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WORLD MYCOTOXIN JOURNAL 17 (2024) 191–203

World Mycotoxin Journal
brill.com/wmj

RESEARCH ARTICLE

# Assessment of urinary biomarkers of mycotoxin exposure in adults from Cameroon

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Received 9 December 2023 | Accepted 18 October 2024 | Published online 22 November 2024 | Published in issue 5 December 2024

**Abstract**

In Cameroon, dietary staples are frequently contaminated with diverse toxic fungal metabolites, known as mycotoxins. Aflatoxins and fumonisins are a public health concern, particularly concerning cancer and/or early life stunting. Mycotoxin mixtures are predicted from food measures; and this study reports the levels and frequencies of urinary mycotoxin biomarkers in Cameroonian adults. A single first void urine sample was collected from 89 adults from Yaoundé, Cameroon. Urine samples 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). 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 (98%) of which were above the limit of quantitation. Aflatoxin $M_1$ was detected in 42% (n.d.-0.21 μg/l) of samples of which about a quarter additionally contained fumonisin $B_1$. Of the remaining toxins deoxynivalenol (78%), zearalenone (99%), ochratoxin A (95%), nivalenol (53%), and citrinin (87%) were present in the samples. Alternariol was not detected in any sample. Mixtures of mycotoxins in the samples were frequently observed with 64 samples (72%) containing more than five mycotoxin exposure biomarkers. Estimates of intake exceeded the TDIs for fumonisin $B_1$ (n = 4), deoxynivalenol (n = 1) and zearalenone (n = 2), no TDI is set for aflatoxin. This study reveals frequent co-exposure of Cameroonian individuals to a complex mixture of toxic and carcinogenic mycotoxins, with mixtures of aflatoxin and fumonisin being a particular priority from a public health standpoint.

Published with license by Koninklijke Brill BV | DOI: 10.1163/18750796-BJA10010
 | ISSN: 1875-0796 (online)

**Keywords**

Cameroon – mycotoxin exposures – urinary biomarkers – food safety – human biomonitoring – LC-MS/MS

## 1 Introduction

Agricultural crops around the globe that are used for food and feed are frequently contaminated during crop growth and/or storage by several toxigenic fungi that produce poisonous secondary metabolites, known as mycotoxins. A combination of poor agricultural practices, favourable temperatures and humidity, and poor and extended storage practices, often exacerbate toxin production, especially the aflatoxins, in sub-Saharan Africa (Bennett and Klich, 2003; Medina *et al.*, 2015; Nji *et al.*, 2022; Richard and Payne, 2003). Many mycotoxins are heat resistant, such that traditional cooking practices with grains or nuts have little impact on plate-ready toxin concentrations (Ezekiel *et al.*, 2019; Karlovsky *et al.*, 2016). In many parts of rural Africa diets are both reliant on some of these high-risk grains and nuts, and exhibit poor diversity, thus, mycotoxin exposure seems inevitable.

Acute and chronic exposures to mycotoxins can be harmful to health, and on occasions fatal (Lewis *et al.*, 2005). Mycotoxins such as aflatoxin $B_1$ ($AFB_1$) are proven human carcinogens (IARC, 2012a). Additionally, $AFB_1$ and other mycotoxins are implicated in a range of other conditions including kwashiorkor in children (Tchana *et al.*, 2010), infant stunting ($AFB_1$ and fumonisin $B_1$ ($FB_1$)) (IARC, 2012b; Kimanya *et al.*, 2010; Turner, 2013), immunosuppression ($AFB_1$ and deoxynivalenol (DON)) (Bondy and Pestka, 2000; Pestka, 2010; Pestka *et al.*, 2004; Pierron *et al.*, 2016; Turner *et al.*, 2003), and neural tube defects ($FB_1$) (Marasas *et al.*, 2004; Missmer *et al.*, 2006). 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 (IARC, 2012b). Maximum limits (MLs) 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 (EC, 2001, 2023; Egmond *et al.*, 2007; FAO, 2004). 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 (SCF, 2003) and DON by the European Food Safety Agency (EFSA, 2017), likewise ZEN (EFSA, 2011); 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 (Abia *et al.*, 2013a, 2007; Ngwegoue *et al.*, 2018; Njobeh *et al.*, 2010, 2013; Tchana *et al.*, 2010). $AFB_1$ and $FB_1$ have been observed in groundnuts- and cereals-based food, and in some cases, the concentrations exceeded MLs stipulated by the Codex Alimentarius Commissions (CAC, 2019; FAO, 2004) and the European Union (EC, 2023). Limited studies are available that measure multiple urinary markers of mycotoxin exposure in Cameroon, these include 175 adults (Abia *et al.*, 2013b) and 220 young children (Njumbe *et al.*, 2013). 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.

## 2 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, approximately 50 ml each, were collected prior to intake of food 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. A priori, the study was neither attempting nor powered to show differences by normal versus hypertensive status. However, sixty-six of the participants were part of a hypertension clinic in Yaounde (Les Promoteurs de la Bonne Sante), who may have slightly different dietary behaviours compared to non-hypertensives. 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 Asam *et al.* (2009) 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 the 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 Sarkanj *et al.* (2018).

### *Sample Preparation*

Urine samples were allowed to reach room temperature and centrifuged for 3 min at 5,600 ×*g*. The supernatant (500 µl) was incubated with 500 µl phosphate-buffered saline (200 mM, pH = 7.4) containing 3,000 U of β-glucuronidase from *E. coli* Type IX-A (Sigma-Aldrich, G7396-2MU) (modified from Turner *et al.*, 2010) for 16 h at 37 °C to allow de-glucuronidation of mycotoxin-glucuronides (e.g. DON-15-glucuronide, ZEN-14-glucuronide, Warth *et al.*, 2013). Following hydrolysis, 1 ml was passed through Oasis PRiME HLB® SPE columns (Waters, Milford, MA, USA), 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 Sarkanj *et al.* (2018).

### *Analysis of urine samples by LC-ESI-MS/MS*

Sample analysis was performed using a Sciex QTrap® 6500+LC-MS/MS system (Foster City, CA, USA) equipped with a Turbo V electrospray ionization (ESI) source interfaced with an Agilent 1290 series Ultra-high performance liquid chromatography (UHPLC) system (Waldbronn, Germany) following the method described by Sarkanj *et al.* (2018) to quantify urinary mycotoxin biomarkers (UMBs). In brief, analytes of interest were separated on an Atlantis T3 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. After injection of 10 µl, the needle was washed for 20 s to minimize carry-over. The column effluent was transferred either to the mass spectrometer (minutes 5 to 22.5) or the waste via a six-port valve. The analytes were separated on a column at 35 °C.

ESI-MS/MS was performed in scheduled multiple reaction monitoring (sMRM) mode, with a 180 s detection window. 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 –4,500 V in negative mode while it was set to 4,500 V in positive mode.

Note: The total concentrations of DON, ZEN, and CIT (expressed in µg/l) were the summed values of the parent compound and its metabolite(s) taking molecular weights of the components into account.'

### *Statistical analysis*

Statistical analysis was performed using GraphPad In Stat version 3.10 for Windows (GraphPad Software Inc, San Diego, CA, USA) and Microsoft Office Excel 2010 (Part of Microsoft Professional Edition, Computer Program), Statistica 13.3. (TibcoSoft, Palo Alto, CA, 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).

## 3 Results

There were no differences by hypertensive status in the age, weight, height or BMI of the participants (see Supplementary 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) |
|---|---|
| Sex: female and male | 49 and 40 |
| Age: mean (range) year | 55.4 (28.0-85.0) |
| Age: median age, year | 52 |
| # of males of age below (or equal and above median value) | 19 (21) |
| # of females of age below (or equal and above median value) | 14 (35) |
| 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) |
| BMI median value kg/m$^2$ | 29.7 |
| # of males with median BMI value and below (above median value) | 22 (18) |
| # of females with median BMI value and below (above median value) | 27 (22) |
| Occupation: | |
| Retired civil servant | 9 |
| Civil servant | 47 |
| Home-care servant | 16 |
| Farmer | 6 |
| Others | 11 |

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 were 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 the samples (LOQ 0.001 μg/l: overall range n.d. –0.210 μg/l), and $FB_1$ was observed above the LOQ in 10% of the samples (LOQ 0.15 μg/l: overall range n.d. – 0.83 μg/l). 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. In the cases of total DON and total ZEN, all glucuronides were de-conjugated before quantitation, thus both free and glucuronide-bound toxins were included in those data.

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.

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 l/day, mean estimates of transfer of the mycotoxins from the diet to urine, and individual body weight. 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, ZEN, and CIT did not exceed the TDI. A few individuals were predicted to exceed the TDI for $FB_1$ (4/89), DON (1/89), ZEN (2/89) and CIT (1/89). No individuals exceeded the TDI for more than one mycotoxin. Individuals with the maximum estimated intake were 2.95, 17.6, 0.48, and 30 times higher than the TDI for $FB_1$, DON, ZEN, and CIT, respectively. Using linear regression there were no significant relationships between estimated intakes for any of the four mycotoxins, data not shown. Despite the high occurrence frequencies of OTA and NIV, their estimated intakes were not calculated considering there is inad-

TABLE 2 Profiles of urinary mycotoxins biomarkers and their derivatives in 89 urine samples from Cameroon[1]

| Analyte | All studied participants (n = 89) | | | | | |
|---|---|---|---|---|---|---|
| | LOD / LOQ (μg/l) | Mean$^p$ (SD) (μg/l) | Median$^p$ (IQR) (μg/l) | n > LOD | n > LOQ | *Mean (max) (μg/l) |
| $AFM_1$ | 0.0005/0.001 | 0.03 (0.05) | 0.01 (0.01-0.03) | 37 (42%) | 37 (42%) | 0.01 (0.21) |
| $FB_1$ | 0.05/0.15 | 0.43 (0.22) | 0.35 (0.24-0.49) | 25 (28%) | 9 (10%) | 0.08 (0.83) |
| DON | 0.05/0.15 | 15.9 (94.4) | 1.91 (1.21-5.91) | 67 (75%) | 64 (72%) | 11.5 (759) |
| 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 | - | 17.8 (80.4) | 1.38 (0.29-5.16) | 72 (78%) | 68 (76%) | 12.1 (760) |
| ZEN | 0.001/0.003 | 0.16 (0.21) | 0.08 (0.05-0.18) | 86 (97%) | 71 (80%) | 0.13 (1.10) |
| α-ZEL | 0.001/0.003 | 0.58 (0.76) | 0.33 (0.09-0.82) | 4 (5%) | 4 (5%) | 0.03 (1.67) |
| 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 | - | 0.17 (0.31) | 0.07 (0.02-0.16) | 88 (99%) | 73 (82%) | 0.17 (1.67) |
| OTA | 0.0003/0.001 | 0.01 (0.01) | 0.004 (0.002-0.008) | 84 (95%) | 71 (80%) | 0.006 (0.090) |
| NIV | 0.033/0.10 | 0.59 (0.79) | 0.33 (0.19-0.77) | 47 (53%) | 32 (36%) | 0.23 (4.36) |
| CIT | 0.01/0.03 | 0.28 (0.39) | 0.14 (0.06-0.33) | 66 (74%) | 51 (57%) | 0.16 (2.15) |
| DHC | 0.003/0.01 | 2.1 (12.0) | 0.40 (0.19-0.73) | 63 (71%) | 63 (71%) | 1.5 (96) |
| Total CIT | - | 2.3 (10.3) | 0.24 (0.05-0.73) | 77 (87%) | 71 (80%) | 1.66 (97.5) |

[1] 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; $AFM_1$ = aflatoxin $M_1$; $FB_1$ = fumonisin $B_1$; DON = deoxynivalenol; DOM-1 = deepoxy-deoxynivalenol; Total DON = $\sum$(DON + DOM-1); ZEN = zearalenone; α-ZEL = α-zearalenol; β-ZEL = β-zearalenol; Total ZEN = $\sum$(ZEN +α-+β-ZEL); OTA = ochratoxin A; NIV = nivalenol; CIT = citrinin; DHC = dihydrocitrinone; Total CIT = $\sum$(CIT + DHC).

equate guidance on the estimated excretory rates for these mycotoxins.

## 4 Discussion

Several studies have reported mixtures of mycotoxins in dietary staples in Cameroon (Abia *et al.*, 2013a, 2017; Tchana *et al.*, 2010). Human biomonitoring (HBM) typically provides more reliable exposure estimates, and as such improve studies assessing the relationships between dietary mycotoxins and human health. This study aimed 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 (Abia *et al.*, 2013b; Ezekiel *et al.*, 2014; Njumbe *et al.*, 2013; Shephard *et al.*, 2013), in roughly similar-sized studies. The mean (maximum) concentration of $AFM_1$ [0.03 (0.21) μg/l; 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; 10%] (Abia *et al.*, 2013b) and urine from households in Nigeria [0.3 (1.5) μg/l; 14.2%] (Ezekiel *et al.*, 2014); however, the $AFM_1$ incidence was higher in our present study than in the two previous reports. Mycotoxins such as aflatoxins are known to have heterogeneous distribution in crops and food stores, and vary by season and climate (IARC, 2012a,b; Medina *et al.*, 2017). The $FB_1$ concentrations were similar in the present study (mean 0.43 (max 0.83) μg/l, 10%) compared to those previously reported (mean 0.33: max 9.54) μg/l, 3%) in Cameroon (Abia *et al.*, 2013b), though the maximum level was somewhat higher. The detected mean $FB_1$ concentration in our study was, however, lower than the mean concentration [4.6 (max 12.8) μg/l; 13.3%] reported in a Nigerian population (Ezekiel *et al.*, 2014). These differences should not be over-interpreted given the relatively small numbers of samples involved. In addition, previous reports from Burkina Faso identified large variations (10-fold differences in mean) for $FB_1$ contamination of maize collected from the same region in one year; and

TABLE 3 Mycotoxin mixtures in 89 urine samples from Cameroon

| No. of mycotoxins | Mycotoxins mixture types[1] | | | | | | | 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 | |

[1] $AFM_1$ = aflatoxin $M_1$; $FB_1$ = fumonisin $B_1$; DON = deoxynivalenol; ZEN = zearalenone; OTA = ochratoxin A; NIV = nivalenol; CIT = citrinin.

additionally in the same village where maize was collected in two separate years (Nikiema *et al.*, 2004, 2008). Thus, significant variations in biomarker data are to be expected in urine from distinct studies even in similar locations with similar reliance on maize as a dietary staple.

DON (and its metabolite 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 (Abia *et al.*, 2013b), and much more frequently than in Nigeria (5%), where children rather than adults dominated the exposure (Ezekiel *et al.*, 2014). In South Africa, a similar high frequency (100%) of total DON was reported as observed in the present study (Shephard *et al.*, 2013). In these earlier studies, the mean concentrations were typically around 5-15 μg/l, and this is in line with many studies in regions outside of Africa (Šarkanj *et al.*, 2013; Turner *et al.*, 2012). However, while the mean [17.8 μg/l] is similar, one individual sample [760 μg/l] 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 (Warth *et al.*, 2012a, 2013) was not measured directly in this study as enzymatic deconjugation was applied (Sarkanj *et al.*, 2018).

The mean (maximum) concentration of OTA and ZEN were relatively lower in this study compared to previously reported data from Cameroon (Abia *et al.*, 2013b) and Nigeria (Ezekiel *et al.*, 2014). 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 (Preindl *et al.*, 2019). Recent studies further highlighted that ZEN is prone to synergistic mixture effects (Vejdovszky *et al.*, 2017a,b) and able to pass the placental barrier and thus exposure of mothers is likely to result in *in utero* exposure of the unborn child (Warth *et al.*, 2019). The impact of these 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 (Sarkanj *et al.*, 2018) was approximately

TABLE 4 Intake estimates based on urinary mycotoxin concentrations

| | Aflatoxin | Fumonisin | Deoxynivalenol | Zearalenone | Citrinin |
|---|---|---|---|---|---|
| Urinary excretion rate [%] | 1-3[a] | 0.3[b] | 72[c]-69[g] | 9.4[d]-36.8[e] | 40[f] |
| Intake Mean (SD) [μg/kg bw/d] [h] | 0.007 (0.02) | 0.44 (0.86) | 0.29 (1.86) | 0.009 (0.019) | 0.095 (0.64) |
| Max intake [μg/kg bw/d] [h] | 0.14 | 5.89 | 17.6 | 0.121 | 6.09 |
| Established TDI [μg/kg bw/d] | None defined | 2 | 1 | 0.25 | 0.2[x] |
| Individuals exceeding TDI [h] | n/a | 4/89 | 1/89 | 0/89 | 1/89 |

[a] Zhu *et al.* (1987).

[b] Average of two mean urinary excretory $FB_1$ values: 0.5% (Riley *et al.*, 2012) and 0.075% (van der Westhuizen *et al.*, 2011).

[c] Turner *et al.* (2010) – and was used as the urinary excretion factor for DON.

[d] Warth *et al.* (2013).

[e] Llorens *et al.* (2018) – and was used as the urinary excretion factor for ZEN considering 24 h urine samples were collected.

[f] Degen *et al.* (2018).

[g] Mengelers *et al.* (2024). Tolerable daily intake (TDI) values for $FB_1$ defined by the Scientific Committee on Food (SCF 2003); DON by the European Food Safety Agency (EFSA 2017), likewise, ZEN (EFSA, 2011). 0.2[x]: Similarly, for CIT, the value used (0.2 μg/kg bw per day) is an assumed preliminary TDI for CIT determined by the EFSA as a level of no concern for nephrotoxicity (EFSA, 2012).

[h] Calculations are based on the individual weight of 89 participants; 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, as reported by (Solfrizzo *et al.* 2014 with modification):

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

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 dietary source of CIT in the studied population in the city of Yaounde is probably from stored grains and other plant-based foods such as fruits and spices (EFSA, 2012). The detected mean (maximum) concentration of total CIT [2.3 (98) μg/l; 80%] in this present study were lower than those in Nigeria [6.0 (241) μg/l; 66%] (Sarkanj *et al.*, 2018), although our study had a 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 two mycotoxins (ZEN and CIT), while 19 combinations of three 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 the combined effects *in vitro* (Assunção *et al.*, 2019; Eze *et al.*, 2019; Fernández-Blanco *et al.*, 2018; Gong *et al.*, 2019; Kouadio *et al.*, 2007; Lin *et al.*, 2019; Marin *et al.*, 2019; Vejdovszky *et al.*, 2016, 2017; Wan *et al.*, 2013a,b,c, 2014), with animal studies being more limited (Przybylska-Gornowicz *et al.*, 2018; Wan *et al.*, 2016). 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 of such longitudinal mycotoxin co-exposure assessment was recently published for an infant that was exclusively fed by breastmilk, which was tested for 29 mycotoxins (Braun *et al.*, 2020). Furthermore, more recent studies from sub-Saharan Africa have revealed potential correlations between the chemical exposome (e.g. total faecal mycotoxin levels) and the gut microbiome (Ayeni *et al.*, 2024; Oesterle *et al.*, 2024). However, it will be even more relevant to consider other food- and environment-related exposures beyond mycotoxins as proposed by the exposome concept (Ayeni *et al.*, 2022; Vermeulen *et al.*, 2020; Warth *et al.*, 2017; Wild, 2005).

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 LOD for concentrations below LOD and half the LOQ for concentrations below LOQ was used. This is generally used in food safety risk assessment (European Food Safety Agency, EFSA) as it provides conservative estimates for calculation of exposure assessment (EFSA, 2010). Dietary $AFM_1$ (hydroxylated metabolite of $AFB_1$, relative potency factor 0.1 with respect to a liver carcinogenic potency of 1 for $AFB_1$) is considered to be of concern, and besides not appropriate to establish TDI, its calculated margin of exposure based on a benchmark-dose level ($BMDL_{10}$) of 0.4 μg/kg bw/d, is below 10,000 (EFSA CONTAM Panel, 2020). 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 bw/d). Based on food measures and urinary markers, aflatoxin, and fumonisin exposure remain a significant concern in sub-Saharan Africa including Cameroon (Abia *et al.*, 2013a,b, 2007; Njumbe *et al.*, 2013; Sarkanj *et al.*, 2018; Tchana, *et al.*, 2010; Warth *et al.*, 2012b). In this study co-exposures to $AFM_1$ and $FB_1$ occurred in about 10% of the samples. For DON, only one individual exceeded the 1.0 μg/kg bw/d TDI (EFSA, 2017), however, this intake estimate, by far exceeded data typically seen in Sub-Saharan Africa at 17.6 μg/kg bw/d and is relatively higher than the previously reported study from Cameroon (Abia *et al.*, 2013b). Likewise, the TDI of ZEN derived by the European Food Safety Agency (EFSA, 2011) was exceeded by the estimated maximum exposure level for total ZEN in urine samples from two individuals (0.30 and 0.48 μg/kg bw/d). For CIT, considering the level of no concern for nephrotoxicity, 0.2 μg/kg bw/d (EFSA, 2012), only one individual's derived CIT exposure estimate exceeded it. Overall, although only a few individuals exceeded TDIs for $FB_1$, DON, ZEN, and CIT, several percent of the study population were not insignificant (Abia *et al.*, 2013b). Furthermore, the spot (early morning) urine samples analysed in this study might not have revealed a complete excretion profile of the mycotoxins relative to a 24 h urine sample which would reflect total daily excretion when considering the case of DON (Alvito *et al.*, 2022; Llorens *et al.*, 2018; Mengelers *et al.*, 2024). In addition, multiple collections over several days would create a more reliable 'typical' exposure assessment. Some additional caution is always required in interpreting intake estimates from small to moderate studies. For example, for toxins such as DON high rates of excretion and a strong correlation between intake has been observed (reviewed by Turner and Snyder, 2021). A somewhat lower excretion rate for $AFM_1$ is reported, however, intake of the toxin and the urinary biomarker concentration are strongly correlated. Thus there is good strength in using these urinary measures to estimate intake for DON and $AFB_1$ (the parent compound of $AFM_1$), albeit with some noise in the precision of a mean intake with this modest-sized study. However, the excretion rate of $FB_1$ in urine is both extremely low and poorly correlated with intake (Alvito *et al.*, 2022; Riley *et al.*, 2012; Turner and Snyder, 2021), thus caution is required for this intake estimate.

## 5 Conclusions

This study has further revealed that mycotoxin exposure is prevalent in the city of Yaounde, Centre Region, Cameroon. This is evident in the detection of 11 mycotoxins (seven mycotoxins representative of AF, FB, DON, ZEN, NIV, OTA and CIT, and four of their metabolites: 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 many as seven mycotoxins in up to 20 different patterns may worsen the scenario and predict potential health risk for the population. Although the TDIs for FB, DON, ZEN, and CIT were not exceeded, the presence of biomarkers of the (genotoxic) human carcinogen (IARC classification Group 1) (aflatoxin) in 42% of the samples and of those about a quarter additionally contained fumonisin $B_1$ (possibly carcinogenic to humans (IARC Group 2B)) 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 (Ankouane *et al.*, 2014; Bigna *et al.*, 2017; Djuidje *et al.*, 2018) and stunting (Nzefa *et al.*, 2019; UNICEF/WHO/WB, 2019).

### Supplementary material

Supplementary material is available online at:
https://doi.org/10.6084/m9.figshare.27332199

**Table S1.** Anthropometric measures of the studied population.

**Acknowledgements**

The authors are thankful to the participants who donated urine samples. In addition, the authors sincerely acknowledge the participating clinic in Yaounde Cameroon (Les Promoteurs de la Bonne Sante) where 66 hypertensive adult participants were recruited into this study. Furthermore, the authors are grateful to the BOKU/IFA-Tulln, Austria, where urinary mycotoxin analyses were done. The authors are also grateful to Queen's University Belfast for the resources made available to the first author to put this manuscript together.

**Authors' contribution**

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, BW analysed and interpreted the urine data. WAA drafted the manuscript with major contributions to writing from BW, PCT, RK, CTE, BS, CNE, PFM. All authors read and approved the final manuscript.

**Conflict of interest**

The authors declare they have no competing financial interests.

**Data availability**

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

**Funding**

The study was partly funded by the Research Modernization Allowance to lecturers of the Ministry of Higher Education of Cameroon, and the City of Vienna Jubilee Funds for the University of Natural Resources and Life Sciences, Vienna (BOKU Research Funding, project *MycoMarker*). PCT was additionally supported by an Institutional Grant from the University of Maryland Grand Challenges Program.

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