN and DOM at 20 and 300 μg/kg, respectively. The fortified sample was kept in the dark for 15 min. DOM was used as internal standard (IS) for DON, 3-AcDON, and 15-AcDON, whereas ZAN was used for the other toxins. Both internal standards were used to correct for small volume changes during sample preparation.

All samples were extracted with 15 mL of extraction solvent methanol/ethyl acetate/water (70:20:10, v/v/v) for 30 min using an end-over-end shaker and centrifuged for 15 min at 3170*g*. The supernatant was transferred into a new extraction tube and evaporated at 40 °C. The residue was reconstituted in 5 mL of methanol/water (85:15, v/v) to which 10 mL of dichloromethane/hexane (30:70, v/v) solution was added. The mixture was shaken for 10 min and centrifuged at 3200*g* for 10 min. The dichloromethane/hexane phase was discarded, whereas the methanol/water phase was kept for further cleanup. The defatted extract (5 mL) was split into two parts of 2.5 mL each, for sample enrichment (cleanup). One part (of the split extract) was cleaned by passing it through a glass fiber filter, whereas the second part was cleaned up using amino propyl (NH_2) cartridges. After the equilibration step with 6 mL of methanol/water (85:15, v/v), the sample extract was loaded onto the SPE cartridge and the eluate collected in a test tube. Both parts (the NH_2 SPE and the glass fiber) of the cleaned extracts were recombined and evaporated at 40 °C. The residue was redissolved in 300 μL of mobile phase consisting of methanol/water/acetic acid (57.2:41.8:1, v/v/v) and 5 mM ammonium acetate. Ultrafree MC centrifugal devices were used to further filter the resulting solution prior to injection into the LC-MS/MS system; this was performed for 15 min at 10000*g*.

LC-MS/MS Method. Both the chromatographic and mass spectrometric conditions and settings were described previously.[21] Briefly, a Symmetry RP-C18 column of 150 mm × 2.1 mm, 5 μm, and a 10 mm × 2.1 mm Sentry guard column of the same material (Waters, Zellik, Belgium) were used for the chromatographic separation. The mobile phase constituted two solvent mixtures both containing 5 mM ammonium acetate. Solvent A was composed of water/methanol/acetic acid (94:5:1, v/v/v), whereas methanol/water/acetic acid (97:2:1, v/v/v) was used as solvent B. The solvent flow rate of 0.3 mL/min was adopted for a total gradient run time of 28 min. A 20 μL injection volume was applied.

Detection and quantitation were performed with a Waters Acquity UPLC apparatus coupled to a Micromass Quattro Micro triple-quadrupole mass spectrometer (Waters, Milford, MA, USA). The mass spectrometry analyses were carried out using selected reaction monitoring (SRM) channels in positive electrospray ionization (ESI +) mode. The following were the instrumental settings: source and desolvation temperatures, 130 and 350 °C, respectively; capillary voltage, 3.2 kV; cone nitrogen and desolvation gas flows, 200 and 800 L/h, respectively.

Commission Decision 2002/657/EC[22] and Commission Regulation 401/2006/EC[20] were used as guidelines for the validation of the analytical method. The performance characteristics of the method were in good agreement with both guidelines as earlier described.[21] Table 2 shows the limit of detection (LOD) and limit of quantitation (LOQ) of the target analytes.

Food Frequency Questionnaire (FFQ). A FFQ was administered by trained interviewers. The FFQ was developed on the basis of the consumption habits regarding the target food matrices. In addition to the target food matrices, other food items such as rice, yams, and plantain were also included to cover as many traditional foods as possible. Consumption frequency was measured by a range starting from less than once a month to three times a day. Portion sizes were chosen for each food item according to usual measures: cups for rice and peanuts, slices for yams, and weights for cereal grains and cassava products (pictures depicting the different portion sizes were presented during the interview). The FFQ was applied to 30 randomly selected households living in each village. A total of 300 households (520 individuals) from the 10 villages investigated were collected. The 300 households were evenly distributed throughout the two sampling seasons. Each participating household was expected to provide at least one of the three dietary staples. The body weight of a child and that of an adult (from the same household) were recorded together with the food intake and were used for risk assessment using the deterministic approach.

Table 2. Limit of Detection (LOD) and Limit of Quantitation (LOQ) of the Target Analytes

	LOD (μg/kg)			LOQ (μg/kg)		
analyte	maize	cassava	peanut	maize	cassava	peanut
NIV	35	31	25	106	93	76
DON	9	15	12	27	46	37
PA	8	8	8	25	25	24
NEO	5	8	21	16	15	64
FUS-X	14	5	23	43	15	70
ΣAcDON	10	10	9	30	30	27
AFG_2	1	2	0.3	4	6	1
AFG_1	2	1	1	5	2	3
AFB_2	0.5	1	1	2	3	4
ALT	1	1	1	3	2	2
AFB_1	2	0.3	2	6	0.8	6
DAS	1	0.4	0.1	2	1	0.4
ALT	3	3	2	10	8	5
HT-2	13	9	11	39	28	34
FB_1	6	6	5	20	20	15
T2	0.4	0.1	0.3	1	0.4	1
FB_3	15	15	15	45	45	45
OTA	0.1	0.3	0.1	0.3	0.8	0.3
FB_2	15	15	15	45	45	45
AME	2	1	1	6	4	4
BEA	5	3	0.2	15	10	1
STE	2	3	1	5	9	2
ROQC	0.3	0.1	0.2	1	0.3	1
ZEA	9	6	10	27	20	30

Statistical Analysis. The SPSS software version 20.0 (SPSS Inc., Chicago, IL, USA) program was used for statistical analysis. For exposure assessment, the mean of the entire sample population was used for the calculations with samples <LOD given a value of LOD/2. A nonparametric Mann–Whitney test for two independent samples and a Kruskal–Wallis test for k independent samples were used to evaluate possible differences in the mean (mean rank) mycotoxin levels across the different agro-ecological zones and sampling sessions, after evaluation of the homogeneity of variances with Levene's test. In addition, a contingency test was used to evaluate the possible differences between the incidences of each mycotoxin within the different sample categories. The mean values were calculated for the different sample categories, taking into consideration the negative samples. A p value of 0.05 was used to determine the statistical significance.

RESULTS AND DISCUSSION

General Mycotoxin Contamination Pattern. Of all food samples, 51% (215/420) were found to be contaminated with at least one of the 25 mycotoxins. The contamination rates for each sample matrix were as follows: 74, 62, and 24% for maize, peanut, and cassava products, respectively (Table 3).

Occurrence of Mycotoxins in Maize Samples. DON and its acetylated derivatives (sum of 3-AcDON and 15-AcDON (∑AcDON)), ZEA, ROQC, BEA, aflatoxins (AFB_1 and AFB_2), and fumonisins (FB_1, FB_2, and FB_3)) were detected in the maize samples. The fumonisins (sum of FB_1, FB_2, and FB_3) had the highest occurrence rate (74%, 122/165) in maize samples (Table 3) with the concentration FB_1 in all positive samples being greater than those of FB_2 and FB_3. The highest concentrations of FB_1, FB_2, and FB_3 detected were 5412, 2890, and 2180 μg/kg, respectively (Table 4), which were higher than the levels recently reported (2313 μg/kg) from the same country.[12]

Table 3. Occurrence of Mycotoxins in Different Sample Matrices[a]

sample matrix/mycotoxin[b]	no. of positive samples[c]	% positive samples
maize		
$FB_1 + FB_2 + FB_3$	122/165	74
AFB_1	37/165	22
ROQC	32/165	19
AFB_2	30/165	18
ZEA	23/165	14
DON	20/165	12
∑AcDON	17/165	10
BEA	16/165	10
peanut		
AFB_1	26/90	29
OTA	12/90	13
cassava		
AFB_1	42/165	25
PA	10/165	6

[a]Data summarized from the two sampling periods. [b]DON, deoxynivalenol; PA, penicillic acid; 3-AcDON, 3-acetyldeoxynivalenol; 15-AcDON acetyldeoxynivalenol; AFB_2, aflatoxin B_2; AFB_1, aflatoxin B_1; FB_1, fumonisin B_1; FB_3, fumonisin B_3; OTA, ochratoxin A; FB_2, fumonisin B_2; BEA, beauvericin; ROQC, roquefortine C; ZEA, zearalenone; ΣAcDON, sum of 3-AcDON and 15-AcDON. [c]Ratio of positive samples to the total number of samples analyzed for that particular matrix.

For every DON positive sample, co-occurrence of 3-AcDON and/or 15-AcDON was also detected except in five samples for which the levels of ∑AcDON were below the method LOQ (16 μg/kg). The presence of DON (20/165; 27–3842 μg/kg) and its metabolites (∑AcDON (17/165)) in maize has often been associated with samples originating from temperate regions such as northern Europe and North America.[23] However, reports emerging from tropical countries, especially from sub-Saharan Africa, continue to reveal the occurrence of DON in maize and maize products.[24] Furthermore, the detection of DON in the urine of toddlers (1.5–5 years) from Cameroon[13] also affirms its presence in food commodities originating from sub-Saharan Africa. The maximum level for DON in unprocessed maize was set by Commission Regulation 1881/2006[16] at 1750 μg/kg. Despite its relatively low occurrence rate (12%), eight samples were contaminated above the maximum level set by the European Commission.[20]

AFB_1 and AFB_2 were detected in maize samples at rates of 22 and 18%, respectively, and mean levels and the range detected for AFB_1 and AFB_2 are presented in Table 4. Between the different years of sampling, there were no statistically significant differences in the mean concentrations; 111 and 127 μg/kg for AFB_1 and 23 and 18 μg/kg for AFB_2 in sampling periods 1 and 2, respectively. A total of 35% (13/37) and 24% (9/37) of the aflatoxin positive samples obtained in sampling periods 1 and 2, respectively, exceeded the maximum level of 10 μg/kg of total aflatoxins in maize as specified in Commission Regulation 1881/2006.[16]

Members of the *Penicillium* spp. are well-known colonizers of maize silages, leading to the production of ROQC. Recently, ROQC production in maize fields prior to harvesting was demonstrated, contradicting the belief that *Penicillium* toxin formation occurs exclusively during storage.[25] This might possibly explain the occurrence of ROQC in maize samples originating from Cameroon. Its presence in 19% (32/165; 1–

Table 4. Mycotoxin Pattern in Cameroon from Three Agro-ecological Regions through Maize, Peanut, and Cassava Products

	mean concentration (CI, 95%) range (μg/kg) of mycotoxins detected in the different staples[a]							
	sampling period 1: 2009				sampling period 2: 2010–2011			
sample matrix/ mycotoxin[b]	HFM	HFB	WH	mean	HFM	HFB	WH	mean
maize								
DON	207 (27–2141)	60 (212–918)	275(27–2741)	181	612(27–2411)	161(27–1084)	452 (218–3842)	408
FB_1	1418 (75–3716)	468 (20–1418)	2102 (112–5412)	1329	2601 (20–4030)	665 (314–2841)	2949 (20–3212)	2072
FB_2	1157 (112–2268)	370 (50–843)	965 (75–2882)	831	2555 (10–2890)	663 (50–915)	1742 (112–1846)	1653
FB_3	398 (50–1442)	156 (75–482)	357 (65–412)	303	993 (112–2180)	604 (132–864)	376 (50–698)	657
AFB_1	22 (6–184)	59 (6–345)	26 (6–195)	35	100 (6–645)	96 (6–216)	47 (6–210)	81
AFB_2	7 (2–108)	8 (2–215)	6 (2–85)	7	23 (2–225)	14 (2–120)	29 (2–75)	22
ZEA	61 (75–279)	58 (27–228)	65 (85–262)	61	113 (35–334)	111 (27–242)	86 (55–286)	103
∑AcDON	44 (65–231)	36 (30–187)	47 (54–170)	42	71 (30–115)	62 (30–176)	46 (30–186)	60
ROQC	20 (1–94)	15 (1–137)	25 (1–145)	20	48 (1–84)	44 (1–118)	55 (1–181)	49
BEA	39 (15–412)	33 (15–384)	35 (15–264)	35	69 (15–312)	66 (15–284)	51 (15–385)	62
peanut								
OTA	ns	ns	ns	ns	5 (0.3–12)	3 (0.3–10)	4 (0.3–4)	4
AFB_1	ns	ns	ns	ns	26 (6–125)	22 (6–77)	22 (6–110)	23
cassava								
AFB_1	12 (6–194)	2 (6–95)	5 (6–193)	6	10 (6–125)	1 (6–32)	10 (6–141)	7
PA	7 (25–184)	3 (25–96)	3 (25–72)	4	4 (25–76)	2 (25–46)	4 (25–44)	4

[a]Samples < LOD were given the LOD/2 value. CI, confidence interval; HFM, humid forest region with monomodal rainfall; HFB, humid forest region with bimodal rainfall; WH, western highland; ns, no samples. [b]DON, deoxynivalenol; PA, penicillic acid; 3-AcDON, 3-acetyldeoxynivalenol; 15-AcDON, 15-acetyldeoxynivalenol; AFB_2, aflatoxin B_2; AFB_1, aflatoxin B_1; FB_1, fumonisin B_1.

181 μg/kg) (Tables 3 and 4) of the maize samples should not be underestimated.

BEA was detected in 16/165 (10%) of the maize samples at levels of 15–412 μg/kg. BEA, a cyclic hexadepsipeptide, is produced by various *Fusarium* spp. such as *Fusarium avenaceum*, *Fusarium lateritium*, *Fusarium scirpi*, and *Fusarium oxysporum* and structurally relates to the enniatins.[26−28] Three percent of maize samples originating from southern Nigeria were found to be contaminated with the enniatins.[29] In this study, the majority of the BEA positive samples were detected in the HFM. Considering the close geographical location of both southern Nigeria and the HFM of Cameroon (latitude 0–10° N) the presence of BEA in maize samples from Cameroon further strengthens earlier reports that BEA and enniatins are natural contaminants in maize samples originating from sub-Saharan Africa. Recently, a 93% occurrence rate of these mycotoxins was found in maize beer from Cameroon.[12] With very little or no information on its potential toxic effects in humans and no legislation with regard to its maximum level, the potential health risk associated with its exposure cannot be estimated.

The occurrence of ZEA in agricultural commodities has not been rigorously investigated in sub-Saharan Africa. It was first reported in South African maize and subsequently recovered in maize and other commodities elsewhere on the continent.[30] The maize samples analyzed in this study showed a 14% (23/165) occurrence rate for ZEA, with none of the samples exceeding the maximum level of 350 μg/kg for unprocessed maize products.[20] The detected concentration range was 27–334 μg/kg (Table 4). Compared to other reports on the occurrence of ZEA in maize samples from sub-Saharan Africa, the levels detected in this study were lower than levels previously reported[29] in maize samples from Nigeria (up to 779 μg/kg). ZEA was also reported in maize samples from Cameroon with a mean of 68 μg/kg and a maximum concentration of 309 μg/kg.[12]

Occurrence of Mycotoxins in Peanut Samples. AFB_1 and OTA were the main contaminants in peanut samples. The occurrence rate and levels of AFB_1 (26/90, 6–125 μg/kg) were considerably higher than those of OTA (12/90, 0.3–12 μg/kg) (Tables 3 and 4). From this study, the concentrations of aflatoxins detected in peanut samples can be said to be comparatively low when compared with data from other sub-Saharan African countries. Similar results have been reported in Kenya, where 96% of raw podded peanuts had levels <4 μg/kg and only 4% had levels >10 μg/kg.[31] Meanwhile, in Botswana, concentrations of 12–329 mg/kg in raw peanuts were reported.[32]

The European Commission has fixed stringent maximum levels for total aflatoxins and AFB_1 in peanuts intended for direct human consumption at 2 and 4 μg/kg, respectively, which has led to a serious impact (export products have plummeted) on the economy of several African countries.[33] The Codex Alimentarius Commission also adopted the limit for total aflatoxins at 15 μg/kg in peanuts;[34] nevertheless, the WHO prescribed the maximum level of 5 μg/kg for AFB_1 in various foodstuffs.[35] The levels of AFB_1 in 13 of the 26 AFB_1-positive samples exceeded the maximum level as specified in Commission Regulation No. 1881/2006[16] with 8 samples exceeding 100 μg/kg.

Occurrence of Mycotoxins in Cassava Products. Fermented cassava (flakes) had a higher contamination rate (32%) than cassava chips (nonfermented products) (15%). For cassava flakes, AFB_1 and PA were detected at concentrations that ranged between 6 and 194 μg/kg and between 25 and 184 μg/kg, respectively (Table 4), whereas for the nonfermented cassava products, AFB_1 was the sole contaminant with levels of 6–95 μg/kg. PA was not detected in any of the nonfermented products, suggesting that cassava flakes constitute a suitable substrate for growth of *Penicillium* spp. and hence mycotoxin production. Significant levels of other acidic mycotoxins such as tenuazonic acid, cyclopiazonic acid, and patulin have also been

reported[36] in dried cassava products originating from Ghana. The levels of PA reported herein (25−184 μg/kg) were lower than levels (60−230 μg/kg) previously reported[36] from Ghana.

The occurrence of aflatoxins in cassava products is still a topic of debate. Studies conducted in Tanzania,[37] Nigeria,[38] and Benin[39,40] reported no aflatoxins in market cassava products. In contrast, a recent survey conducted in Tanzania and the Republic of Congo showed the occurrence of AFB_1 in market cassava samples.[41] Furthermore, studies with dried cassava products (Kokonte) from markets in Ghana[36] showed the presence of STE, a precursor of AFB_1. This necessitates further research be carried out in other African countries on cassava products to fully comprehend the aflatoxin problem in cassava products. Cassava fermentation is thought to lower the microbial load and hence mycotoxin contamination. However, if appropriate storage systems are not implemented, this might lead to fungal growth and possibly mycotoxin contamination.[42]

Given that only 3/65 nonfermented cassava samples had AFB_1 above 10 μg/kg, it can be said that consumption of nonfermented cassava products might not pose a serious risk to the populations concerned, compared with maize and peanut. However, continuous monitoring and surveys are needed to mitigate any future disease outbreaks and adverse health effects. At this moment, there are no existing regulations on mycotoxins in cassava products.

Differences in the Mycotoxin Pattern across the Different Agro-ecological Zones and Sampling Years. The mean (geometric mean) and concentration range of each of the detected toxins for a given sample matrix and agro-ecological zone are given in Table 4. To understand the distribution of mycotoxins across the different agro-ecological zones, a pairwise comparison of the mean mycotoxin concentration from a given agro-ecological zone and sample matrix was compared with the levels detected across the other two agro-ecological zones. Differences between the two different sampling years were also evaluated.

Maize Samples. Irrespective of the year of sampling, the mean FB_1 levels in samples from the HFM and WH regions were significantly higher (p = 0.01 for HFM and 0.032 for WH) than the mean levels detected in samples from the HFB (Table 4). The same trend was also observed when the sum of the fumonisins was considered. Although differences in climatic conditions across the different agro-ecological regions can explain the observed distribution of fumonisins, other determinants such as differences in preferred storage conditions across the different regions cannot be ruled out. The HFB is a transition zone from rain forest to savanna, which is characterized by four seasons, unlike the two other agro-ecological regions. Taking into consideration the prevailing weather conditions and agricultural practices in the HFB region, one would anticipate a high rate of mycotoxin occurrence as this region is characterized by two periods of seasonal rainfall during which harvesting is carried out under intense rainy conditions. Furthermore, data extracted from prevalidated questionnaires revealed that 84% of the households harvested the matured cobs while still fresh, with the remaining 16% of the cobs left in the farms to dry before being harvested. A combination of the prevailing weather conditions (four seasons), agricultural practices such as harvesting under intense rainy conditions, and allowing the cobs to dry in the field could have favored potential mold growth, but it was not the case in this study. Samples from this region showed lower levels of FB_1 contamination than samples from the two other regions.

With regard to the different years of sampling, significant differences were observed in the mean fumonisin levels (sum of FB_1 + FB_2 + FB_3) between the first and second years of sampling (p = 0.02), with significantly higher levels in the second year than in the first year of sampling. This can be explained by the long and heavy seasonal rains, which occurred from February to September 2010, whereas in 2009 the duration of seasonal rains was considered to be moderate for that time of the year.[43] Similarly, the mean levels of DON and ∑AcDON were significantly higher (p = 0.001 for DON and 0.01 for ∑AcDON) in the second year than in samples obtained during the first year of sampling. Just like the fumonisins, heavy rains, insufficient drying conditions to reach the safe water content levels, and poor storage conditions could be the contributing factors that accounted for these differences in contamination between the two sampling periods. Because *Fusarium* spp. are primarily field mycotoxin producers, mycotoxin production could have occurred in the field.

With regard to aflatoxin contamination in maize samples, the mean concentration of aflatoxins in samples from the HFB was significantly higher (p > 0.05) than those detected in samples collected from the two other regions (Table 4). This trend was not observed for samples obtained during the second sampling year.

No significant differences were observed in the mean levels of ROQC, ZEA, and BEA between the different sampling years (p > 0.05). With respect to the agro-ecological zones, a trend was observed; the mean levels of ROQC in samples from the WH region were significantly higher than those obtained from HFM and HFB. For ZEA and BEA, no significant differences were found across the different agro-ecological zones (Table 4).

Mycotoxins in Peanut and Cassava Products. The mean AFB_1 levels detected in peanut samples from the HFM were significantly higher than those detected in samples from the HFB (p = 0.012), but no significant differences (p > 0.05) were observed when levels of the toxins detected in peanut samples from the WH and the HFM were compared (Table 4). Likewise, no significant differences were observed in the mean OTA levels in peanut (p > 0.05) across the different agro-ecological zones.

Given that cassava is a root crop, the levels of mycotoxins in cassava are less known to be associated with seasonal and climatic conditions. Hence, contamination could occur only during and/or after processing.

Significant differences in the mean AFB_1 levels were observed across the different agro-ecological zones. Fermented cassava products (flakes) are mostly consumed in the WH and HFM regions, whereas cassava chips are predominantly eaten in the HFB. Irrespective of the sampling year, the mean AFB_1 levels in the fermented cassava products from the WH and HFM regions were significantly higher (p = 0.021 and 0.015, respectively) than those detected in nonfermented samples. This observation suggests that fermentation increases the microbial load, which might have led to AFB_1 production. Similar observations highlighted the fact that the distribution of aflatoxin in positive samples depended on parameters such as pH, moisture content, storage duration, type of chips (fermented cassava), size of chips, level of contamination by aflatoxin-producing fungi, processing practices, and storage facilities.[9] No significant differences (p > 0.05) were found in the mean AFB_1 levels in cassava samples (both cassava chips

and flakes) originating from both the WH and tHFM regions. As previously mentioned, PA was not detected in the nonfermented cassava products.

Consumer Risk Assessment. Exposure to mycotoxins of the different subpopulations was estimated by considering the consumption data obtained from the FFQ and the mean concentrations of mycotoxin (including samples with concentrations <LOD) for the different agro-ecological zones (deterministic approach). Data were computed for infants as well as for adults. The calculated exposure was compared with health-based guidance values (tolerable daily intake (TDI))[44−49] and also expressed as percentage of the analyte-specific TDI exceeded (%TDI calculated as ((calculated exposure/TDI) × 100)). %TDI < 100 implies the TDI for the specific mycotoxin was not exceeded.

Because AFB_1 is a genotoxic carcinogen, there is no set TDI. However, a provisional range of 0.11−0.19 ng/kg bw/day was proposed[49] for the African and Asian populations that might also be predisposed to hepatitis B virus (HBV). For the purpose of this study and for comparing the risk of the different populations, a mean value of 0.15 ng/kg bw/day was used.

Portion sizes of 0.03 and 0.2 kg per day were considered average for an infant and adult, respectively (average intake from the FFQ). Median weights of 60 and 15.2 kg were also assumed to be representative for an adult and infant (<2 years), respectively.

The calculated average exposure for all of the mycotoxins for households in the HFM and WH were in general higher than the average exposure for households in the HFB except for the aflatoxins (Tables 5 and 6). For the aflatoxins, exposure was in the order HFB > WH > HFM and HFM > HFB > WH for years 1 and 2, respectively, for both adults and infants. With regard to maize intake, the calculated exposure for FB_1 calculated for both adults and infants in both the HFM and WH regions exceeded the TDI (Tables 5 and 6). In general, exposure was higher in adults than in infants despite the higher body weight of the adults. Likewise, the TDI for AFB_1 was exceeded in the range of 10^4−10^5% TDI (Tables 5 and 6) irrespective of the agro-ecological region and growth category (infant or adult). The TDIs for ZEA, OTA, DON, and ΣAcDON were not exceeded (<100% TDI) for both the infant and adult populations for the different sampling seasons and agro-ecological regions.

With respect to peanut consumption, exposure to AFB_1 calculated for both the infant and adult populations was in the range of 2.8×10^3−5.7×10^4% TDI. On the other hand, exposure calculated for OTA was not exceeded (<100% TDI) for both the infant and adult populations (Tables 5 and 6). With respect to cassava consumption, data from Tables 5 and 6 reveal that the TDI for AFB_1 was exceeded (1.3×10^3−1.3×10^4% TDI) by both the infant and the adult populations in all three agro-ecological regions and during the different sampling seasons. The potential consequences that might arise following chronic exposure to these mycotoxins should not be underestimated.

A putative mycotoxin contamination pattern from three agro-ecological regions in Cameroon using maize, peanut, and cassava products as food models was established. Whereas significant differences in the mean mycotoxin levels were observed across the different agro-ecological zones, exposure was significantly higher in the HFM and WH regions and lowest in the HFB (but not for the aflatoxins), irrespective of the sampling period. Furthermore, the mean concentration of

Table 5. Risk Associated with Adults Following Consumption of Dietary Staples

		calculated exposure[a] (%TDI[b])						
		sampling period 1			sampling period 2			
matrix/mycotoxin		HFM	HFB	WH	HFM	HFB	WH	health-based guidance values; TDI[c]
maize								
	DON + ∑AcDON	0.7 (69)	0.2 (20)	0.9 (92)	2 (204)	1.81 (54)	1.5 (151)	1 μg/kg bw/day[d]
		4.7 (236)	1.6 (78)	7 (350)	9 (434)	1.45 (112)	9.8 (491)	2 μg/kg bw/day[e]
	FB_1	$0.07(5 \times 10^4)$	$0.2\ (1.3 \times 10^3)$	$0.1\ (6 \times 10^4)$	$0.3\ (2.2 \times 10^5)$	$0.34\ (2.1 \times 10^5)$	$0.2\ (1.0 \times 10^5)$	0.15 ng/kg bw/day[f]
	AFB_1	0.2 (81)	0.2 (77)	0.2 (87)	0.4 (151)	0.58 (148)	0.3 (115)	0.25 μg/kg bw/day[g]
	ZEA							
peanut								
	OTA	NA[i]	NA	NA	0.02 (98)	0.01 (59)	0.013 (78)	0.017 μg/kg bw/day[h]
	AFB_1	NA	NA	NA	0.1 (5.7×10^5)	0.19 (4.8×10^5)	0.07 (48×10^5)	0.15 ng/kg bw/day[f]
cassava								
	AFB_1	$0.04\ (2.6 \times 10^4)$	$0.01\ (4 \times 10^3)$	$0.02\ (1.1 \times 10^4)$	$0.03\ (2.2 \times 10^4)$	$0.002\ (2.2 \times 10^3)$	$0.04\ (2.2 \times 10^4)$	0.15 ng/kg bw/day[f]

[a]Calculated exposure expressed as μg/kg bw/day. [b]%TDI calculated as (calculated exposure/TDI) × 100. %TDI < 100 implies the TDI for the specific mycotoxin was not exceeded. HFM, humid forest with monomodal rainfall; HFB, humid forest with bimodal rainfall; WH, western highland. [c]Tolerable daily intake (TDI). [d]Based on scientific opinion released by SCF, 2002. [e]Levels based on scientific opinion released by SCF, 2000. [f]Based on Kuiper Goodman et al.[49] [g]Based on scientific opinion released by EFSA, 2011. [h]Based on toxicity levels based on scientific opinion released by EFSA, 2006. [i]NA, not applicable.

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Table 6. Risk Associated with Infants Following Consumption of Dietary Staples

	calculated exposure[a] (%TDI[b])						
	sampling period 1			sampling period 2			
matrix/mycotoxin	HFM	HFB	WH	HFM	HFB	WH	health-based guidance values; TDI[c]
maize							
DON + ∑AcDON	0.4 (237)	0.1 (46)	0.5 (309)	1.2 (120)	0.3 (32)	4.81 (89)	1 μg/kg bw/day[d]
FB_1	2.8 (145)	0.9 (54)	4 (215)	5.1 (257)	1.3 (66)	5.24 (291)	2 μg/kg bw/day[e]
AFB_1	0.04 (24)	0.1 (71)	0.05 (20)	0.2 (1.3×10^5)	0.20 (1.2×10^5)	0.17 (6.1×10^4)	0.15 ng/kg bw/day[f]
ZEA	0.1 (133)	0.1 (151)	0.13 (149)	0.2 (89)	0.2 (87)	0.53 (67)	0.25 μg/kg bw/day[g]
peanut							
OTA	NA[i]	NA	NA	0.01 (592)	0.006 (35)	0.008 (47)	0.017 μg/kg bw/day[h]
AFB_1	NA	NA	NA	0.05 (3.4×10^4)	0.043 (2.8×10^4)	0.043 (2.8×10^4)	0.15 ng/kg bw/day[f]
cassava							
AFB_1	0.02 (23)	0.002 (10)	0.01 (21)	0.02 (1.3×10^4)	0.002 (1.3×10^3)	0.02 (1.3×10^4)	0.15 ng/kg bw/day[f]

[a]Calculated exposure expressed as μg/kg bw/day. [b]%TDI, calculated as (calculated exposure/TDI) × 100. %TDI < 100 implies the TDI for the specific mycotoxin was not exceeded. HFM, humid forest with monomodal rainfall; HFB, humid forest with bimodal rainfall; WH, western highland. [c]Tolerable daily intake (TDI). [d]Based on scientific opinion released by SCF, 2002. [e]Levels based on scientific opinion released by SCF, 2000. [f]Based on Kuiper Goodman et al.[49] [g]Based on scientific opinion released by EFSA, 2011. [h]Based on toxicity levels based on scientific opinion released by EFSA, 2006. [i]NA, not applicable.

the mycotoxins detected in maize samples obtained from the second year (2010−2011) of sampling was significantly higher than those of the first year (2009) of sampling, which suggests a possible influence of preharvest (e.g., climatic changes) and/or postharvest (e.g., storage practices) practices on mycotoxin contamination from year to year. To summarise, a total of 51% of the samples were contaminated with one or more mycotoxins. The rates of contamination were 74, 62, and 24% for maize, peanut, and cassava products, respectively. Hence, mycotoxin contamination constitutes a major problem in agricultural staples from Cameroon. The relative abundance of the mycotoxins was as follows: FB1 > FB2 > FB3 > AFB1 > AFB2 > ROQC > ZEA > OTA > DON > ∑AcDON > BEA > PA.

Data from the deterministic risk assessment showed that the calculated exposure for infants as well as adults exceeded the TDI through maize, peanut, and cassava consumption. On the basis of the calculated exposure, the TDI was exceeded for FB_1 and AFB_1 following maize consumption. Likewise, exposure to AFB_1 through cassava consumption was also exceeded. Particular attention should be paid to AFB_1, especially in populations with a very high prevalence of HBV (10%). Vulnerable groups and/or individuals (such as elderly or immune-compromised people and pregnant women) living in these study zones should be alerted to the potential danger arising from the consumption of mycotoxin-contaminated foodstuffs. There is a need to carry out periodic surveys and awareness campaigns in the high-risk regions (HFM and WH) to educate subsistence farmers as well as other agricultural stakeholders on the merits of good agricultural practices in relation to reducing mycotoxin exposure.

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Notes

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