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Quantitation of Mycotoxins in Food and Feed from Burkina Faso and Mozambique Using a Modern LC-MS/MS Multitoxin Method

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Supporting Information

ABSTRACT: In this study an LC-MS/MS multitoxin method covering a total of 247 fungal and bacterial metabolites was applied to the analysis of different foods and feedstuffs from Burkina Faso and Mozambique. Overall, 63 metabolites were determined in 122 samples of mainly maize and groundnuts and a few samples of sorghum, millet, rice, wheat, soy, dried fruits, other processed foods and animal feeds. Aflatoxin B_1 was observed more frequently in maize (Burkina Faso, 50% incidence, median = 23.6 μg/kg; Mozambique, 46% incidence, median = 69.9 μg/kg) than in groundnuts (Burkina Faso, 22% incidence, median = 10.5 μg/kg; Mozambique, 14% incidence, median = 3.4 μg/kg). Fumonisin B_1 concentrations in maize were higher in Mozambique (92% incidence, median = 869 μg/kg) than in Burkina Faso (81% incidence, median = 269 μg/kg). In addition, ochratoxin A, zearalenone, deoxynivalenol, nivalenol, and other less reported mycotoxins such as citrinin, alternariol, cyclopiazonic acid, sterigmatocystin, moniliformin, beauvericin, and enniatins were detected. Up to 28 toxic fungal metabolites were quantitated in a single sample, emphasizing the great variety of mycotoxin coexposure. Most mycotoxins have not been reported before in either country.

KEYWORDS: *natural contaminants, Africa, aflatoxin, fumonisin, dietary exposure assessment, emerging mycotoxin, food safety, corn, peanut, bacterial and fungal metabolites*

INTRODUCTION

Mycotoxins are toxic secondary metabolites produced by various molds and frequently contaminate food and feed worldwide. Their incidence depends on various factors, such as the commodity, climatic conditions, agricultural practices, storage conditions, and seasonal variances. Mycotoxins have the potential to seriously affect human health by acute and chronic effects such as the induction of hepatocellular carcinoma (HCC) or sudden death due to acute toxicity in the case of aflatoxins.[1] Fumonisins have been linked with esophageal cancer in the former Transkei, South Africa.[2] In addition, immune modulation effects of some mycotoxins intensifying health impacts of major diseases troubling Africa such as malaria, kwashiorkor, and HIV/AIDS have been suggested.[3] It is evident that especially rural sub-Saharan populations are at extraordinarily high risk for chronic dietary mycotoxin exposure because they often consume affected crops as a staple diet and because crops in tropical and subtropical regions are more susceptible to contamination due to favorable climatic conditions.[4,5]

Besides severe health issues, economic losses and trade barriers pose another important problem to agriculture in the affected countries. Since many legislative bodies such as the European Commission[6] established regulatory limits for major food mycotoxins, exports of agricultural products, especially groundnuts (*Arachis hypogaea*), decreased dramatically.[7] However, in sub-Saharan Africa regulatory limits are rarely in place or not properly implemented, and regular surveillance is often a major issue. In 2003, FAO performed a survey regarding worldwide mycotoxin regulations in which Mozambique stated a maximum level for total aflatoxins with a tolerated concentration of 10 μg/kg in groundnuts, groundnut milk, and feedstuff. In contrast, Burkina Faso does not have mycotoxin regulations in law at all.[8] The lack of regulation is partly due to missing information on the occurrence of certain toxins, which is a prerequisite for effective mitigation. Recent studies from Benin[9] and Nigeria[10−12] elucidated the great power and potential of advanced multimycotoxin LC-MS/MS methods for the simultaneous analysis of a multitude of different toxins.

Whereas in some African countries such as South Africa, Nigeria, or Ghana significant research, especially on aflatoxins and fumonisins, has been published,[10−14] very little work has been performed in the countries addressed in this study. In Burkina Faso, Nikiema and co-workers[15] reported high incidence of fumonisins in maize (*Zea mays*) from the western part of the country (Kenedougou province). All 124 samples analyzed showed detectable amounts of fumonisins, with the highest concentrations found in maize from local markets in Bobo Dioulasso (mean = 2900 μg/kg, range = 130−16040 μg/kg). The samples were not screened for other toxins, but the

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authors stated that aflatoxin contamination is endemic in Burkina Faso. A similar observation stated that aflatoxin contamination of groundnut has always been of concern in Burkina Faso.[16]

During 1968−1974 a major survey on aflatoxin contamination in prepared foodstuffs (n = 2183)[17] and raw cereals (n = 509)[18] was conducted in Mozambique. The investigated province Imhambane was identified as a high-risk area for hepatocellular carcinoma, and the relationship between dietary aflatoxin exposure and HCC incidents was studied. Groundnuts were identified as the main source of aflatoxin with an average concentration of 1036 μg/kg (n = 153), whereas maize was much less contaminated with an average concentration of 2.4 μg/kg (n = 168). In 8% of all prepared food samples, aflatoxins were detected with a mean value of 38 μg/kg for positive samples (maximum = 1317 μg/kg). Those samples predominantly contained aflatoxins B_1 (89%) and B_2 (6%), whereas aflatoxins G_1 and G_2 were much less frequently detected. In 1996, three maize samples were analyzed for fumonisin and zearalenone contamination. All samples contained fumonisins (range = 240−295 μg/kg) but no zearalenone.[19] Otherwise, no comprehensive data on mycotoxin occurrence have been published in the scientific literature to the best of our knowledge, highlighting the need for more research in both countries.

The aim of this study was to screen multiple food and feed matrices for their fungal and bacterial metabolite pattern and to quantitate their respective concentrations using a highly sensitive and selective LC-MS/MS multimycotoxin method. By this, information on the occurrence and concentration ranges was gained to serve food safety initiatives in Burkina Faso and Mozambique. The co-occurrence of mycotoxins was exemplified for six samples for which the exact quantities of each toxin were listed.

MATERIALS AND METHODS

Chemicals and Reagents. Methanol (LC gradient grade) and glacial acetic acid (p.a.) were purchased from Merck (Darmstadt, Germany), acetonitrile (LC gradient grade) was from VWR (Leuven, Belgium), and ammonium acetate (MS grade) was from Sigma-Aldrich (Vienna, Austria). Water was purified by an Elga Purelab ultra analytic system (Veolia Water, Bucks, UK). Standards of fungal and bacterial metabolites were obtained either as gifts from research groups or from commercial sources: Romerlabs (Tulln, Austria), Sigma-Aldrich (Vienna, Austria), Iris Biotech GmbH (Marktredwitz, Germany), Axxora Europe (Lausanne, Switzerland), BioAustralis (Smithfield, Australia), BioViotica (Göttingen, Germany), and LGC Promochem GmbH (Wesel, Germany). Stock solutions of each analyte were prepared by dissolving the solid substance in acetonitrile, methanol, water, or mixtures of those (1:1, v/v). Combined working solutions were prepared prior to the spiking experiments by mixing the stock solutions of the corresponding analytes, followed by a further dilution in appropriate solvent. All solutions were stored at −20 °C and allowed to reach room temperature before use.

Samples. In total, 122 samples were collected from different locations in Burkina Faso (n = 69) and Mozambique (n = 53) (see the Supporting Information). The samples obtained in Burkina Faso were taken from various local markets, cereal traders, food/feed-processing enterprises, grain mills, and small-scale farmers in the cities of Ouagadougou, Bobo Dioulasso, and Boromo as well as in surrounding villages. In Mozambique, samples were received from the same branches/origins in the local capital and rural villages in Nampula province, northern Mozambique. The sampled locations, matrices, and numbers of sample types were uneven and based on availability and the needs and requests of the locals. Main matrices were maize (Burkina Faso n = 26; Mozambique n = 13), groundnuts (Burkina Faso n = 9; Mozambique n = 23), and feed (Burkina Faso n = 4; Mozambique n = 10). Additional matrices were tested to a lesser extent and are referred to as "others" (Burkina Faso n = 30; Mozambique n = 7): other grains [sorghum (*Sorghum* ssp.; 7/0), millet (*Pennisetum glaucum*; 3/2), rice (*Oryza* ssp.; 3/0), sesame (*Sesame indicum*; 2/0), and wheat (*Triticum* spp.; 1/0)]; grain-based processed foods [infant food formulations (3/0), mixed cuscus (3/0), cornflakes (2/0), and cookies (2/0)]; soy (*Glycine max*; 0/3); dried fruits (4/0), and waste product from feed production (0/2), which was intended for disposal. Feed producers claimed that those samples will be discharged, but it cannot be excluded that the samples might find their way back as feed, for example, for chickens located next to the production facilities. Three samples with known high mycotoxin contamination levels were delivered by interested locals who had analyzed the samples previously, using less sophisticated methods such as thin layer chromatography. Samples are referred to as "suspected samples" (two rice samples from Burkina Faso and one groundnut sample from Mozambique) and were not included for average calculations. All samples originated in the country of sampling except the two suspected rice samples mentioned above, which were assumed to come from Asia. Some feed samples were not typical mixed-feed formulations, but consisted of only maize or groundnuts that were obviously of bad quality and, therefore, intended for animal feeding. The identities of villages, markets, vendors, and commercial manufacturers of foods and feeds are not disclosed due to the confidential nature of this information.

Samples were collected during April 2010 (Burkina Faso) and May 2010 (Mozambique) and taken as a bulk sample (500−1000 g), which was prepared from at least four subsamples of equal weight. The subsamples were obtained from random points in storage bags, farmer's basin, or other storage containers and mixed to form the bulk. In some cases, wrapped processed food was sampled. A representative (200 g) aliquot of each bulk sample was shipped to Austria at ambient temperature for quantitative measurements of multimycotoxin contamination by LC-MS/MS. Samples were stored at 4 °C in the African countries and at −20 °C in Austria until analysis.

Sample Preparation, LC-MS/MS Parameters, and Estimation of Matrix Effects. The representative, homogenized samples were weighed into a 50 mL polypropylene tube and covered by extraction solvent consisting of acetonitrile/water/acetic acid (79:20:1, v/v/v) in a ratio of 4 mL solvent/g sample. For spiking experiments 0.25 g and for all other experiments 5 g of sample were applied for extraction. Samples were extracted for 90 min on a rotary shaker at 180 rpm, diluted with an equal volume of dilution solvent (acetonitrile/water/acetic acid 20:79:1, v/v/v), injected to the LC-MS/MS system, and analyzed as described in detail elsewhere.[20,21]

In brief, a QTrap 4000 LC-MS/MS system (Applied Biosystems, Foster City, CA) equipped with a TurboIonSpray electrospray ionization (ESI) source and an 1100 series HPLC system (Agilent, Waldbronn, Germany) was used for the LC-MS/MS analysis. Chromatographic separation was carried out at 25 °C on a Gemini C_{18} column, 150 × 4.6 mm, 5 μm particle size, equipped with a C_{18} 4 × 3 mm guard cartridge, all from Phenomenex (Torrance, CA, USA). The chromatographic method and chromatographic and mass spectrometric parameters for 186 of the investigated analytes were as described in the literature.[22] ESI-MS/MS was done in the time-scheduled selected reaction monitoring (SRM) mode, in both positive and negative polarities in two separate chromatographic runs per sample by scanning two fragmentation reactions per analyte. Confirmation of positive analyte identification was obtained by the acquisition of two MRMs per analyte, which yielded 4.0 identification points according to Commission Decision 2002/657/EC.[23] In addition, the LC retention time and the intensity ratio of the two MRM transitions agreed with the related values of an authentic standard within 0.1 min and 30% relative abundance, respectively.

The method applied in this study was extended by integration of further analytes into the existing methodology to cover a total of 207 fungal and 40 bacterial metabolites. This updated method has proven its value in several studies and has recently been validated for several food matrices (unpublished results). However, to obtain the

Table 1. Method Performance Characteristics for 63 Metabolites in Maize, Groundnut, Sorghum, and Feed

				recovery[a]			
no.	analyte	spiking level (μg/kg)	LOD[b] (μg/kg)	maize	groundnut	sorghum[c]	feed
1	aflatoxin B_1	34	3.0	0.33 ± 0.04	0.58 ± 0.08	0.36	0.72 ± 0.04
2	aflatoxin B_2	34	6.0	0.36 ± 0.03[d]	0.47 ± 0.03	0.48	0.80 ± 0.10
3	aflatoxin G_1	34	8.0	0.32 ± 0.06	0.52 ± 0.22	0.27	0.61 ± 0.03
4	aflatoxin G_2	34	8.0	0.42 ± 0.18	0.69 ± 0.02	0.26	0.60 ± 0.04
5	aflatoxin M_1	82	4.0	0.72 ± 0.05	0.68 ± 0.06	0.54	0.81 ± 0.02
6	fumonisin B_1 (FB_1)	670	20	0.72 ± 0.08	0.65 ± 0.04	0.80	0.51 ± 0.02
7	fumonisin B_2	670	10	0.77 ± 0.04	0.80 ± 0.06	0.89	0.52 ± 0.02
8	fumonisin B_3	88	20	0.94 ± 0.04[d]	0.75 ± 0.06	1.04	0.52 ± 0.02
9	fumonisin B_4		nd[e]	nd[e]	nd[e]	nd[e]	nd[e]
10	hydrolyzed FB_1	255	0.8	0.96 ± 0.13	1.06 ± 0.16	1.03	1.14 ± 0.06
11	ochratoxin A	144	5.0	0.92 ± 0.08	1.00 ± 0.12	0.95	0.91 ± 0.04
12	ochratoxin B	29	5.0	0.84 ± 0.08[d]	0.78 ± 0.02	0.61	0.96 ± 0.07
13	deoxynivalenol	179	20	0.75 ± 0.04[d]	0.93 ± 0.07	0.74	0.92 ± 0.01
14	DON-glucoside	120	10	1.05 ± 0.21[d]	0.74 ± 0.18	1.01	0.38 ± 0.02
15	nivalenol	179	20	0.95 ± 0.02[d]	1.15 ± 0.01	1.05	0.74 ± 0.03
16	xearalenone	181	10	0.83 ± 0.05[d]	0.94 ± 0.05	0.44	0.78 ± 0.02
17	moniliformin	446	40	0.94 ± 0.01	1.91 ± 0.10	1.09	0.79 ± 0.07
18	3-nitropropionic acid	559	80	0.31 ± 0.01	0.47 ± 0.09	0.35	0.70 ± 0.06
19	cyclopiazonic acid	447	200	0.72 ± 0.06	0.94 ± 0.04	0.89	nd
20	citrinin	266	250	0.34 ± 0.11	0.38 ± 0.04	0.24	nd
21	alternariol (AOH)	89	5.0	0.71 ± 0.07	0.82 ± 0.09	0.91	0.78 ± 0.01
22	AOH methyl ether	90	8.0	0.93 ± 0.06	0.66 ± 0.15	0.84	0.83 ± 0.04
23	altertoxin I	1120	3.0	0.58 ± 0.05	1.03 ± 0.01	0.51	nd
24	altersolanol	410	80	3.09 ± 0.59	2.86 ± 0.17	2.03	nd
25	altenusin	464	80	nd	nd	0.68	nd
26	tentoxin	35	0.4	2.75 ± 0.03	2.33 ± 0.24	2.73	1.12 ± 0.14
27	citreoviridin	136	25	1.22 ± 0.07	1.01 ± 0.03	1.07	nd
28	macrosporin A	306	10	2.18 ± 0.16	1.38 ± 0.08	0.77	0.92[c]
29	fusaric acid	547	50	0.89 ± 0.14	0.61 ± 0.01	nd	nd
30	enniatin A	0.30	0.20	0.98 ± 0.55[d]	1.08 ± 0.21	0.80	1.11 ± 0.05
31	enniatin A_1	1.93	0.10	0.79 ± 0.16[d]	0.90 ± 0.08	0.76	0.93 ± 0.08
32	enniatin B	2.03	0.05	0.91 ± 0.09	1.04 ± 0.02	1.12	1.00 ± 0.09
33	enniatin B_1	5.48	0.05	0.82 ± 0.19[d]	1.01 ± 0.06	0.97	0.92 ± 0.06
34	enniatin B_2	2.96	0.20	0.79 ± 0.11	1.00 ± 0.07	0.89	0.91 ± 0.09
35	enniatin B_3	5.1	0.005	0.60 ± 0.02[d]	1.01 ± 0.15	0.85	0.89 ± 0.09
36	beauvericin	5.1	0.05	0.92 ± 0.08	0.99 ± 0.09	0.85	0.76 ± 0.14
37	apicidin	18	0.10	0.96 ± 0.01	1.44 ± 0.09	1.37	0.99 ± 0.03
38	aurofusarin	12	20	0.98 ± 0.22[d]	0.84 ± 0.19	0.97	0.31[c]
39	chlamydosporol	68	15	0.64 ± 0.06[d]	0.61 ± 0.01	0.59	1.02 ± 0.08
40	equisetin	128	10	1.33 ± 0.27	2.42 ± 0.19	1.10	1.75 ± 0.42
41	kojic acid	2000	100	0.95 ± 0.09	0.95 ± 0.04	0.90	0.78 ± 0.02
42	sterigmatocystin (ST)	89	2.0	0.89 ± 0.12	0.86 ± 0.05	0.87	0.97 ± 0.08
43	*O*-methyl-ST	68	2.0	0.51 ± 0.04[d]	0.77 ± 0.04	0.50	0.95 ± 0.01
44	agroclavin	176	5.0	nd	nd	nd	0.43 ± 0.01
45	chanoclavin	7.1	0.14	0.48 ± 0.02[d]	0.47 ± 0.07	0.83	0.96 ± 0.03
46	elymoclavin	5.9	2.0	1.32 ± 0.11[d]	1.38 ± 0.11	nd	0.95 ± 0.02
47	ergometrine	20	0.50	0.90 ± 0.17[d]	1.30 ± 0.02	0.91	0.91 ± 0.05
48	festuclavin	176	0.20	0.66 ± 0.01	0.89 ± 0.06	0.71	0.95 ± 0.02
49	cyclosporin A	230	1.0	2.13 ± 0.17	2.44 ± 0.05	2.23	nd
50	cyclosporin H	406	10	0.59 ± 0.09[d]	1.03 ± 0.03	0.76	0.63 ± 0.01
51	cytochalasin J	254	10	0.66 ± 0.05	0.73 ± 0.06	0.71	0.66[c]
52	curvularin	102	3.0	1.12 ± 0.02	1.68 ± 0.25	1.60	0.58[c]
53	cycloaspeptide A	102	2.0	0.78 ± 0.01	1.01 ± 0.11	1.03	nd
54	emodin	90	10	0.58 ± 0.28	0.64 ± 0.12	0.37	1.04[c]
55	physcion	460	60	1.26 ± 0.32	0.99 ± 0.08	0.78	nd
56	malformin C	266	5.0	1.48 ± 0.19	1.94 ± 0.02	1.43	nd
57	terphenyllin	68	5.0	1.40 ± 0.03	2.17 ± 0.04	0.83	nd
58	calphostin C	32	15	0.86 ± 0.01	0.96 ± 0.15	0.78	nd
59	NG012	266	5.0	1.07 ± 0.11	1.37 ± 0.15	1.23	nd

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Table 1. continued

no.	analyte	spiking level (μg/kg)	LOD[b] (μg/kg)	recovery[a] maize	groundnut	sorghum[c]	feed
60	bacitracin	10000[f]	100[f]	nd	nd	nd	nd
61	anisomycin	35	0.30	0.71 ± 0.01[d]	0.89 ± 0.02	0.98	nd
62	radicicol	53	2.0	2.40 ± 0.12	2.90 ± 0.26	1.90	0.37 ± 0.05
63	valinomycin	25	0.20	0.97 ± 0.02[d]	1.02 ± 0.05	0.89	0.39 ± 0.02

[a]Apparent recovery ± standard deviation calculated from spiking experiments of three different samples; 1.00 is equivalent to an apparent recovery of 100%. [b]LOD, limit of detection [S/N = 3:1] expressed as μg/kg sample. [c]Calculation was restricted to one spiked sample because the others exhibited background concentrations of the related metabolite or to save multimix standard solution (sorghum). [d]Calculation was restricted to two spiked samples. [e]No standard available; estimation of concentration based on response and recovery of fumonisin B_2. [f]LOD was calculated from standard in pure solvent.

Table 2. Occurrence and Concentration of 27 Regulated and Other Mycotoxins in Tested Samples Originating from Burkina Faso (n = 69)

	maize (n = 26)		groundnuts (n = 9)		feed (n = 4)		others (n = 30)	
	F (a)[a]	median (range)[b] (μg/kg)	F (a)[a]	median (range)[b] (μg/kg)	F (a)[a]	median (range)[b] (μg/kg)	F (a)[a]	median (range)[b] (μg/kg)
aflatoxin B_1	13 (50)	23.6 (3.4–636)	2 (22)	10.5 (5.6–15.5)	4 (100)	280 (68.4–557)	4 (14)	11.3 (3.1–19.1)
aflatoxin B_2	4 (15)	9.7 (7.4–46.3)	nd	–[c]	4 (100)	57.5 (28.4–83.6)	nd	–
aflatoxin G_1	7 (27)	20.5 (12.3–56.8)	nd	–	4 (100)	260 (12.9–328)	nd	–
aflatoxin G_2	1 (4)	13.2	nd	–	2 (50)	20.2 (17.8–22.5)	nd	–
aflatoxin M_1	1 (4)	8.1	nd	–	2 (50)	9.0 (6.3–11.6)	nd	–
fumonisin B_1	21 (81)	269 (22.5–1343)	nd	–	3 (75)	3236 (578–3390)	1 (4)	73.8
fumonisin B_2	18 (69)	107 (11.3–589)	nd	–	3 (75)	1225 (186–1235)	1 (4)	28.2
fumonisin B_3	12 (46)	62.5 (23.2–274)	nd	–	3 (75)	310 (70.0–362)	nd	–
ochratoxin A	1 (4)	18.6	nd	–	2 (50)	35.1 (28.0–42.3)	1 (4)	13.8
deoxynivalenol (DON)	1 (4)	31.4	nd	–	nd	–	10 (36)	60.3 (22.3–250)
DON-glucoside	nd	–	nd	–	nd	–	2 (7)	31.7 (23.6–39.7)
nivalenol	nd	–	nd	–	nd	–	1 (4)	40.2
zearalenone	2 (8)	13.4 (11.0–15.8)	nd	–	2 (50)	49.1 (43.9–54.3)	8 (29)	16.5 (12.3–17.0)
moniliformin	2 (8)	719 (413–1025)	nd	–	1 (25)	48	5 (18)	83.6 (70.2–320)
3-nitropropionic acid	7 (27)	330 (161–951)	nd	–	1 (25)	1294	6 (21)	161 (85.6–1629)
cyclopiazonic acid	nd	–	nd	–	2 (50)	247 (224–270)	nd	–
citrinin	3 (12)	1784 (531–5074)	nd	–	1 (25)	341	nd	–
alternariol	3 (12)	6.9 (5.1–16.0)	nd	–	3 (75)	15.1 (5.1–25.4)	nd	–
alternariol methyl ether	1 (4)	18	nd	–	1 (25)	11.1	3 (11)	16.0 (12.4–17.2)
altertoxin I	2 (8)	7.1 (3.4–10.8)	nd	–	3 (75)	10.5 (3.0–13.0)	1 (4)	3.1
enniatin A	nd	–	nd	–	nd	–	6 (21)	0.5 (0.3–1.4)
enniatin A_1	nd	–	nd	–	1 (25%)	0.1	8 (29)	2.9 (0.2–9.1)
enniatin B	nd	–	nd	–	nd	–	8 (29)	6.7 (1.2–16.4)
enniatin B_1	1 (4%)	0.2	nd	–	nd	–	8 (29)	5.7 (0.9–21.4)
enniatin B_2	nd	–	nd	–	nd	–	4 (14)	0.5 (0.2–0.8)
beauvericin	14 (54)	0.5 (0.1–5.9)	1 (11)	0.1	3 (75)	25.4 (4.5–31.7)	16 (57)	0.4 (0.1–47.0)
sterigmatocystin	2 (8)	2.3 (2.2–2.5)	nd	–	3 (75)	6.5 (4.3–40.1)	2 (7)	6.7 (4.8–8.6)

[a]Number of positive samples (percentage in parentheses). [b]Median (range) refers to the median of values >LOD and the range from min to max value. [c]–, not detected, i.e., below LOD.

performance parameters for the main matrices of this study, the apparent recoveries and detection limits were estimated preliminarily by spiking experiments. Each three maize and groundnut samples not contaminated with the major mycotoxins, or contaminated to only a minor extent, were spiked using a multianalyte standard solution at a medium concentration level (Table 1). In the case of low contamination, the initial concentration was subtracted. If the background level was higher than the spiked concentration, the value was excluded from average calculation. For sorghum, only a single sample was spiked to save limited multimycotoxin spiking solution, because the number of samples analyzed was significantly lower than for maize or groundnuts. For feed samples, apparent recoveries determined in a similar study[10] were used for correction of results. The spiked samples were stored overnight at room temperature to allow evaporation of the solvent and to establish equilibrium between the analytes and the sample. Extraction, dilution, and analysis were performed as described above. The corresponding peak areas of the spiked samples were used for estimation of the apparent recovery by comparison to a standard of the same concentration prepared by dilution in pure solvent:

$$R_A\ (\%) = 100 \times \text{peak area}_{\text{spiked samples}} / \text{peak area}_{\text{liquid standards}} \quad (1)$$

As a consequence of this preliminary estimation of matrix effects, results were corrected by the calculated correction factor for maize, groundnuts, sorghum, and feed. Other matrices were not corrected. Limit of detection (LOD) values were calculated according to the

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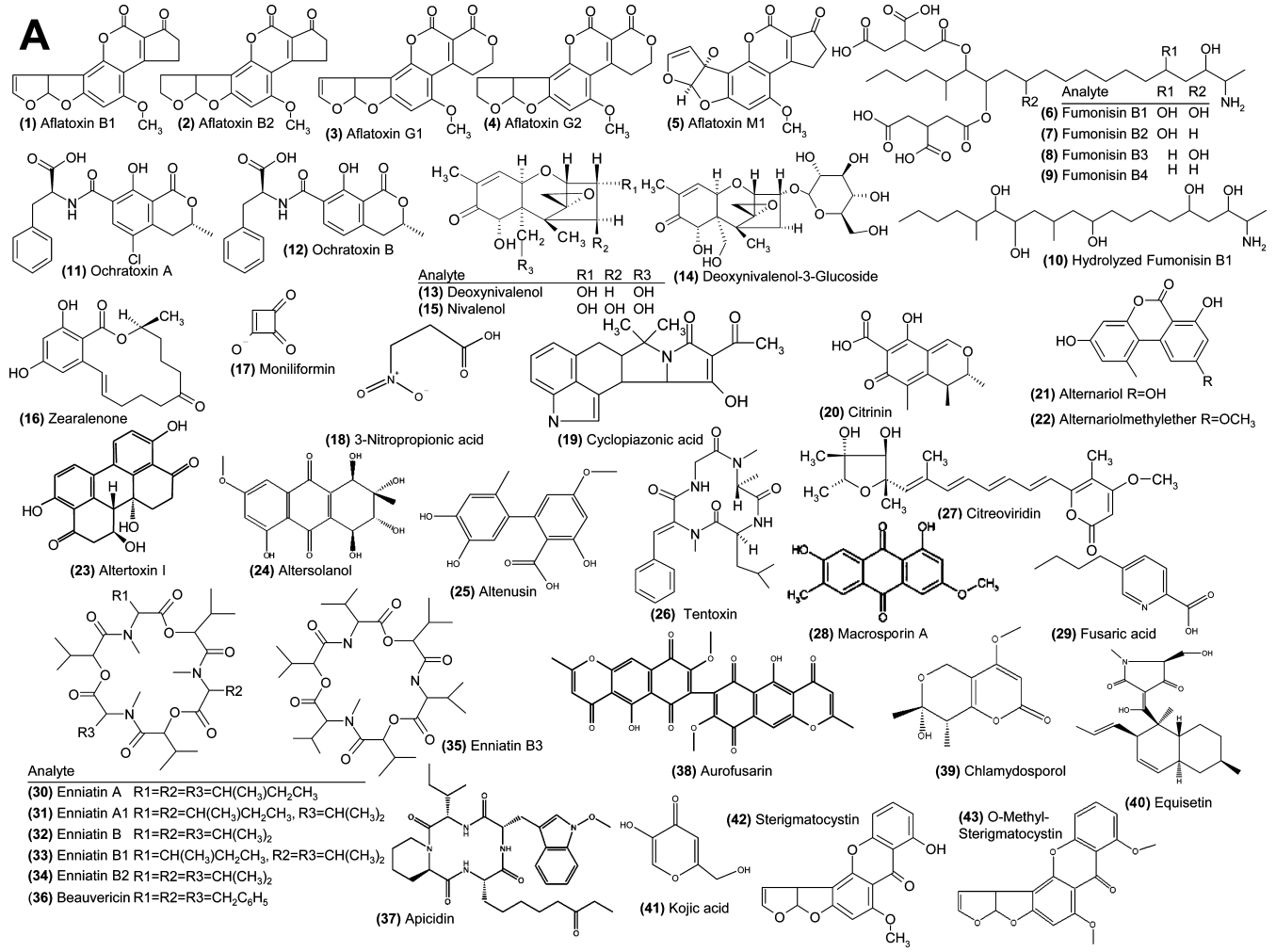

Figure 1. continued

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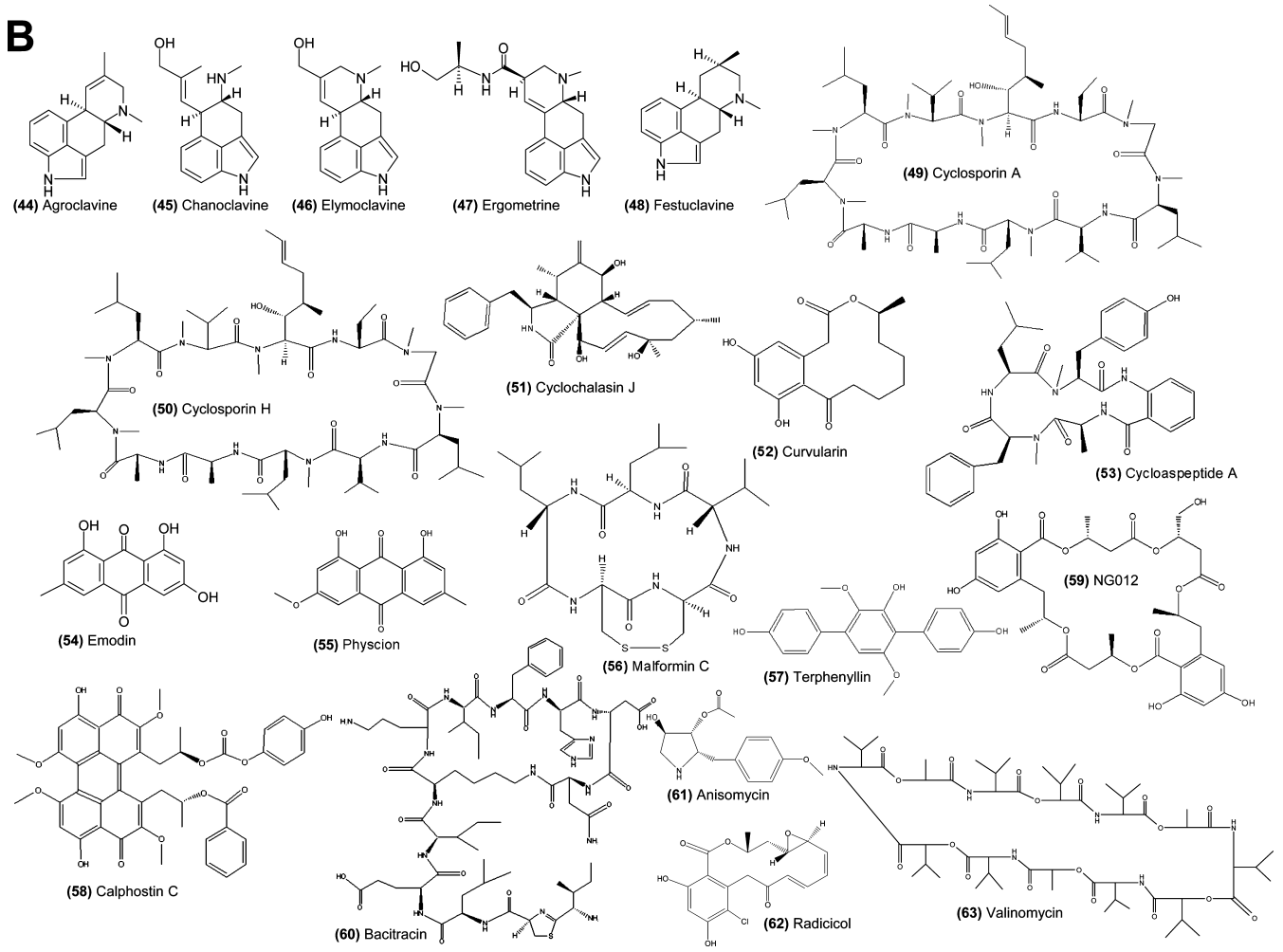

Figure 1. Structures of the 60 fungal and 3 bacterial metabolites that were detected in the investigated samples obtained from Burkina Faso and Mozambique: (A) metabolites 1−43; (B) metabolites 44−63.

software tool of Analyst (version 1.5, AB Sciex), which was based on a signal-to-noise ratio of 3:1 in the spiked samples.

■ RESULTS AND DISCUSSION

Analytical Method Quality Assurance. The performance characteristics of the analytical method established from spiked blank samples are presented in Table 1. The limits of detection (LOD) ranged from 0.005 to 250 μg/kg for enniatin B_3 and citrinin, respectively. Apparent recoveries were low, particularly for aflatoxins and fumonisins. This agrees with our former studies as fumonisins are not quantitatively extracted by the extraction solvent used, which was optimized as a compromise between polar, apolar, acidic, and basic components. Also, the reduced recoveries of aflatoxins, especially in maize, had been observed in former studies because they are particularly prone to matrix effects during the electrospray ionization process.[20,21] However, low relative standard deviations (RSD) of apparent recoveries were calculated for aflatoxins and fumonisins, which were used to adjust the results for the combined effects of extraction efficiency and matrix effects. Overall, the RSD exceeded 10% for about one-third of analytes. This is acceptable in view of the fact that three different samples from a complex matrix have been used in spiking experiments. The quality of the method and the accuracy of quantitation were also ensured by continuous participation in ring trials determining major mycotoxins in different food and feed matrices.

Occurrence of Regulated and Other Mycotoxins in Burkina Faso. *Aflatoxins.* Occurrence data of regulated and other important mycotoxins are reported in Table 2. Because no mycotoxin regulations are in place in Burkina Faso, the limits permitted in the European Union are used as a basis for discussion. Mycotoxins that are regarded as relevant by means of toxicity and occurrence but not regulated yet are merged into a group called "other mycotoxins". Overall, 2 bacterial (anisomycin and valinomycin) and 52 fungal metabolites (see Figure 1) including common mycotoxins were detected in the 69 samples from Burkina Faso. Aflatoxins were detected in 23 samples (33%). In maize intended for human consumption (n = 26), half (50%) was contaminated with aflatoxin B_1 at concentration levels ranging up to 636 μg/kg. Other aflatoxins occurred less frequently, as expected, with aflatoxin G_1 being the next most abundant, positive in 27% of maize samples. With regard to maximum tolerable levels/limits (MTL) established by the European Commission,[6] all 13 samples (50%) in which aflatoxins were detected exceeded the MTL of 4 μg/kg, which was implemented for total aflatoxin concentration. In contrast, only one of nine groundnut samples exceeded the MTL of 10 μg/kg, with three samples being positive for aflatoxin B_1 but none of the other aflatoxins. This is in contrast to recent studies

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Table 3. Occurrence and Concentration of 27 Regulated and Other Mycotoxins in Tested Samples Originating from Mozambique (n = 53)

	maize (n = 13)		groundnuts (n = 23)		feed (n = 10)		others (n = 7)	
	F (a)[a]	median (range)[b] (μg/kg)	F (a)[a]	median (range)[b] (μg/kg)	F (a)[a]	median (range)[b] (μg/kg)	F (a)[a]	median (range)[b] (μg/kg)
aflatoxin B_1	6 (46)	69.9 (16.3−363)	3 (14)	3.4 (3.4−123)	6 (60)	109 (24.0−297)	3 (43)	4.6 (3.8−427)
aflatoxin B_2	4 (31)	15.9 (6.9−31.4)	1 (5)	19.5	5 (50)	24.4 (21.7−29.8)	1 (14)	51.3
aflatoxin G_1	6 (46)	42.7 (19.7−256)	1 (5)	30.3	5 (50)	124 (24.4−236)	1 (14)	382
aflatoxin G_2	4 (31)	25.0 (9.6−40.2)	nd	−[c]	5 (50)	28.9 (8.7−47.8)	1 (14)	48.6
aflatoxin M_1	3 (23)	5.7 (5.6−6.0)	nd	−	4 (40)	7.2 (4.4−9.4)	1 (14)	6.4
fumonisin B_1	12 (92)	869 (159−7615)	nd	−	7 (70)	2124 (810−20579)	3 (43)	3862 (273−45450)
fumonisin B_2	12 (92)	288 (27.7−3061)	nd	−	8 (80)	694 (13.5−7088)	4 (57)	440 (11.5−15254)
fumonisin B_3	11 (85)	93 (26.6−777)	nd	−	7 (70)	197 (94.3−2264)	3 (43)	307 (74.8−5115)
ochratoxin A	nd	−	nd	−	3 (30)	6.9 (5.4−12.4)	1 (14)	5.7
deoxynivalenol (DON)	2 (15)	120 (116−124)	nd	−	5 (50)	464 (99.1−697)	1 (14)	145
DON-glucoside	3 (23)	22.0 (12.6−32.5)	nd	−	3 (30)	33.3 (17.6−84.0)	nd	−
nivalenol	4 (31)	34.1 (20.2−45.9)	nd	−	2 (20)	47.7 (42.7−52.7)	2 (29)	95.0 (76.8−113)
zearalenone	3 (23)	13.8 (10.9−18.1)	nd	−	6 (60)	17.1 (11.2−28.2)	3 (43)	81.2 (78.8−318)
moniliformin	7 (54)	241 (98−1305)	nd	−	8 (80)	135 (61.0−1601)	4 (57)	338 (46.8−1923)
3-nitropropionic acid	6 (46)	1446 (205−3553)	2 (9)	786 (223−1349)	3 (30)	360 (201−6931)	2 (29)	1661 (95.0−3228)
cyclopiazonic acid	1 (8)	606	1 (5)	763	nd	−	1 (14)	789
citrinin	6 (46)	545 (276−5074)	nd	−	4 (40)	332 (306−25487)	1 (14)	7061
alternariol	nd	−	nd	−	1 (10)	5.8	2 (29)	18.2 (8.0−28.4)
alternariol methyl ether	nd	−	nd	−	1 (10)	12.6	3 (43)	24.3 (9.0−44.5)
altertoxin I	nd	−	nd	−	nd	−	1 (14)	10.1
enniatin A	nd	−	nd	−	4 (40)	3.6 (0.6−7.9)	2 (29)	1.1 (0.2−2.0)
enniatin A_1	2 (15)	0.1 (0.1−0.1)	nd	−	4 (40)	22.0 (3.4−43.9)	2 (29)	2.1 (0.2−4.1)
enniatin B	nd	−	nd	−	4 (40)	39.3 (2.2−114)	1 (14)	0.9
enniatin B_1	1 (8)	0.1	1 (5)	0.3	7 (70)	6.0 (0.1−94.4)	1 (14)	4.1
enniatin B_2	nd	−	nd	−	3 (30)	4.8 (0.9−9.1)	nd	−
beauvericin	11 (85)	4.7 (0.1−35.6)	16 (73)	0.5 (0.1−24.0)	10 (100)	12.9 (3.3−418)	7 (100)	11.5 (3.5−486)
sterigmatocystin	1 (8)	2.7	1 (5)	9.7	1 (10)	11	2 (29)	26.1 (3.0−49.2)

[a]Number of positive samples (percentage in parentheses). [b]Median (range) refers to the median of values >LOD and the range from min to max value. [c]−, not detected, i.e., below LOD.

from Nigeria,[11] where aflatoxin B_1 concentrations exceeded the U.S. maximum limit of 20 μg/kg in about 90% of 29 groundnut cake samples (maximum 2820 μg/kg), and from Benin[9], where 15 samples of the same matrix had a total aflatoxin content between 10 and 346 μg/kg.

Likewise, in the "other" food matrices aflatoxin B_1 was found in only one sorghum (16.2 μg/kg) sample and one rice (3.1 μg/kg) sample, and, critically, in two of three infant food formulations (6.3 and 19.1 μg/kg). In view of the rigorous MTL set in place for baby food, which is as low as 0.1 μg/kg, this value was exceeded by approximately factors of 60 and 190, respectively. All four tested feed samples were highly contaminated with all types of aflatoxins. Maximum concentrations for aflatoxin B_1 and aflatoxin G_1 were 557 μg/kg (mean = 296 μg/kg; range = 68−557 μg/kg) and 328 μg/kg (mean = 215 μg/kg; range = 13−328 μg/kg), respectively. These concentrations are in accordance with a recent study from Nigeria for aflatoxin B_1 that reported aflatoxin contamination in 76% of feedstuffs (n = 58) with aflatoxin B_1 concentrations ranging from 6 to 1067 μg/kg (mean = 198 μg/kg), but not for aflatoxin G_1 (mean = 45 μg/kg; range = 8−235 μg/kg), which had lower concentrations in the Nigerian study.[10]

Fumonisins. Fumonisins were detected frequently in the investigated maize samples with mean concentrations of 348 μg/kg (range = 23−1343 μg/kg) for fumonisin B_1 and 128 μg/kg (range = 11−589 μg/kg) and 89 μg/kg (range = 23−274 μg/kg) for fumonisin B_2 and fumonisin B_3, respectively. These concentrations are in agreement with those reported by Nikiema et al.[15] Results confirm the conclusion of the authors that chronic exposure to fumonisins is likely in Burkina Faso. The MTL for total fumonisins (Σ fumonisin B_1 + fumonisin B_2; 1000 μg/kg) in the recent study was exceeded in two maize samples (n = 26; 8%). No fumonisins were found in groundnuts, as expected, and only one "other" food sample (infant food; 102 μg/kg Σ fumonisin B_1 + fumonisin B_2) was contaminated. As the MTL for infant food is 200 μg/kg, this baby food did not exceed the limit. Three maize-based feed samples contained significant levels of fumonisin B_1 (mean = 2401 μg/kg; range = 578−3390 μg/kg), fumonisin B_2 (mean = 882 μg/kg; range = 186−1235 μg/kg), and fumonisin B_3 (mean = 247 μg/kg; range = 70−362 μg/kg). The levels reported here are higher than in feed from Nigeria.[10]

Ochratoxin A, Deoxynivalenol, Zearalenone, and Nivalenol. Ochratoxin A was detected in one maize sample (4%) at a concentration of 18.6 μg/kg. As the MTL for ochratoxin A in unprocessed cereals is 5 μg/kg, this sample exceeded the limit. In an infant food sample (13.8 μg/kg), the MTL of 0.5 μg/kg was exceeded significantly, by a factor of 28. Two feed samples (n = 4) were also contaminated, with a mean concentration of 35.1 μg/kg. Deoxynivalenol, zearalenone, and nivalenol were not frequently encountered, and all samples had concentrations below the MTL: Deoxynivalenol was found in one maize

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sample (13 μg/kg) and some "other" foodstuffs. One sample each of millet (163 μg/kg), sorghum (67 μg/kg), and wheat (250 μg/kg) were contaminated. In addition, low amounts were found in two infant foods (22 and 42 μg/kg) and processed cereal-based foodstuff (31−103 μg/kg). Zearalenone was detected in two maize (11−16 μg/kg), two feed (44−54 μg/kg), and eight "other" food samples (12−17 μg/kg). Seven of those were sorghum/millet (n = 10; 70%; range = 16−17 μg/kg); the other sample was a grain-based cookie (12 μg/kg). Nivalenol occurred only in a single rice sample (40 μg/kg).

Other Mycotoxins. Other important toxins detected in samples obtained from Burkina Faso include moniliformin, 3-nitropropionic acid, cyclopiazonic acid, citrinin, *Alternaria* metabolites, enniatins, and beauvericin. The exact occurrence data and concentration ranges are displayed in Table 2. Remarkably, high concentrations of moniliformin (maximum 1025 μg/kg) and 3-nitropropionic acid (maximum 951 μg/kg) were detected in maize intended for human consumption. Furthermore, the nephrotoxic and carcinogenic citrinin, which leads to synergistic effects with ochratoxin A,[24] was found in three maize samples with a very high maximum concentration of 5074 μg/kg. Alternariol, its methyl ether, and altertoxin I were present in low quantities and concentrations in all matrices except groundnuts. Enniatins were frequently found in grain-based processed foods containing wheat, with highest concentrations for enniatin B_1 (21 μg/kg). However, concentrations were much lower than in other surveys conducted mainly in northern Europe and the Mediterranean.[25] Beauvericin was an abundant metabolite in maize, processed foods, and feed and was also found in one groundnut sample. The highest concentration was determined in a millet sample at a concentration of 47 μg/kg. This is, however, much lower than the beauvericin levels determined in cereals from Spain, ranging from 510 to 1178 μg/kg.[26]

Occurrence of Regulated and Other Mycotoxins in Mozambique. *Aflatoxins.* Occurrence data on regulated and emerging toxins in Mozambique are reported in Table 3; in total, 3 bacterial (bacitracin, anisomycin, and valinomycin) and 55 fungal metabolites including many mycotoxins were detected in the 53 samples. Aflatoxins occurred frequently in maize, with aflatoxin B_1 and aflatoxin G_1 being present in 46% of samples (n = 13). Average values were higher in Mozambique than in Burkina Faso (aflatoxin B_1, 114 vs 75 μg/kg; aflatoxin G_1, 73 vs 30 μg/kg). Interestingly, aflatoxin G_1 had a greater contribution to total aflatoxin contamination in Mozambique. The European MTL was significantly exceeded for all positive maize samples. For groundnuts the European MTL, which is equal to the one established in Mozambique (10 μg/kg), was exceeded for two samples only (n = 23; 9%), one of which was delivered suspected to be heavily contaminated. However, both samples were seriously contaminated, one with a total aflatoxin concentration of 173 μg/kg (123 μg/kg aflatoxin B_1, 20 μg/kg aflatoxin B_2, 30 μg/kg aflatoxin G_1) and the other at 643 μg/kg (85 μg/kg aflatoxin B_1, 18 μg/kg aflatoxin B_2, 467 μg/kg aflatoxin G_1, 73 μg/kg aflatoxin G_2). The high prevalence and concentrations of aflatoxins in maize as well as its minor prominence in groundnuts are contradictory to those found about 40 years ago.[18] It is well-known that the prevalence of aflatoxin varies in time and space.[3,4]

Also, we found much higher contributions of aflatoxins G_1 and G_2 to overall aflatoxin concentrations than the other study. This is typical for infection by *Aspergillus parasiticus* or strain S_{BG} belonging to an unnamed *Aspergillus* taxon[27] instead of *Aspergillus flavus*. Of the three "other" samples contaminated with aflatoxins, one was millet (4 μg/kg) and the other two were defined as waste products from feed production (5 and 861 μg/kg $\sum$ AFs). In contrast to maize and groundnut samples, average aflatoxin concentrations in feed were below those in Burkina Faso (aflatoxin B_1, 129 vs 296 μg/kg), but 60% still exceeded the national MTL considerably.

Fumonisins. Fumonisins were detected in 12 of 13 maize samples (92%) intended for human consumption with maximum concentrations of 7615 μg/kg (fumonisin B_1), 3061 μg/kg (fumonisin B_2), 777 μg/kg (fumonisin B_3), and 570 μg/kg (fumonisin B_4), resulting in $\sum$ fumonisins of 12024 μg/kg. Seven samples (54%) exceeded the European MTL, and the observed incidence as well as concentrations was higher than in Burkina Faso. Likewise, concentrations in feed (average $\sum$ fumonisin B_1 + fumonisin B_2 = 6625 μg/kg, range = 823−27667 μg/kg) and "other" matrices were elevated. Especially one feed waste product had exceptionally high concentrations, resulting in $\sum$ fumonisin B_1 + fumonisin B_2 of 60704 μg/kg (the same that contained 861 μg/kg $\sum$ aflatoxins). In addition, one of the two millet samples clearly exceeded the MTL with $\sum$ fumonisin B_1 + fumonisin B_2 = 4634 μg/kg.

Ochratoxin A, Deoxynivalenol, Zearalenone, and Nivalenol. Ochratoxin A was detected in neither maize nor groundnuts. One soy sample showed low contamination (5.7 μg/kg) as did three feeds (average = 6.9 μg/kg), suggesting a general low exposure. The zearalenone concentration exceeded the MTL in a millet sample (318 μg/kg; MTL = 100 μg/kg), which was additionally contaminated by nivalenol (113 μg/kg). Zearalenone was also found in the second millet sample to a minor extent (81 μg/kg), in one waste product (79 μg/kg), in maize (23%, average = 14 μg/kg, range = 11−18 μg/kg), and in feed (60%, average = 18 μg/kg, range = 11−28 μg/kg). Besides the mentioned millet sample, nivalenol occurred in low concentrations in maize (31%, average = 34 μg/kg, range = 20−46 μg/kg), feed (20%, average = 48 μg/kg, range = 43−53 μg/kg) and in one waste sample (77 μg/kg). Deoxynivalenol was not of great importance in Mozambique, with limited incidences and quite low concentrations in maize (15%, average = 120 μg/kg), feed (50%, average = 373 μg/kg, range = 99−697 μg/kg) and one waste sample (145 μg/kg).

Other Mycotoxins. Other toxins were detected in high numbers and concentrations in samples from Mozambique. Moniliformin, 3-nitropropionic acid, and citrinin were detected in about half of the maize samples at concentrations as high as 5074 μg/kg in the case of citrinin. Furthermore, they were found in feed and waste samples. Moniliformin (601 μg/kg) and 3-nitropropionic acid (95 μg/kg) were also present in the millet sample mentioned above. In contrast to Burkina Faso, 3-nitropropionic acid (average = 786 μg/kg, range = 223−1349 μg/kg) and cyclopiazonic acid (763 μg/kg) were also present in groundnut, albeit in low concentrations. *Alternaria* toxins were less frequently observed than in Burkina Faso, particularly in millet, feed, and feed waste. Enniatins were predominantly found in feed, with a maximum concentration of 114 μg/kg enniatin B. Concentrations were higher than those observed in Burkina Faso samples but still below those reported by Santini and co-workers.[25] Beauvericin was ubiquitous in all matrices with a 100% incidence in feed and "others" and at 85 and 73% in maize and groundnuts, respectively. The highest concentration of 486 μg/kg was determined in a waste sample.

Occurrence of Rare Fungal and Bacterial Metabolites and Toxins in Burkina Faso and Mozambique. Besides the

27 more prominent mycotoxins reported above and in Tables 2 and 3, also 33 other less known fungal and 3 bacterial toxins and metabolites occurred frequently in the samples from Burkina Faso and Mozambique (see Supporting Information). *Fusarium* metabolites such as fusaric acid, fumonisin B_4, hydrolyzed fumonisin B_1, or aurofusarin typically occurred corresponding to main *Fusarium* toxins fumonisin B_1 and fumonisin B_2. Likewise, kojic acid and curvularin are markers for *Aspergillus* and *Penicillium* contamination, respectively. Maximum concentrations of those metabolites were 34286 and 1708 μg/kg, respectively. Another mycotoxin produced by *Penicillium*, citreoviridin, was quantified in maize-based food and feed samples with a maximum concentration of 554 μg/kg. This is critical in view of a new hypothesis indicating that this toxin may initiate an endemic cardiomyopathy called Keshan disease.[28]

Eleven of the analytes discussed in this section were detected in a maximum of three samples. The respective maximum concentrations and matrices are as follows: elymoclavin and agroclavine (59 and 69 μg/kg; infant food), altersolanol (18 μg/kg, millet), altenusine (258 μg/kg, millet), calphostin C (18 μg/kg, millet), ochratoxin B (12 μg/kg, feed), enniatin B_3 (0.03 μg/kg, waste product), cycloaspeptide A (14 μg/kg, feed), NG012 (14 μg/kg, maize), anisomycin (3 μg/kg, millet), and bacitracin (60082 μg/kg, waste product). The extremely high concentration of bacitracin is remarkable as this metabolite produced by *Bacillus subtilis* is an antibiotic that is suspected to cause clinically relevant allergic reactions and near-fatal anaphylaxis.[29] To verify this uncommon finding (presence of bacitracin), a product ion scan was additionally performed in the EPI mode.

Examples of Exposure to Multimycotoxin Cocktails. The strength of the multimycotoxin method applied in this study is to investigate exposure to cocktails of different, potentially synergistically acting, toxins. By this approach the real burden of fungal toxins can be quantitated easily and help to estimate food quality as well as potential health issues. Six examples of severe coexposure to multiple mycotoxins, three each from Burkina Faso (Figure 2) and Mozambique (Figure 3), are given in the following section:

Maize from a Large-Scale Trader in Burkina Faso. The sample was taken in a big commercial storehouse with good management and advanced sanitary level. LC-MS/MS analysis revealed the presence of 19 mycotoxins in detectable amounts (numbers in parentheses give the concentration in μg/kg in the rest of the Results and Discussion): aflatoxin B_1 (636), aflatoxin B_2 (46), aflatoxin M_1 (8), fumonisin B_1 (1167), fumonisin B_2 (244), fumonisin B_3 (274), fumonisin B_4 (57), kojic acid (19328), *O*-methylsterigmatocystin (17), citreoviridin (554), chanoclavin (0.2), 3-nitropropionic acid (951), emodin (76), macrosporin A (98), radicicol (25), alternariol (16), alternariol methyl ether (18), altertoxin I (3), and NG012 (14). Aflatoxins and fumonisins exceeded the MTLs of 4 and 1000 μg/kg, respectively. Concentrations of all analytes are displayed in Figure 2A.

Infant Food from Burkina Faso. The sample was a commercially distributed instant cereal-based baby food that needs to be prepared only by the addition of boiling water. The producer declared that it consists of several ingredients such as milk powder and various grains. Ten mycotoxins were determined simultaneously (Figure 2B): aflatoxin B_1 (19), fumonisin B_1 (74), fumonisin B_2 (28), ochratoxin A (14), beauvericin (0.2), kojic acid (1366), 3-nitropropionic acid (86),

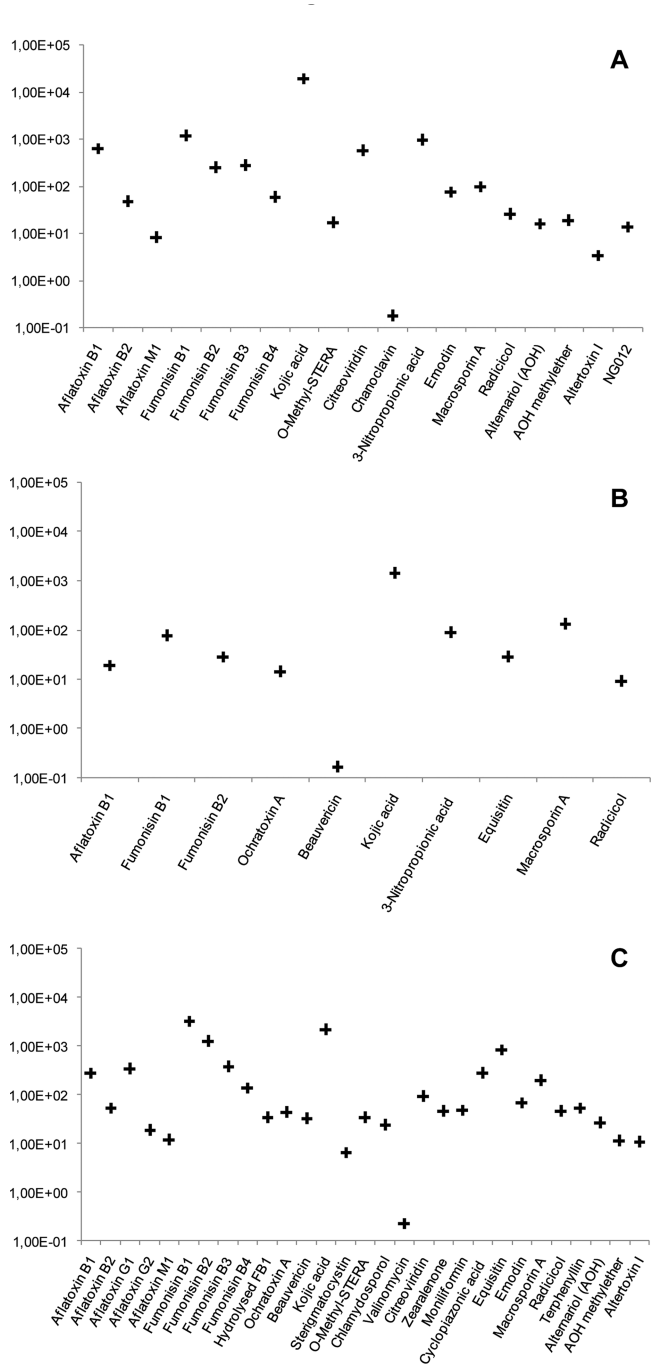

Figure 2. Mycotoxin cocontamination in selected samples obtained from Burkina Faso: (A) maize; (B) infant food; (C) feed. Concentrations are given in micrograms per kilogram.

equisetin (28), macrosporin A (127), and radicicol (9). This sample vastly exceeded the rigorous limits set for infant food by factors of 190 for aflatoxins (MTL = 0.1 μg/kg) and 28 for ochratoxin A (MTL = 0.5 μg/kg).

Feed from a Commercial Maize Mill in Burkina Faso. This feed sample originated from a maize mill where the husks were separated and used for feeding purpose. In all, 29 different analytes were found (Figure 2C): aflatoxin B_1 (275), aflatoxin B_2 (53), aflatoxin G_1 (328), aflatoxin G_2 (18), aflatoxin M_1 (12), fumonisin B_1 (3235), fumonisin B_2 (1224), fumonisin B_3 (362), fumonisin B_4 (138), hydrolyzed fumonisin B_1 (33), ochratoxin A (42), beauvericin (32), kojic acid (2067),

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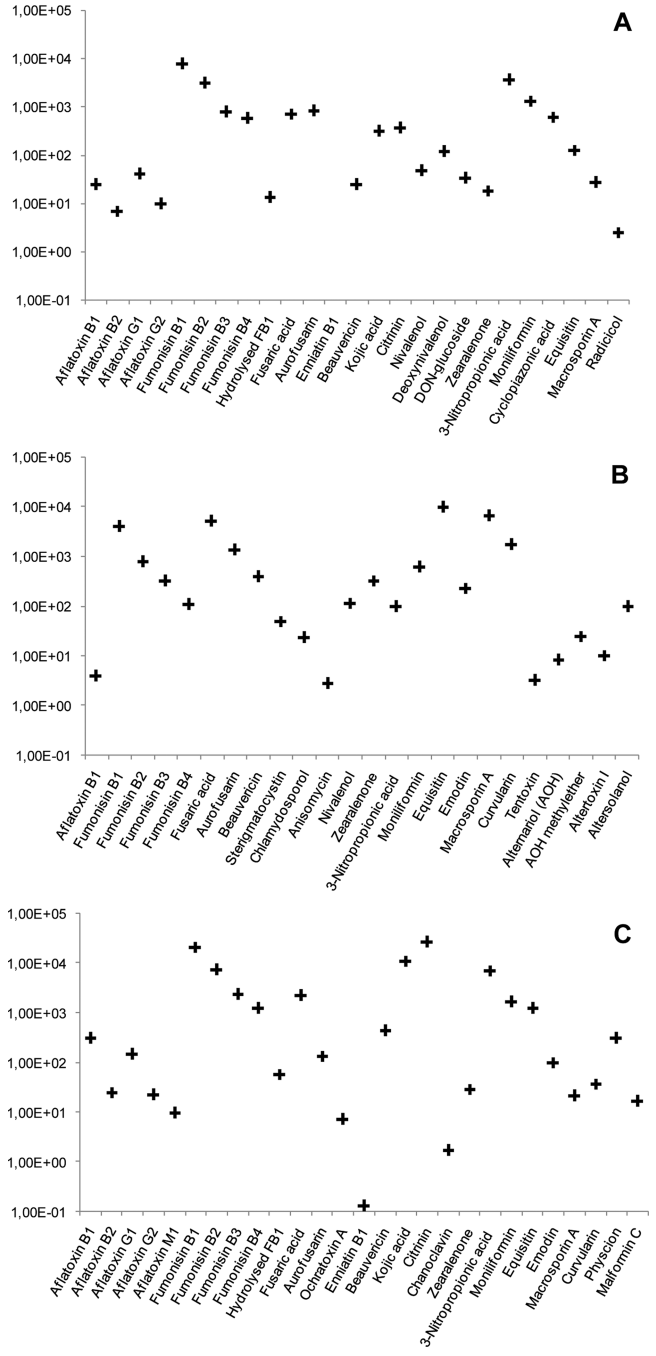

Figure 3. Mycotoxin cocontamination in selected samples obtained from Mozambique: (A) maize; (B) millet; (C) feed. Concentrations are given in micrograms per kilogram.

sterigmatocystin (6), *O*-methylsterigmatocystin (33), citreoviridin (91), chlamydosporol (23), valinomycin (0.2), zearalenone (44), moniliformin (48), cyclopiazonic acid (270), equisetin (829), emodin (68), macrosporin A (190), radicicol (44), terphenyllin (53), alternariol (25), alternariol methyl ether (11), and altertoxin I (11). On the basis of the study by Pedrosa and Borutova,[30] it can be concluded that many of the detected mycotoxins are likely to act in an additive or synergistic way, causing adverse effects on animals.

Maize from a Local Market in Mozambique. This sample was purchased at a local market in Nampula city. The seller declared that this maize was of low grade and consequently cheaper than another maize that was also sampled and contained only nine mycotoxins, in contrast to the bad-quality maize (Figure 3A): aflatoxin B_1 (24), aflatoxin B_2 (7), aflatoxin G_1 (40), aflatoxin G_2 (10), fumonisin B_1 (7615), fumonisin B_2 (3061), fumonisin B_3 (777), fumonisin B_4 (570), hydrolyzed fumonisin B_1 (13), fusaric acid (710), aurofusarin (803), enniatin B_1 (0.06), beauvericin (24), kojic acid (308), citrinin (353), nivalenol (46), deoxynivalenol (116), deoxynivalenol-glucoside (32), zearalenone (18), 3-nitropropionic acid (3553), moniliformin (1304), cyclopiazonic acid (606), equisetin (125), macrosporin A (26), and radicicol (2.5). Aflatoxins and fumonisins exceeded the MTLs of 4 and 1000 μg/kg, whereas deoxynivalenol and zearalenone were quantitated below their regulatory limits.

Millet from a Subsistence Farmer in Mozambique. The millet sample was harvested in a small rural village in Nampula province in 2009 and stored for several months. The 24 analytes aflatoxin B_1 (3.8), fumonisin B_1 (3862), fumonisin B_2 (772), fumonisin B_3 (307), fumonisin B_4 (105), fusaric acid (5049), aurofusarin (1317), beauvericin (383), sterigmatocystin (49), chlamydosporol (23), anisomycin (3), nivalenol (113), zearalenone (318), 3-nitropropionic acid (95), moniliformin (601), equisetin (9382), emodin (225), macrosporin A (6460), curvularin (1708), tentoxin (3), alternariol (8), alternariol methyl ether (24), altertoxin I (10), and altersolanol (96) have been determined in the sample (Figure 3B), with aflatoxin B_1, fumonisins, and zearalenone exceeding the MTLs. Also, the high concentrations of *Fusarium* metabolites aurofusarin, beauvericin, equisetin, macrosporin A, and curvularin and the four *Alternaria* metabolites are notable.

Feed from a Commercial Feed Producer in Mozambique. This sample was maize of bad quality intended as a feeding material for poultry. A total of 28 mycotoxins were determined (Figure 3C): aflatoxin B_1 (297), aflatoxin B_2 (24), aflatoxin G_1 (140), aflatoxin G_2 (22), aflatoxin M_1 (9), fumonisin B_1 (20579), fumonisin B_2 (7088), fumonisin B_3 (2264), fumonisin B_4 (1191), hydrolyzed fumonisin B_1 (56), fusaric acid (2169), aurofusarin (132), ochratoxin A (7), enniatin B_1 (0.13), beauvericin (418), kojic acid (10,672), citrinin (25,486), chanoclavin (1.6), zearalenone (28), 3-nitropropionic acid (6931), moniliformin (1601), equisitin (1208), emodin (98), macrosporin A (21), curvularin (36), physcion (302), and malformin-C (16). Besides the high levels of aflatoxins and fumonisins, the co-occurrence of ochratoxin A with the exceptionally high concentration of citrinin should be highlighted as a synergistic effect was described.[24,30]

Potential Health Risks for Consumers. As many of the analyzed food items contained significant amounts of various mycotoxins, and most persons in rural areas consume the tested cereals as staple food, a high average exposure to a multitude of mycotoxins is likely. Maize was the food matrix exhibiting highest contamination levels of various mycotoxins in both countries. Besides the aflatoxins and fumonisins, other potent mycotoxins such as ochratoxin A, deoxynivalenol, zearalenone, nivalenol, moniliformin, 3-nitropropionic acid, citrinin, and citreoviridin were detected in maize. Additionally, it was identified as a main source of dietary mycotoxin exposure because MTL values were exceeded as follows: aflatoxins (50% Burkina Faso, 46% Mozambique), fumonisins (8% Burkina Faso, 54% Mozambique), ochratoxin A (4% Burkina Faso). Neither deoxynivalenol nor zearalenone exceeded the limits in maize. Interestingly, and in contrast to other studies,[9,18] groundnuts showed less and lower contamination with

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aflatoxins as well as other toxins. The aflatoxin MTL was exceeded for one randomly collected groundnut sample each in Burkina Faso and Mozambique (11% Burkina Faso, 5% Mozambique). None of the other mycotoxins exceeded the limit in groundnuts.

In conclusion, a comprehensive screening for regulated as well as other mycotoxins was conducted in various food and feed matrices from Burkina Faso and Mozambique. The power of the applied LC-MS/MS method revealed simultaneous contamination of up to 28 mycotoxins within a single sample. This emphasizes the need to routinely analyze also mycotoxins that are not addressed by regulations to get a comprehensive picture of the pattern of toxic fungal metabolites. Further studies on greater sample numbers of the single matrices are encouraged as this study was rather intended as a pilot screening survey and not a comprehensive investigation with the aim of exact surveillance mapping.

■ ASSOCIATED CONTENT

Supporting Information

One table presents an overview of the numbers and types of samples investigated; the occurrence and concentrations found for 25 nonregulated and rare fungal and bacterial metabolites from Burkina Faso and Mozambique are provided in two additional tables. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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■ ABBREVIATIONS USED

HCC, hepatocellular carcinoma; FAO, Food and Agriculture Organization; LC-MS/MS, liquid chromatography−tandem mass spectrometry; ESI, electrospray ionization; MRM, multiple-reaction monitoring; LOD, limit of detection; MTL, maximum tolerable limit.

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NOTE ADDED AFTER ASAP PUBLICATION

This paper was published August 27, 2012, with an error to Figure 1. The corrected version was reposted on August 31, 2012.