Assessment of urinary biomarkers of mycotoxin exposure in adults from Cameroon

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Abstract

Background

In Cameroon dietary staples are contaminated with diverse toxic fungal metabolites, known as mycotoxins. Aflatoxins and fumonisins are of particular public health concern, particularly in relation to cancer and/or early life stunting. Mixtures of these toxins are predicted from food measures, and in this work, the levels and frequencies of urinary mycotoxin biomarkers are reported in Cameroonian adults.

Methods

A single first void urine sample was collected from 89 adults (aged range: 28–85, male 39, female 50) from the city of Yaoundé, Centre Region, Cameroon. Urines were tested for eight distinct mycotoxins using measures of both parent compounds and/or their metabolites by Liquid Chromatography tandem mass spectrometry (LC-MS/MS).

Results

Altogether seven distinct mycotoxins, aflatoxin, fumonisin, deoxynivalenol, zearalenone, nivalenol, ochratoxin A and citrinin, (or their metabolites) were observed in urine samples. At least one mycotoxin was detected in all of the urine samples, 87 (97.8%) of which were above the method's quantification limit. Aflatoxin M_1 was detected in 42% (n.d. – 0.21 μg L^{-1}) of samples of which about a quarter additionally contained fumonisin B_1. Of the remaining toxins deoxynivalenol, zearalenone, ochratoxin A, nivalenol and citrinin were present in 78%, 99% 95%, 53%, and 87% of the samples respectively. Alternariol was not detected in any sample. Mixtures of mycotoxins in the samples were frequently observed with 64 samples (72%) containing more than five mycotoxins. Estimates of intake exceeded the TDIs for fumonisin (n = 4), deoxynivalenol (n = 1) and zearalenone (n = 2), no TDI is set for aflatoxin.

Conclusions

This study reveals frequent co-exposure of Cameroonian individuals to a complex mixture of toxic and carcinogenic mycotoxins, with mixtures of aflatoxin and fumonisin a particular priority from a public health standpoint.

Background

Foodstuffs around the world are frequently contaminated during crop growth and/or storage by several toxigenic fungi that produce poisonous secondary metabolites, known as mycotoxins. Poor agricultural techniques, under favourable conditions including temperature and relative humidity often exacerbate

toxin production in many world regions [1, 2]. In sub-Saharan Africa, poor and prolonged storage additionally contribute to the burden of crop contamination for some mycotoxins, such as aflatoxins. Many mycotoxins are heat resistant, such that traditional cooking practices with grains or nuts have little impact on plate-ready concentrations [3, 4]. Consequently, mycotoxin exposure seems inevitable in many parts of rural Africa.

Acute and chronic ingestion of mycotoxins can be harmful to health, and on occasions be fatal [5]. Mycotoxins such as aflatoxin B_1 (AFB_1) are proven human carcinogens [6]. Additionally, AFB_1 and other mycotoxins are implicated in a range of other conditions including kwashiorkor in children [7], infant stunting (AFB_1 and fumonisin B_1, FB_1) [8, 9, 10], immunosuppression (AFB_1 and deoxynivalenol, DON) [11, 12, 13, 14, 15], and neural tube defects (FB_1) [16, 17]. The diversity of these health effects creates significant concerns regarding the need to monitor and assess the potential risks posed to consumers of mycotoxin-tainted foods in regions where mycotoxin-prone crops are frequently consumed [8]. Maximum tolerable limits (MTLs) in food for some mycotoxins such as aflatoxins (AFB_1, and total AFs), fumonisins (FB_1, and total FBs), ochratoxin A (OTA), deoxynivalenol (DON), and zearalenone (ZEN) have been established [18, 19, 20, 21]. Tolerable daily intake (TDI) levels for some of the frequently occurring toxins including FB_1 has also been defined by the Scientific Committee on Food [22] and DON by the European Food Safety Agency[23], likewise ZEN [24]; but as a proven carcinogen, no recommended TDI is possible for AFB_1.

In Cameroon, reports on mycotoxin contamination of raw and cooked foods are increasing in the past decade [7, 25, 26, 27 28, 29]. AFB_1 and FB_1 have been observed in food, and in some cases the concentrations exceeded MTLs stipulated by the Codex Alimentarius [18] and the European Union [20]. Limited data are available that measure multiple urinary markers of mycotoxin in exposure in Cameroon, including 175 adults [30] and 220 young children [28]. Here we report data from 89 Cameroon adults by a highly sensitive LC-MS/MS method to add to the growing data sets on individual mycotoxin measurements in Sub-Saharan Africa.

Materials And Methods

Study populations, recruitment of participants and sample collection

This study was carried out in 2013 in the city of Yaoundé, Centre Region, Cameroon. Targeted sub-populations were informed about the nature of the study. Signed informed consent forms were obtained from 89 adult male (n = 39) and female (n = 50) volunteers (age range: 28–85 years, body weight (range: 55–129 kg)) recruited in this study. Ethical approval was received from the Cameroon National Ethics Committee of Research for Human Health (Authorisation No. 2013/05/252/CNERSH/SP). First morning urine samples (50 mL each) were collected from each recruited individual in sealed mailing urine bottles. The urine samples were immediately frozen and transported on dry ice to BOKU/IFA-Tulln, Austria, where

they were stored at − 20 °C until analysis. Sixty-six of the participants were part of a hypertension clinic in Yaounde (Les Promoteurs de la Bonne Sante). Additional data on age, weight, height or body mass index (BMI) of the hypertensive versus non hypertensive participants are provided in Supplementary Table S1.

Reagents and Chemicals

Methanol (MeOH; LC gradient grade), acetonitrile (ACN; LC gradient grade), and glacial acetic acid (HAc; MS grade) were purchased from Merck (Darmstadt, Germany). Mycotoxin standards were purchased from Romer Labs Diagnostic GmbH Tulln, Austria, including: nivalenol (NIV), ^{13}C-NIV, DON, ^{13}C-DON, deepoxy-DON (DOM-1), OTA, ^{13}C-OTA, AFM_1,^{13}C- AFM_1, citrinin (CIT), FB_1, ^{13}C-FB_1, ^{13}C-ZEN or Sigma, Vienna, Austria (ZEN, α- and β-zearalenol (ZEL)). The deuterated [$^2H_4$] alternariol (AOH) was synthesized by [31] and kindly provided by Prof. Michael Rychlik, TU Munich. Solid standard substances were dissolved in pure ACN (α- and β-ZEL; [$^2H_4$] AOH). All other standards were delivered in either ACN or ACN/H_2O (FB_1) and stored at -20 °C. A combined multi-standard working solution for preparation of calibrants and spiking experiments was prepared in ACN, and also, fresh mixture of ^{13}C and deuterated [$^2H_4$] stable isotope standards were prepared as described by [32].

Sample Preparation

Urine samples were allowed to reach room temperature, and centrifuged for 3 min at 5600 x *g*. The supernatant (500 µL) was incubated with 500 µL PBS (200 mM, pH = 7.4) containing 3000 U of β-glucuronidase from *E. coli* Type IX-A (Sigma-Aldrich, G7396-2MU) (modified from [33]) for 16 h at 37 °C to allow de-glucuronidation of mycotoxin-glucuronides (e.g. DON-15-glucuronide, ZEN-14-glucuronide [34]). Following hydrolysis, 1 mL was passed through Oasis PRiME HLB® SPE columns (Waters, Milford, MA), pre-equilibrated with 1 mL MeOH, and then 1 mL H_2O. After washing twice with 500 µL H_2O, mycotoxins were eluted with 200 µL ACN, three times. Extracts were evaporated under nitrogen at room temperature, reconstituted with 470 µL dilution solvent (10% ACN, 0.1% HAc) and fortified with 30 µL of the IS mixture as described by [32].

Analysis of urine samples by liquid chromatography-electrospray ionization-tandem mass spectrometry (LC–ESI–MS/MS)

Sample analysis was performed using a Sciex QTrap®6500 + LC-MS/MS system (Foster City, CA) equipped with a Turbo V electro spray ionization (ESI) source interfaced with an Agilent 1290 series Ultra-high performance liquid chromatography (UHPLC) system (Waldbronn, Germany) following the method described by [32] to quantify urinary mycotoxins biomarkers (UMBs) and biomeasures. In brief, analytes of interest were separated on an AtlantisT3 HSS column (2.1 × 100 mm; Waters, Wexford, Ireland) with 1.8 µm particle size. Eluent A (water) and eluent B (ACN) were both acidified with 0.1% HAc. After an initial period of 2 min at 90% A, the percentage of B was linearly raised to 50% until minute 15. Then,

eluent B was raised to 95% until min 18 followed by a hold-time of 4 min and subsequent 3 min column re-equilibration at 90% A. The flow rate was set to 100 µL min^{-1}. After injection of 10 µL the needle was washed for 20 sec to minimize carry-over. The column effluent was transferred either to the mass spectrometer (minutes 5 to 22.5) or to the waste via a six-port valve. The analytes were separated on column at 35 °C.

ESI-MS/MS was performed in scheduled multiple reaction monitoring (sMRM) mode, with a 180 sec detection windows. At least two individual transitions were monitored for each analyte. One chromatographic run consisted of two MS/MS experiments where both ionization modes run simultaneously using fast polarity switching. All measurements were conducted using: source temperature 550 °C, curtain gas 30 psi (69 kPa of max. 99.5% nitrogen), ion source gas 1 (sheath gas) 80 psi (345 kPa of nitrogen), ion source gas 2 (drying gas) 80 psi (345 kPa of nitrogen), collision gas (nitrogen) high. Ion spray voltage was − 4500 V in negative mode while it was set to 4500 V in positive mode.

Analysis of creatinine in urine samples

The concentration of urinary creatinine was determined on the same instrument by the rapid method described in [32]. Urinary mycotoxin concentrations were adjusted for creatinine and expressed as microgram of analyte per gram (µg g^{-1}) creatinine.

Statistical analysis

Statistical analysis was performed using GraphPad In Stat version 3.10 for windows (GraphPad Software Inc, San Diego, California, USA) and Microsoft Office Excel 2010 (Part of Microsoft Professional Edition, Computer Program), Statistica 13.3. (TibcoSoft, Palo Alto, California, USA). For comparisons between (sex) groups, the Mann Whitney U test was used since the data is not normally distributed (tested by Shapiro-Wilk's W test).

Results

There were no differences by hypertensive status in the age, weight, height or BMI of the participants (see supplement Table S1). In Table 1 basic anthropometric parameters are provided for all study participants.

Table 1
Anthropometric measures of the studied population, n = 89

	Participants (N = 89)
Age: mean (range) year	55.4 (28.0–85.0)
Weight: mean (range) kg	84.1 (55.0-129.0)
Height: mean (range) m	1.7 (1.5–1.8)
Body mass index (BMI): mean (range) kg/m^2	30.2 (20.3–54.4)

Mycotoxin concentrations were measured in urine samples from 89 adult Cameroonians (males, 39 and females, 50). The LC-MS/MS method measured seven parent mycotoxins and five mycotoxin metabolites in the samples, limit of quantitation (LOQ) and limit of detection (LOD) values vary for each toxin and metabolite. The parent mycotoxins were FB_1, OTA, DON, ZEN, NIV, CIT, and AOH, whilst AFM_1 (from AFB_1), DOM-1 (from DON), α-ZEL and *β*-ZEL (from ZEN), and DHC (from CIT) constituted the metabolic products. One or more of the mycotoxins or metabolites was observed in all 89 samples, with 87/89 samples having at least one of the toxins at a concentration above the LOQ (Table 2). AOH was not detected in any of the urine samples. AFM_1 was observed above the LOQ in 42% of samples (LOQ 0.001 μg L^{-1}: overall range n.d. – 0.210 μg L^{-1}), and FB_1 was observed above the LOQ in 10% of samples (LOQ 0.15 μg L^{-1}: overall range n.d. – 0.83 μg L^{-1}). Of the other mycotoxins, total DON (72%), total CIT (80%), OTA (80%) and total ZEN (82%) were observed most frequently above the LOQs, see Table 2.

Table 2
Profiles of urinary mycotoxins biomarkers and their derivatives in 89 urine samples from Cameroon.

Analyte	All studied participants (N = 89)					
	LOD LOQ ($\mu g\ L^{-1}$)	Mean^p (SD) ($\mu g\ L^{-1}$)	Median^p (IQR) ($\mu g\ L^{-1}$)	n > LOD	n > LOQ	*Mean (max) ($\mu g\ L^{-1}$)
Aflatoxin M_1 (AFM_1)	0.0005 0.001	0.03 (0.05)	0.01 (0.01–0.03)	37 (42%)	37 (42%)	0.01 (0.21)
Fumonisin B_1 (FB_1)	0.05 0.15	0.43 (0.22)	0.35 (0.24–0.49)	25 (28%)	9 (10%)	0.08 (0.83)
Deoxynivalenol (DON)	0.05 0.15	15.9 (94.4)	1.91 (1.21–5.91)	67 (75%)	64 (72%)	11.5 (759)
Deepoxy-deoxynivalenol (DOM-1)	0.05 0.15	4.5 (8.3)	1.25 (0.76–2.07)	15 (17%)	13 (15%)	0.67 (27.4)
Total DON i.e. ∑(DON + DOM-1)	-	17.8 (80.4)	1.38 (0.29–5.16)	72 (78%)	68 (76%)	12.1 (760)
Zearalenone (ZEN)	0.001 0.003	0.16 (0.21)	0.08 (0.05–0.18)	86 (97%)	71 (80%)	0.13 (1.10)
Alpha-Zearalenol (α-ZEL)	0.001 0.003	0.58 (0.76)	0.33 (0.09–0.82)	4 (5%)	4 (5%)	0.03 (1.67)
Beta-Zearalenol (β-ZEL)	0.001 0.003	0.30 (0.13)	0.32 (0.22–0.40)	6 (7%)	4 (5%)	0.01 (0.42)
Total ZEN i.e. ∑(ZEN + α-+β-ZEL)	-	0.17 (0.31)	0.07 (0.02–0.16)	88 (99%)	73 (82%)	0.17 (1.67)
Ochratoxin A (OTA)	0.0003 0.001	0.01 (0.01)	0.004 (0.002–0.008)	84 (95%)	71 (80%)	0.006 (0.090)

LOD: limit of detection; **LOQ**: limit of quantitation; **Mean^p**: mean of samples > LOQ; **Median^p**: median of samples > LOQ; **SD**: Standard Deviation; **IQR**: Interquartile range; **Max**: Maximum; **%**: Percentage; **nd**: Not detected; ***Mean** (max): Mean (maximum) all samples - values calculated with half LOD values used for samples < LOD and half LOQ used for values < LOQ.

Analyte	All studied participants (N = 89)					
	LOD LOQ (µg L^{-1})	Mean^{p} (SD) (µg L^{-1})	Median^{p} (IQR) (µg L^{-1})	n > LOD	n > LOQ	*Mean (max) (µg L^{-1})
Nivalenol (NIV)	0.033 0.10	0.59 (0.79)	0.33 (0.19–0.77)	47 (53%)	32 (36%)	0.23 (4.36)
Citrinin (CIT)	0.01 0.03	0.28 (0.39)	0.14 (0.06–0.33)	66 (74%)	51 (57%)	0.16 (2.15)
Dihydrocitrinone (DHC)	0.003 0.01	2.1 (12.0)	0.40 (0.19–0.73)	63 (71%)	63 (71%)	1.5 (96)
Total CIT i.e. ∑(CIT + DHC)	-	2.3 (10.3)	0.24 (0.05–0.73)	77 (87%)	71 (80%)	1.66 (97.5)

LOD: limit of detection; **LOQ**: limit of quantitation; **Mean^{p}**: mean of samples > LOQ; **Median^{p}**: median of samples > LOQ; **SD**: Standard Deviation; **IQR**: Interquartile range; **Max**: Maximum; **%**: Percentage; **nd**: Not detected; ***Mean** (max): Mean (maximum) all samples - values calculated with half LOD values used for samples < LOD and half LOQ used for values < LOQ.

The co-existence of mycotoxins was observed in human urine samples from Cameroon. A total of 88 (98.9%) of the 89 studied urine samples had two or more mycotoxins at detectable concentrations (Table 3). Altogether, 20 different patterns of urinary mycotoxin mixtures were observed. The majority (64; 72%) of the 89 studied urine samples contained five to seven different mycotoxin combinations constituting 10 of the 20 observed patterns. AFM_1 was in 9/20 different mixture combinations of urinary mycotoxins, including six combinations where urine contained five or more toxins. AFM_1 and FB_1 were co-observed in three distinct urinary mixtures. ZEN (19/20, 95%) and OTA (19/20; 95%) were the most frequent mycotoxins represented in the mixtures, while FB_1 was the least (7/20, 35%), see Table 3.

Table 3
Mycotoxin mixtures in 89 urine samples from Cameroon

No. of mycotoxins	Mycotoxins mixture types							Frequency
	AFM_1	FB_1	DON	ZEN	OTA	NIV	CIT	
7	+	+	+	+	+	+	+	6 (6.7%)
6	+	-	+	+	+	+	+	10 (11.2%)
	+	+	+	+	+	-	+	2 (2.2%)
	-	+	+	+	+	+	+	11 (12.4%)
5	+	+	+	+	+	-	-	1 (1.1%)
	+	-	-	+	+	+	+	3 (3.4%)
	+	-	+	+	+	-	+	11 (12.4%)
	-	+	-	+	+	+	+	4 (4.5%)
	-	+	+	+	+	-	+	3 (3.4%)
	-	-	+	+	+	+	+	13 (14.6%)
4	+	-	-	+	+	+	-	1 (1.1%)
	+	-	-	+	+	-	+	2 (2.2%)
	-	+	-	+	+	-	+	1 (1.1%)
	-	-	+	+	+	-	+	14 (15.7%)
	-	-	-	+	+	+	+	1 (1.1%)
	-	-	+	+	+	+	-	1 (1.1%)
3	+	-	-	-	+	-	+	1 (1.1%)
	-	-	-	+	+	-	+	2 (2.2%)
	-	-	-	+	+	+	-	1 (1.1%)
2	-	-	-	+	-	-	+	1 (1.1%)
Occurrence (n = 20)	9	7	10	19	19	10	16	
%	45	35	50	95	95	50	80	

Intakes of each mycotoxin can be roughly estimated using the individual mycotoxin concentration (including parent and metabolites), an estimated average urinary output of 1.5 litre per day, mean estimates of transfer of the mycotoxins from the diet to urine, and individual bodyweight. In Table 4 the mean estimated intakes are compared to established TDIs for four mycotoxins where the transfer

percentage has been established. There is no TDI for aflatoxins, and the overall mean estimated intake for FB_1, DON and ZEN did not exceed the TDI. A few individuals were predicted to exceed the TDI for FB_1 (4/89), DON (1/89) and ZEN (2/89). No individuals exceeded the TDI for more than one mycotoxin. Individuals with the maximum estimated intake were 2.95, 17.6 and 1.92 times higher than the TDI for FB_1, DON and ZEN, respectively. Using linear regression there were no significant relationships between estimated intakes for any of the four mycotoxins, data not shown.

Table 4
Intake estimates based on urinary mycotoxin concentrations.

	Aflatoxin	Fumonisin	Deoxynivalenol	Zearalenone
Urinary excretion rate [%]	1-3[a]	0.3[b]	72[c]	9.4[d]
[#]Intake Mean (SD) [µg kg^{-1} bw/d]	0.007 (0.02)	0.44 (0.86)	0.29 (1.86)	0.034 (0.07)
[#]Max Intake [µg kg^{-1} bw/d]	0.14	5.89	17.6	0.48
Established TDI [µg kg^{-1} bw/d]	None defined	2	1	0.25
[#]Individuals Exceeding TDI	n/a	4/89	1/89	2/89

[a]: [35]. [b]: Average of two mean urinary excretory FB_1 values: 0.5% [36] and 0.075% [37]. [c]: [33]. [d]: [34]. Tolerable daily intake (TDI) values for FB_1 defined by the Scientific Committee on Food [22]; DON by the European Food Safety Agency [23], likewise, ZEN [24]. [#]: Calculations are based on the average body weight (84 kg)'; An assumed daily urine excretion of 1.5 L for all sub-populations; and Mean (Standard Deviation, SD; likewise, maximum, max) all samples - values calculated with half LOD values used for samples < LOD and half LOQ used for values < LOQ, using the equation below:

$$\text{Mean (or Maximum) Estimated Exposure } (\mu g\ kg^{-1}\ bw/day) = \frac{100 \times (\text{Mean mycotoxin in } \mu g\ L^{-1}) \times (\text{Assumed daily urine excretion of 1.5 L})}{(\text{Mean urinary excretory rate in \%}) \times (\text{Mean body weight of 89 adults in kg})}$$

Discussion

Several studies have reported mixtures of mycotoxins in dietary staples in Cameroon [7, 26, 27]. Human biomonitoring (HBM) typically provides more reliable exposure estimates, and as such improve studies assessing the relationships between dietary mycotoxins and human health. The aim of this study was to determine the levels of urinary biomarkers of mycotoxin exposures in male and female adults in the city of Yaounde, Centre Region, Cameroon.

This study supports recent observations of frequent mycotoxin co-exposures in African populations based on urinary measures. This study observed 11 mycotoxin analytes, in 89 urine samples, while earlier studies in Nigeria, Cameroon, South Africa and Cameroon reported eight, eleven, four and seven mycotoxin analytes, respectively [38, 30, 39, 28], in roughly similar sized studies. The mean (maximum)

concentration of AFM_1 [0.03 (0.21) μg L^{-1}; 42%] in urine analysed in the present study was similar, albeit lower, compared with the mean (maximum) levels of AFM_1 previously reported in adult urine from Cameroon [0.05 (1.38) μg L^{-1}; 10%] [30] and urine from households in Nigeria [0.3 (1.5) μg L^{-1}; 14.2%] [38]; however, the AFM_1 incidence was higher in our present study than in the two previous reports. The FB_1 concentrations were also similar in the present study (mean 0.43 (max 0.83) μg L^{-1}, 10%) compared to (mean 0.33 (max 9.54) μg L^{-1}, 3%) previously reported in Cameroon [30], though the maximum level was somewhat higher. The detected mean (maximum) amounts of FB_1 in our study were, however, lower than the mean (maximum) levels of FB_1 [4.6 (12.8) μg L^{-1}; 13.3%] reported in a Nigerian population [38]. These differences should not be over-interpreted given the relatively small numbers of samples involved.

DON (and its derivative DOM-1), ZEN (and its metabolites: α-ZEL and β-ZEL) and OTA were detected in urine, typically at higher frequencies than AFM_1 and FB_1. Total DON was detected about twice as frequently (76%) in this study compared to an earlier Cameroon study [30], and much more frequently than in Nigeria (5%), where children rather than adults dominated the exposure [38]. In South Africa, a similar high frequency (100%) of total DON was reported as observed in the current study [39]. In these earlier studies, the mean concentrations were typically around 5–15 μg L^{-1}, and this is in line with many studies in regions outside of Africa [40, 41]. However, while the mean [17.8 μg L^{-1}] is similar, one individual sample [760 μg L^{-1}] was notably higher in the current study than most previously reported HBM studies. Notwithstanding, the major metabolite of DON in human urine, DON-15-glucuronide [42, 34] was not measured directly in this study as enzymatic deconjugation was applied [32].

The mean (maximum) concentration of OTA and ZEN were relatively lower in this study compared to previously reported data from Cameroon [30] and Nigeria [38]. However, the extremely high detection rate of 82% for total ZEN is somehow worrisome given the high xenoestrogenic potential of ZEN and its phase I biotransformation products [43]. Recent studies further highlighted that ZEN is prone to synergistic mixture effects [44, 45] and able to pass the placental barrier and thus exposure of mothers is likely to result in *in utero* exposure of the unborn child [46]. The impact of this chronic low-dose exposures on the endocrine system and related disease should be investigated in future studies.

The mean NIV level recently reported in a Nigerian study [32] was approximately 10 times greater than the level reported here for the Cameroonian population. Urinary CIT and its metabolite, DHC, were quantified in this study for the first time in Cameroon. The detected mean (maximum) concentration of total CIT [2.3 (98) μg L^{-1}; 80%] in this present study were lower than those in Nigeria [6.0 (241) μg L^{-1}; 66%] [32], although our study had higher incidence. Comparison of urinary mycotoxin concentrations by either sex, or by hypertensive status did not reveal any significant differences ($p < 0.05$), noting limited study size would preclude meaningful comparisons.

One urine sample contained only one mycotoxin, while 20 combinations of two or up to seven mycotoxin urinary biomarkers were observed; more than 70% of the urines contained five or more different mycotoxins. Complex mixture toxicology remains poorly examined though several groups have recently

examined combined effects *in vitro* [47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 44, 45], with animal studies being more limited [58, 59]. These studies remain hard to interpret for public health decisions, but some suggest more than additive effects, thus the mixtures reported here and elsewhere highlight significant knowledge gaps. It will be important to conduct longitudinal studies to better understand typical patterns and seasonal variation to better inform our understanding of mixture exposures. An interesting example for such longitudinal mycotoxin co-exposure assessment was recently published for an infant that was exclusively fed by breastmilk, which was tested for 29 mycotoxins [60]. However, it will be even more relevant to consider other food- and environment-related exposures beyond mycotoxins as proposed by the exposome concept [61, 62, 63].

From the mean (maximum) levels of some of the major urinary mycotoxins in this present study, an estimated average dietary exposure was calculated on the basis of each participant's estimated dietary exposure using each participant's urine mycotoxin exposure amount, individual weight, an assumed 1.5 L urinary output per day and estimated urine excretion rate for each mycotoxin. For data with urinary concentration below the LOQ, either half the LOQ or half the LOD was used. This is generally used in food safety risk assessment (e.g. by European Food Safety Agency, EFSA) as it provides conservative estimates for calculation of exposure assessment [64]. Any dietary AFM_1 is considered to be of concern, as no exposure level of AFM_1 is tolerated based on the conclusions of the Scientific Committee on Food [65, 22]. For FB_1, DON and ZEN the mean estimated intakes were all less than the TDIs, suggesting modest exposures occurred for most. However, in this limited study, seven individuals (i.e. 8%) of the study population exceeded one of the TDIs. For FB_1, 4/89 (4.49%) individuals had estimated intakes above the TDI (range: 2.3–5.9 μg kg^{-1} bw/d). Based on food measures and urinary markers, aflatoxin and fumonisin exposure remain a significant concern in sub-Saharan Africa including Cameroon [7, 26, 30, 27, 28, 32, 66]. In this study co-exposures to AFM_1 and FB_1 occurred in about 10% of samples. For DON, only one individual exceeded the 1.0 μg kg^{-1} bw/d TDI [23], however, this intake estimate by far exceeded data typically seen in Sub-Saharan Africa at 17.6 μg kg^{-1} bw/d and is relatively higher than the previously reported study from Cameroon [30]. Likewise, the TDI of ZEN fixed by the European Food Safety Agency [24] was exceeded by the estimated maximum exposure level for total ZEN in urine samples from two individuals (0.30 and 0.48 μg kg^{-1} bw/d). Overall, although only few individuals exceeded TDIs for FB_1, DON and ZEN, several percent of the study population were not insignificant [30].

Conclusion

This study has further revealed that mycotoxin exposure is prevalent in city of Yaounde, Centre Region, Cameroon. This is evident by the detection of 11 mycotoxins (seven mycotoxins representative of AF, FB, DON, ZEN, NIV, OTA and CIT, and four of their derivatives: α/*β*-ZEL, DOM-1 and DHC) in 89 adult urines in this region. Most importantly is that every single urine sample contained at least one mycotoxin. For the first time urinary CIT and its metabolite, DHC, were quantified in urine samples from Cameroon. The co-existence of as much as seven mycotoxins in up to 20 different patterns worsens the scenario and predicts potential health risk for the population. The presence of aflatoxin biomarkers in 42% of samples

and of those about a quarter additionally contained fumonisin B_1 is a concern. The potential risk derived from additional mixture effects remains poorly defined, but as further studies add to these data sets their putative contributions may be understood, while aflatoxins and fumonisins remain a priority in populations such as Cameroon with a high incidence of liver disease [67, 68, 69] and stunting [70, 71].

Abbreviations

LC/MS/MS
Liquid Chromatography tandem mass spectrometry
MTLs
Maximum tolerable limits
AFs
aflatoxins
AFB_1
aflatoxin B_1
total AFs
total aflatoxins
FBs
fumonisins
FB_1
fumonisin B_1
total FBs
total fumonisins
DON
deoxynivalenol
DOM-1
deepoxy-DON
OTA
ochratoxin A
ZEN
zearalenone
α-ZEL
alpha-zearalenol
β-ZEL
beta-zearalenol
NIV
nivalenol
CIT
citrinin
DHC

Dihydrocitrinone
AOH
alternariol
TDI
Tolerable daily intake
BMI
body mass index
MeOH
Methanol
LC
Liquid chromatography
CAN
acetonitrile
HAc
glacial acetic acid
MS
Mass spectrometry
ACN/H_2O
acetonitrile/water
LC-ESI-MS/MS
liquid chromatography-electrospray ionization-tandem mass spectrometry
ESI
electro spray ionization (ESI)
UHPLC
Ultra-high performance liquid chromatography
UMBs
urinary mycotoxins biomarkers
sMRM
scheduled multiple reaction monitoring
LOD
limit of detection
LOQ
limit of quantitation
SD
Standard Deviation
IQR
Interquartile range
Max
Maximum
%

Percentage
nd
Not detected
HBM
Human biomonitoring
EFSA
European Food Safety Agency

Declarations

Ethics approval and consent to participate

Ethical approval was received from the Cameroon National Ethics Committee of Research for Human Health (Authorisation No. 2013/05/252/CNERSH/SP). Signed informed consent forms were obtained from 89 adult male (n = 39) and female (n = 50) volunteers (age range: 28–85 years, body weight (range: 55–129 kg)) recruited in this study.

Consent for publication

Not applicable.

Availability of data and material

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Competing interests

The authors declare they have no competing financial interests.

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Authors’ contributions

DD, PFM, ANT, EYM surveyed and recruited participants. EYM managed the participants in his clinic. WAA, BS, CNE analysed urinary mycotoxin biomarkers. WAA, BS, PCT analysed and interpreted the urine data. WAA drafted the manuscript with major contributions to writing from BW, RK, CTE, BS, CNE, PFM. All authors read and approved the final manuscript.

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