

Fungal and bacterial metabolites of stored maize (*Zea mays*, L.) from five agro-ecological zones of Nigeria

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Abstract Seventy composite samples of maize grains stored in five agro-ecological zones (AEZs) of Nigeria where maize is predominantly produced were evaluated for the presence of microbial metabolites with the LC-MS/MS technique. The possible relationships between the storage structures and levels of mycotoxin contamination were also evaluated. Sixty-two fungal and four bacterial metabolites were extracted from the grains, 54 of which have not been documented for maize in Nigeria. Aflatoxin B₁ and fumonisin B₁ were quantified in 67.1 and 92.9 % of the grains, while 64.1 and 57.1 % exceeded the European Union Commission maximum acceptable limit (MAL) for aflatoxin B₁ and fumonisins, respectively. The concentration of deoxynivalenol was, however, below the MAL with occurrence levels of 100 and 10 % for its masked metabolite, deoxynivalenol glucoside. The bacterial metabolites had low concentrations and were not a source of

concern. The storage structures significantly correlated positively or negatively ($p < 0.01$ and $p < 0.05$), respectively with the levels of grain contamination. Consumption of maize grains, a staple Nigerian diet, may therefore expose the population to mycotoxin contamination. There is need for an immediate action plan for mycotoxin mitigation in Nigeria, especially in the Derived Savannah zone, in view of the economic and public health importance of the toxins.

Keywords Mycotoxin · Aflatoxin · Microbial metabolite · Maize

Introduction

Maize (*Zea mays*, L., Poaceae family) also known as corn, is the third most traded cereal after wheat and rice with a total production of 745 billion kg in over 1.60 million m² in 2008 (FAOSTAT 2013). Nigeria is the world's 8th largest producer of maize with production increasing from 5,570 kg in 2004 to 9,200 kg in 2012 (FAOSTAT 2013). The consumption pattern of maize-based diets by African adults is about 400 g/person/day, whereas, in the developed world, maize intakes are commonly less than 10 g/person/day (Shephard 2004). Maize can be cultivated in Nigeria for human consumption (78 %), feed and residual uses (17 %), and a small percentage set aside for re-planting (United States Department of Agriculture, 2012). A 4-week nationwide survey conducted in Nigeria between 2001 and 2003 showed that maize is the most widely consumed staple food in the country with an overall percentage frequency of consumption of 20.1 % (Maziya-Dixon et al. 2012).

A number of toxic microbial metabolites abound in agricultural products due to the diversity of fungal and bacterial species that colonise products from field to store. There is ample evidence that the inhabitants of sub-Saharan Africa

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(SSA) are experiencing heavy dietary exposure to mycotoxins particularly aflatoxins and fumonisins (Gong et al. 2002, 2003; Turner et al. 2007). Mycotoxin contamination of foods begins with fungal infestation in the field which is carried into the store, and is further aggravated by poor storage conditions, leading to production of large amounts of toxic metabolites in many parts of SSA. A large number of mycotoxins can also co-occur in maize in SSA (Warth et al. 2012a; Abia et al. 2013). Literature is replete with information on the presence of aflatoxins, fumonisins, trichothecenes and some other *Fusarium* mycotoxins in Nigerian maize (Udoh et al. 2000; Afolabi et al. 2006; Adejumo et al. 2007a, b; Atehnkeng et al. 2008; El-Imam et al. 2012) including ochratoxin A that was only recently isolated from Niger State (Makun et al. 2013), but is lacking concerning other microbial metabolites. However, an array of microbial metabolites has been found in commercial poultry feeds in Nigeria, of which maize is a major constituent (Ezekiel et al. 2012).

In Nigeria, agricultural products are mostly produced by small-scale farmers and the products sold in local markets where “caveat emptor” remains the basic rule (Bandyopadhyay et al. 2007). Since these products rarely enter official channels of sales, surveillance of the level of toxin contamination rarely happens.

This study was conducted to screen maize matrices for fungal and bacterial metabolite pattern in order to quantify the respective concentrations of the stored grains in the various agro-ecological zones of the country, and to establish possible relationships between storage structures and levels of mycotoxin contamination of the grains. Information on the occurrence, distribution and concentration of the contaminated grains will benefit food safety initiatives and enhance necessary interventions by relevant regulatory and health agencies in Nigeria.

Materials and methods

Study area

Surveys were conducted between August 2011 and February 2012 in five out of the seven AEZs of Nigeria where maize is predominantly produced (Adetunji, unpublished, 2013). The zones (Fig. 1) are: Sudan Savanna (Kano and Sokoto States), Northern Guinea Savanna (Kaduna State), Southern Guinea Savanna (Niger State), Derived Savanna (Ondo, Ekiti, Osun, Oyo and Nasarawa States) and Humid Forest (Lagos and Ogun States). The Sahel Savanna and Mid Altitude zones do not produce appreciable quantities of maize. The geographical location, temperature and rainfall pattern of the zones had been documented by previous workers (Udoh et al. 2000; Atehnkeng et al. 2008).

Sampling and sample preparation

Information about farmers who produce and store the grains in large quantities and their locations in the states was collected from the Agricultural Development Programme (ADP) offices of the various states. The modified EU (2002a) method was used for sampling and sample preparation in order to reduce variability. Briefly, the states were divided into ADP zones (based on climatic conditions and types of agricultural produce) which were further subdivided into agricultural blocks (local government) from which two local governments that produce maize in the highest amounts were selected (Table 1). A cell (village or town) that produce maize in the highest amount was selected from each of the two local governments, and maize grains randomly collected from three farmers in each cell.

Shape files of state boundaries, roads and major towns were loaded into Arcview GIS 3.2, where final map embellishments were carried out and sampling locations overlaid on them (Fig. 1). The grains that had been stored for a minimum of 1 month (except for grains in Lagos State which were stored for 2 weeks) were collected from the top, middle and bottom portions of the storage structures in the cells and the grains mixed together to form a composite sample per cell. The compositing of the maize grains was based on their storage structures. Maize grains with the same storage structure were pulled together as a composite sample, otherwise they were treated as a separate batch. The number of samples per state was not even, but depended on the number of ADP zones in the state and the type of storage structures found in each cell. The storage structures of the farmers were also correlated with mycotoxin contamination and the AEZs. A total of 70 composite samples were collected from 33 ADP zones and 55 cells (Table 1) made up of 11 samples each from the Sudan Savanna (SS), Northern Guinea Savanna (NGS) and Southern Guinea Savanna (SGS), 33 from Derived Savanna (DS) and 4 from Humid Forest (HF) zone. The samples were kept in well-labelled sterile polyethylene bags and transported to the laboratory for analysis.

The total weight of each composite sample was 3 kg. The samples were hand-mixed, coarse-ground and allowed to pass through a No.14-mesh screen. Subsamples of 500 g were taken from each lot, ground with a milling machine (Greiffenberger Antriebstechnik, Germany) and further sieved with a 1-mm mesh. Subsamples of 50 g were further taken from the lots and placed in zip lock envelopes for fungal identification (unpublished data) and for multi-metabolite analysis by the LC-MS/MS technique. Samples used for the metabolite analysis were stored at -20°C prior to analyses.

Chemicals

Methanol (LC gradient grade) and glacial acetic acid (p.a.) were purchased from Merck (Darmstadt, Germany),

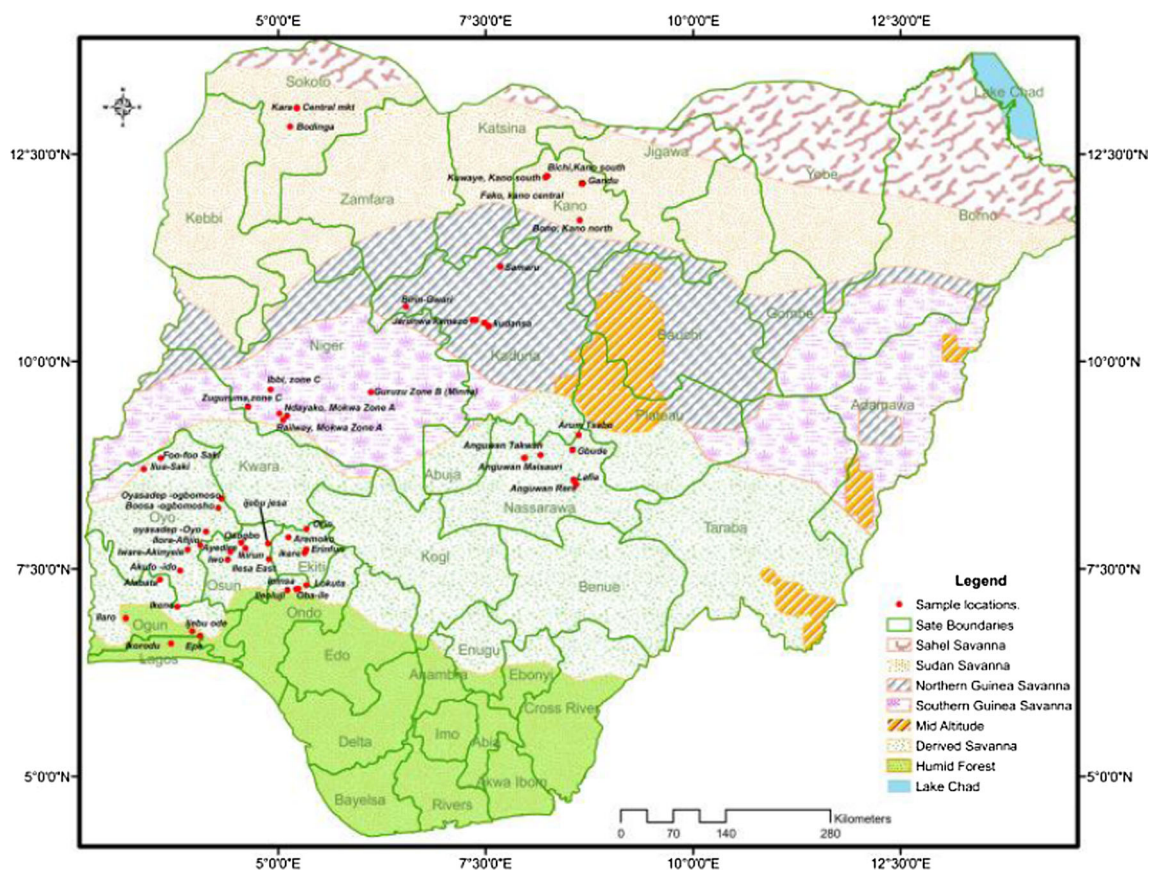


Fig. 1 Sampling locations of maize grains

acetonitrile (LC gradient grade) from VWR (Leuven, Belgium), and ammonium acetate (MS grade) from Sigma-Aldrich (Vienna, Austria). Standards for fungal and bacterial metabolites were obtained from various research groups or from commercial sources. Water was purified successively by reverse osmosis with an Elga Purelab ultra analytic system from Veolia Water (Bucks, UK).

Extraction of maize grains and estimation of matrix effects

Five grams of each representative sample were weighed into a 50-ml polypropylene tube (Sarstedt, Nümbrecht, Germany) and 20 ml of the extraction solvent (acetonitrile/water/acetic acid 79:20:1, v/v/v) added. For spiking experiments, 0.25 g samples were used for extraction. Samples were extracted for 90 min on a GFL 3017 rotary shaker (GFL, Burgwedel, Germany) and diluted with the same volume of dilution solvent (acetonitrile/water/acetic acid 79:20:1, v/v/v), and the diluted extracts injected (Sulyok et al. 2007). Centrifugation was not necessary due to sufficient sedimentation by gravity.

Apparent recoveries of the analytes were cross-checked by spiking a sample that was not contaminated with mycotoxins with a multi-analyte standard on one concentration level, since previously generated maize data is available (Warth et al.

2012a; Abia et al. 2013). The spiked sample was stored overnight at ambient temperature to allow evaporation of the solvent and to establish equilibrium between the analytes and the sample. The extraction, dilution and analysis were as described earlier. The corresponding peak areas of the spiked samples were used for the estimation of apparent recoveries by comparison to a standard prepared and diluted in neat solvent. All concentrations of the naturally contaminated samples were corrected by a factor equivalent to the reciprocal of apparent recovery ($1/R$; where R is the apparent recovery value) of each analyte.

LC-MS/MS parameters

LC-MS/MS screening of target microbial metabolites was performed with a QTrap 5500 LC-MS/MS System (Applied Biosystems, Foster City, CA, USA) equipped with TurboIonSpray electrospray ionization (ESI) source and a 1290 Series HPLC System (Agilent, Waldbronn, Germany). Chromatographic separation was performed at 25 °C on a Gemini[®] C₁₈-column, 150 × 4.6 mm i.d., 5 μm particle size, equipped with a C₁₈ 4 × 3 mm i.d. security guard cartridge (Phenomenex, Torrance, CA, USA). The chromatographic method, chromatographic and mass spectrometric parameters of 186 analytes under investigation are described elsewhere

Table 1 Sample location and storage structures of maize grains from five agroecological zones of Nigeria

S/No	State	Agricultural zone	Agricultural block	Cell	Storage structure	No of composite sample examined (<i>n</i>)
1	Ogun	Ilaro	Ilaro	Ilaro	Straw huts	1
		Ijebu-ode	Ijebu-ode	Ayepe-Ijebu	Stores (bare floors)	1
		Abeokuta	Odeda	Alabata	Straw huts, wooden platforms	2
		Ikenne	Ikenne	Ikene	Stores (bare floors)	1
2	Ondo	Zone I :	Akure North	Ipinsa (Akure North)	Cribs	1
		Owo	Akure South	Oba-Ile (Akure South)	Cribs, stores (bare floors)	2
		Zone II :	Ondo	Lokuta	Kitchen roofs	1
		Ondo	Ile-Oluji	Ile oluji farm settlement	Stores (bare floors)	1
3	Osun	Iwo	Iwo	Agric- Iwo	Cribs, stores (bare floors)	2
			Aiyedire	Aiyedire	Cribs, stores (bare floors)	2
		Oshogbo	Oshogbo	Oke-Osun Farm settlement	Cribs, stores (bare floors)	2
			Ikirun	Ikirun	Stores (bare floors)	1
		Ifè/Ijesha	Oriade	Oriade : Ijebu-Jesa	Stores (bare floors)	1
			Ilesa-East	Ilesa-East	Stores (bare floors)	1
4	Oyo	Ibadan/Ibarapa	Akinyele	Akinyele : Iware	Cribs	1
			Ido	Ido : Akufo F/S	Cribs	1
		Oyo	Oyo West	Oyo West : OYASADEP	Stores (bare floors)	1
			Afijio	Afijio: Ilora	Stores (bare floors)	1
		Ogbomosho	Ogbomosho West	Ogbomosho West	Cribs, stores (bare floors)	2
			Oriire	Oriire: Boosa	Wooden platforms	1
5	Ekiti	Saki	Saki West	Saki West	Cribs, straw huts	2
		Zone I: Iddo/Osi	Iddo-Osi	Orin farm settlement	Cribs	11
		Aramoko	Aramoko	Aramoko	Stores (bare floors)	1
		Zone II: Ikere	Ikere-Ekiti	Ikere-Ekiti	Living rooms	1
6	Lagos	Ado-Ekiti	Ado-Ekiti	Erinfun	Cribs	1
		Epe	Epe	Araga Farm settlemet	Stores (bare floors)	1
7	Kano	IKorodu	IKorodu	Shorefarm	Stores (bare floors)	1
		Kano Central	Menjibir	Gandu	Stores (bare floors), “Rhumbu”	2
8	Sokoto	Kano Central	Fako	Fako,	“Rhumbu”	1
		Kano South	Bichi	Bichi	Wooden platform, stores (cemented floors)	2
		Kano South	Kuwaye	Kuwaye	Stores (bare floors)	1
		Kano North	Bunkure	Bono	Stores (cemented floors)	1
9	Kaduna	Sokoto Central	Ilela	Central market	Stores (bare floors)	1
		Sokoto South	Kara	Kara	Stores (bare floors)	1
		Sokoto South	Bodinga	Bodinga	Stores (bare floors)	1
10	Nasarawa	Jarunwa kamazo	Chukwun	Jarunwa kamazo	Stores (bare floors), “Rhumbu”	2
		Kaduna North	Igebi	Naira	“Rhumbu”, stores (bare floors)	2
		Kaduna North	Megigiyan	Megigiyan,	“Rhumbu”, stores (bare floors)	2
		Kaduna South	Maraba-rido	Kudansa	Kitchen roofs, “Rhumbu”	2
		Birin-Gwari	Birin-Gwari	Birin-Gwari	Stores (bare floors), stores (cemented floors)	2
		Zaria	Samaru	Samaru	Stores (bare floors), stores (cemented floors)	2
10	Nasarawa	Nasarawa South	Shabu	Shabu	Stores (bare floors)	1
		Nasarawa South	Lafia	Anguwan Rere	Stores (bare floors)	1
		Nasarawa North	Wamba	Arum Tsabo	kitchen roofs	1
		Nasarawa North	Gbude	Gbude	Kitchen roofs	1
		Nasarawa West	Kokona	Anguwan Maisauri	Stores (bare floors), kitchen roofs	2
		Nasarawa West	Takwan	Anguwan Takwan	Kitchen roofs	1

Table 1 (continued)

S/No	State	Agricultural zone	Agricultural block	Cell	Storage structure	No of composite sample examined (<i>n</i>)
11	Niger	Zone A	Mokwa	Railway,	Stores (bare floors)	1
		Zone A	Mokwa	Yagbagba	Stores (cemented floors)	1
		Zone A	Ndayako	Ndayako,	Stores (bare floors)	1
		Zone B	Bosso	Guruzu	Stores (bare floors)	1
		Zone B	Bosso	New Guruzu	Stores (bare floors)	1
		Zone C	Mashegu	Zuguruma,	“Rhumbu”	1
		Zone C	Ibbi	Ibbi	“Rhumbu”	1
						<i>n</i> =70

(Vishwanath et al. 2009). At present, this method has been transferred to another instrument and further expanded to 320 metabolites (unpublished data).

ESI-MS/MS was performed in the time-scheduled multiple reaction monitoring (MRM) mode both in positive and negative polarities in two separate chromatographic runs per sample by scanning two fragmentation reactions per analyte. The MRM detection window of each analyte was set to its expected retention time ± 27 and ± 48 s in the positive and the negative modes, respectively. Confirmation of positive analyte identification was obtained by the acquisition of two MRMs per analyte (with the exception of moniliformin and 3-nitropropionic acid, which exhibited only one fragment ion). This yielded 4.0 identification points according to European Union Commission decision 2002/657 (EU 2002b). 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 % rel., respectively.

Relationship between storage structures and mycotoxin concentration of maize grains

The Pearson's correlation method was used to establish relationship between identified storage structures and levels of mycotoxin contamination of the maize grains across the AEZs.

Statistical analysis

The levels of mycotoxin contamination of the maize grains were analysed with the SPSS for windows v.16.0 (SPSS, Chicago, IL, USA). The Duncan's Multiple Range test (DMRT) was used to separate the means ($p < 0.05$). Simple descriptive analysis was used to evaluate the occurrence and concentration of fungal and microbial metabolites across the AEZs.

Results and discussions

LC-MS/MS quality assurance

Table 2 shows the performance characteristics data for the analytical method used as established from one spiked blank sample of maize. The limits of detection (LOD) ranged between 0.04 $\mu\text{g}/\text{kg}$ (chloramphenicol) and 80 $\mu\text{g}/\text{kg}$ (fusaproliferin). Quantitative standards for averufin, nidurufin, norsolorinic acid, versicolorins A and C were not available; these compounds were therefore semi-quantified using the response factor of averantin. Andrastin A was qualitatively determined in the maize samples from the peak area due to lack of internal standard. Apparent recoveries of other metabolites from the spiked samples were in the range of 60–160 % except for ophiobolin A, chanoclavine, skyrin and rugulosin which exhibited lower values.

Occurrence and distribution of microbial metabolites of stored maize grains in Nigeria

A total of 66 microbial (62 fungal and 4 bacterial) metabolites were detected in the maize grains (moisture content of between 12.41 and 13.29 %) across the five AEZs of Nigeria (Tables 3, 4). The large number of microbial metabolites detected in the grains point to the diversity of fungal and bacterial species that colonised the grains from field to the store. The metabolite concentrations ranged between 0.1 and 132,909 $\mu\text{g}/\text{kg}$, and the largest proportion of contaminated grains (aflatoxins including aflatoxin M₁, fumonisins and deoxynivalenol) was found in the DS zone (Tables 3, 4) while the lowest was in the HF zone. The low amount of rainfall (650–1,300 mm), the prevailing high temperature (26–40 °C) (Atehnkeng et al. 2008) and long periods of dry season (6–9 months) in the SS and NGS zones (Udo et al. 2000; Atehnkeng et al. 2008) may be responsible for the low concentration of aflatoxins found in maize grains in these regions. Udo et al. (2000) did not detect aflatoxins in farmers' stores in the NGS zone and attributed it to the fact that farmers usually store

Table 2 LC-MS/MS method performance characteristics of analytes of stored maize grains from five agro-ecological zones of Nigeria

Analyte	LOD ^a (µg/kg)	R ^b	Analyte	LOD ^a (µg/kg)	R ^b
15-Hydroxyculmorin	10.00	92.8	Fumonisin B ₂	0.80	71.6
3 Nitropropionic acid	2.00	75.6	Fumonisin B ₃	8.00	69.9
3, mono-acetyldeoxynivalenol	1.20	99.0	Fusaproliferin	80.00	100.0
Aflatoxin B ₁	0.40	62.0	Fusaric acid	10.00	74.4
Aflatoxin B ₂	0.60	59.6	Geldanamycin	0.20	62.6
Aflatoxin G ₁	0.60	63.8	Hydrolyzed FB ₁	0.10	76.1
Aflatoxin G ₂	0.50	69.3	Kojic acid	40.00	94.2
Aflatoxin M ₁	0.40	78.6	Macrosporin A	0.20	93.5
Agroclavine	0.05	62.3	Malformin C	0.25	103.8
Alpha-Zearalenol	0.80	76.9	Monactin	0.10	87.4
Altemariol	0.60	71.8	Moniliformin	0.80	100.0
Altemariolmethylether	0.05	82.1	Monoacetoxyscirpeno	1.50	84.6
Andrastin A	- ^c	n.d	Monocerin	0.50	94.7
Aurofusarin	5.00	85.5	Nidurufin	0.10	61.2
Averantin	0.05	89.5	Nivalenol	0.80	89.1
Averufanin	0.05	89.5	Nonactin	0.10	83.5
Averufin	0.05	89.5	Norsolorinic acid	0.05	89.5
Beauvericin	0.15	110.4	Ochratoxin A	0.80	93.3
Beta Zearalenol	0.80	83.0	Ochratoxin alpha	0.10	57.0
Butenolid	10.00	66.1	Ochratoxin B	0.70	95.3
Chanoclavine	0.05	26.1	O-Methylsterigmatocystin	0.15	77.2
Chloramphenicol	0.04	103.3	Ophiobolin A	0.80	6.6
Citreoviridin	4.00	91.4	Penicillic acid	4.00	67.6
Culmorin	10.00	101.9	Pestalotin	1.00	84.6
Curvularin	0.15	107.0	Phyiscion	5.00	71.4
Cycloseptide A	2.00	99.4	Radiciol	1.00	154.5
Deoxynivalenol	0.40	96.8	Rugulosin	10.00	50.7
Deoxynivalenol glucoside	0.10	88.1	Secalonic acid D	4.00	118.7
Diacetoxyscirpenol	0.40	78.4	Skyrin	1.50	50.5
Emodin	0.07	85.2	Sterigmatocystin	0.40	97.8
Equisetin	0.50	158.6	Versicolorin A	0.05	89.5
Festoclavine	0.05	64.2	Versicolorin C	0.05	89.5
Fumonisin B ₁	7.00	61.6	Zearalenone	0.30	81.5

n.d. not detected

^a LOD: limit of detection [$s/n=3:1$] expressed as µg/kg sample

^b Recovery, calculated from spiking experiment of a single sample of maize grains

^c No standard available; estimation of concentration based on peak area

their grains in “rhumbu” (local granary) in this zone. The low concentration of aflatoxins in grains stored in the HF zone despite its high rainfall pattern (1,300–2,000 mm) and suitable temperature (26–28 °C) for growth of mycotoxigenic fungi (Udo et al. 2000) may be due to the fact that farmers in the zone do not usually store their maize for long periods, as they sell their maize in the fresh state because the zone is highly urbanised. The grains are usually consumed by the populace as snacks and used by local industries either for feed production or as raw material in other industrial purposes. On the other hand, the high concentration of aflatoxin observed in the DS

zone, especially in Ondo and Nasarawa States, is probably due to the high amount (1,300–1,500 mm) of bimodal rainfall (Atehnkeng et al. 2008) usually recorded in this largest zone. Of the 66 microbial metabolites, 25 are regarded as mycotoxins addressed by regulations, and their derivatives and other notable mycotoxins were targeted by several analytical methods (Table 3), while the remaining 41 are rarely investigated (Table 4). Among the microbial metabolites detected in the stored maize, only the fungal metabolites, aflatoxins, diacetoxyscirpenol, deoxynivalenol, 3,mono-acetyldeoxynivalenol, fumonisins, ochratoxin A, zearalenone,

Table 3 Occurrence and concentration of regulated mycotoxins in stored maize grains from five AEZs^a of Nigeria

Mycotoxin	<i>n</i> ^b	<i>P</i> ^c	Concentration (µg/kg)					Proportion of contaminated maize grains across agro-ecological zones ^a				
			Min	Max	Median	Mean	SD	HF (<i>n</i> =4)	DS (<i>n</i> =33)	SGS (<i>n</i> =11)	NGS (<i>n</i> =11)	SS (<i>n</i> =11)
3-nitropropionic acid	59	84.3	6.5	2,844	42.5	244	530	3	28	10	9	9
Aflatoxin B ₁	47	67.1	0.4	6,738	74	394	1,033	1	25	8	4	9
Aflatoxin B ₂	38	54.3	1	644	12	44	108	1	20	7	2	8
Aflatoxin G ₁	11	15.7	1	264	16	47	83	0	5	2	1	3
Aflatoxin G ₂	4	5.7	0.7	52	6	16	24	0	1	1	0	2
Aflatoxin M ₁	34	48.6	1.2	120	5	14.5	23	1	17	7	2	7
Alternariolmethylether	20	28.6	0.04	21	0.6	3	6	0	9	7	2	2
Alternariol	13	18.6	0.8	57	4	10	16	0	6	5	2	0
Beauvericin	55	78.6	0.1	120	1	10	23	1	25	9	10	10
Deoxynivalenol	70	100.0	11	479	49	60	59	4	34	10	11	11
Deoxynivalenol-glucoside ^d	7	10.0	0.1	76	0.4	11	29	0	4	0	2	1
Fumonisin B ₁	65	92.9	1.8	10,447	1,064	1,552	1,934	2	34	10	11	8
Fumonisin B ₂	59	84.3	12.8	3,455	274	442	581	2	31	10	10	6
Fumonisin B ₃	59	84.3	6.4	720	107	161	163	2	31	10	9	7
Hydrolysed fumonisin B ₁	37	52.9	0.4	135	5	11	22	0	20	7	6	4
Fusaproliferin	3	4.3	57.4	263	243.5	188	114	0	2	1	0	0
Moniliformin	54	77.1	0.8	899	11	130	221	3	26	10	8	7
Nivalenol	38	54.3	0.7	164	6	14	28	1	18	6	7	6
Ochratoxin A	7	10.0	4	580	28	111	208	1	5	0	0	1
Ochratoxin-alpha	1	1.4	11	11	11	11	–	0	0	1	0	0
Ochratoxin B	5	7.1	2	26	2	7.5	11	1	3	0	0	1
Sterigmatocystin	26	37.1	0.4	17	0.9	3	5	0	12	6	4	4
Zearalenone	12	17.1	0.4	2,044	4	174	589	0	10	1	1	0
Alpha-zearalenol	1	1.4	17	17	17	17	–	0	1	0	0	0
Beta-zearalenol	1	1.4	13	13	13	13	–	0	1	0	0	0

^a Agro-ecological zones: *HF* Humid Forest, *DS* Derived Savanna, *SGS* Southern Guinea Savanna, *NGS* Northern Guinea Savanna, *SS* Sudan Savanna

^b Quantity of contaminated grains; *n* number of samples examined

^c Percentage of contaminated grains

^d Masked mycotoxin

and alpha-zearalenol, have been previously reported in Nigerian maize (Afolabi et al. 2006; Adejumo et al. 2007a, b; Atehnkeng et al. 2008; El-Imam et al. 2012), while the remaining 54 fungal and bacterial metabolites have not been documented.

Regulated and common mycotoxins of stored maize grains

The mycotoxins detected in the maize grains are shown in Table 3. They are capable of being carcinogenic (aflatoxins, ochratoxin A and fumonisins), mutagenic (aflatoxins), teratogenic (ochratoxin A), estrogenic (zearalenone), haemorrhagic (trichothecenes), immunotoxic (aflatoxins and ochratoxin A), nephrotoxic (ochratoxin A), dermatotoxic (trichothecenes) and neurotoxic (fumonisin B₁) (IARC 1993, 2002; Bondy and Pestka 2000; CAST 2003). Aflatoxin B₁, the most potent and prevalent form of the aflatoxins, was found in 67.2 % of the maize grains while aflatoxin G₁ contaminated only 15.7 % of the grains. Aflatoxin B₁ has been primarily linked to human

primary liver cancer in which it acts synergistically with hepatitis B virus (HBV) infection (Li and Wu 2010). The aflatoxin levels of the stored maize were much higher than what was reported in a previous study in Nigeria: 0–1,506 µg/kg (Atehnkeng et al. 2008); and the DS AEZ had the highest aflatoxin level. This may be due to the fact that the region produces the highest amount of grains in the country. However, it differed from previous reports that implicated the SGS as the region with the highest aflatoxin levels (Atehnkeng et al. 2008); a pointer to regional variation and widespread contamination of maize grains by aflatoxins across the country. About 61.4 and 57.1 % of the maize grains exceeded the maximum acceptable limits (MAL) set by the European Union Commission (EU, 2006), for aflatoxin B₁ (2 µg/kg) and total aflatoxins (4 µg/kg) in maize, respectively (Fig. 2). About 51.4 % of the maize grains had aflatoxin concentrations above the 10 µg/kg MTL recommended for unsorted food commodities by the National Agency for Food

Table 4 Occurrence and concentration of non regulated microbial metabolites in stored maize grains from five AEZs^a of Nigeria

Metabolite	<i>n</i> ^b	<i>P</i> ^c	Concentration (µg/kg)					Proportion of contaminated maize grains across agro-ecological zones ^a				
			Min	Max	Median	Mean	SD	HF (<i>n</i> =4)	DS (<i>n</i> =33)	SGS (<i>n</i> =11)	NGS (<i>n</i> =11)	SS (<i>n</i> =11)
15-Hydroxyculmorin	2	2.9	63	106	84	84	30	0	1	0	1	0
3, mono-acetyldeoxynivalenol	1	1.4	87.5	87.5	87.5	87.5	–	0	0	0	1	0
Andrastatin A	13	18.6	^d	^d	^d	^d	^d	0	4	6	1	2
Aurofusarin	7	10.0	3	2,608	49	541	954	0	5	0	1	1
Averufin	59	84.3	0.1	677	8	37	107	3	26	10	10	10
Averufanin	42	60.0	0.1	31	1	4	6.5	1	22	7	3	9
Averantin	47	67.1	0.5	55	1	3	8	1	25	7	5	9
Agroclavine	1	1.4	0.06	0.06	0.06	0.06	–	0	0	0	0	1
Butenolid	29	41.4	4.4	492	18	64	115	0	20	3	3	3
Chloramphenicol ^e	69	98.6	0.2	0.9	0.3	0.3	0.1	4	34	10	11	11
Citreoviridin	6	8.6	18	1,990	267	575	768	0	2	3	1	0
Chanoclavine	31	44.3	0.05	9.5	0.2	0.8	2	0	22	6	3	0
Culmorin	2	2.9	45	411	228	228	259	0	1	0	1	0
Curvularin	7	10.0	0.3	277	6	49	101	0	3	2	1	1
Cycloaspeptide A	4	5.7	4.6	75	12	26	33	0	3	1	0	0
Diacetoxyscirpenol	13	18.6	3	30	6	10	9	0	4	3	5	1
Emodin	68	97.1	1.4	237	3.3	11	34.5	3	33	10	11	11
Equisetin	69	98.6	1.2	664	13.5	54.5	103	4	33	10	11	11
Festuclovin	2	2.9	0.1	0.4	0.2	0.2	0.2	0	1	0	1	0
Fusaric acid	25	35.7	32.5	368	56	88	75	0	14	7	4	0
Geldanamycin ^e	1	1.4	27	27	27	27	–	0	0	0	1	0
Kojic acid	49	70.0	62.5	132,909	1,822	8,465	21,457	1	26	9	6	7
Macrosporin A	52	74.3	1.3	120.5	2	8	20	2	20	8	11	11
Malfomin C	4	5.7	0.02	0.8	0.5	0.4	0.3	0	3	0	0	1
Monoacetoxyscirpenol	12	17.1	2	28	4	7	9	0	3	4	3	2
Monactin ^e	1	1.4	0.3	0.3	0.3	0.3	–	0	0	0	1	0
Monocerin	21	30.0	0.3	1,724	5	127	375	0	2	4	9	6
Nidurufin	26	37.1	0.04	7	0.4	1	2	0	13	5	2	6
Nonactin ^e	1	1.4	0.19	0.19	0.19	0.19	–	0	0	0	1	0
Norsolorinic acid	36	51.4	0.03	10	0.2	0.7	2	1	18	6	3	8
Ophiobolin A	2	2.9	31	159	95	95	91	0	1	1	0	0
O-Methylsterigmatocystin	31	44.3	0.05	19	0.5	2	4.3	1	17	5	2	6
Penicillic acid	13	18.6	4	19	7.5	9	5	0	10	2	0	1
Pestalotin	50	71.4	1	29	4	6	5	3	24	8	8	7
Physson	14	20.0	4	580	45	109	167	0	5	3	2	4
Radicicol	21	30.0	4	19	6	7	3.4	0	7	5	6	3
Rugulosin	9	12.9	18	1,468	118	276	464	0	6	2	1	0
Secalonic acid D	1	1.4	6	6	6	6	–	0	0	1	0	0
Skyrin	43	61.4	3	694	5	45	120	1	27	8	5	2
Versicolorin A	34	48.6	0.2	47	1	4	9	1	18	6	3	6
Versicolorin C	36	51.4	0.3	75	2	6.4	13	1	17	7	3	8

n number of samples examined

^a Agro-ecological zones: *HF* Humid Forest, *DS* Derived Savanna, *SGS* Southern Guinea Savanna, *NGS* Northern Guinea Savanna, *SS* Sudan Savanna

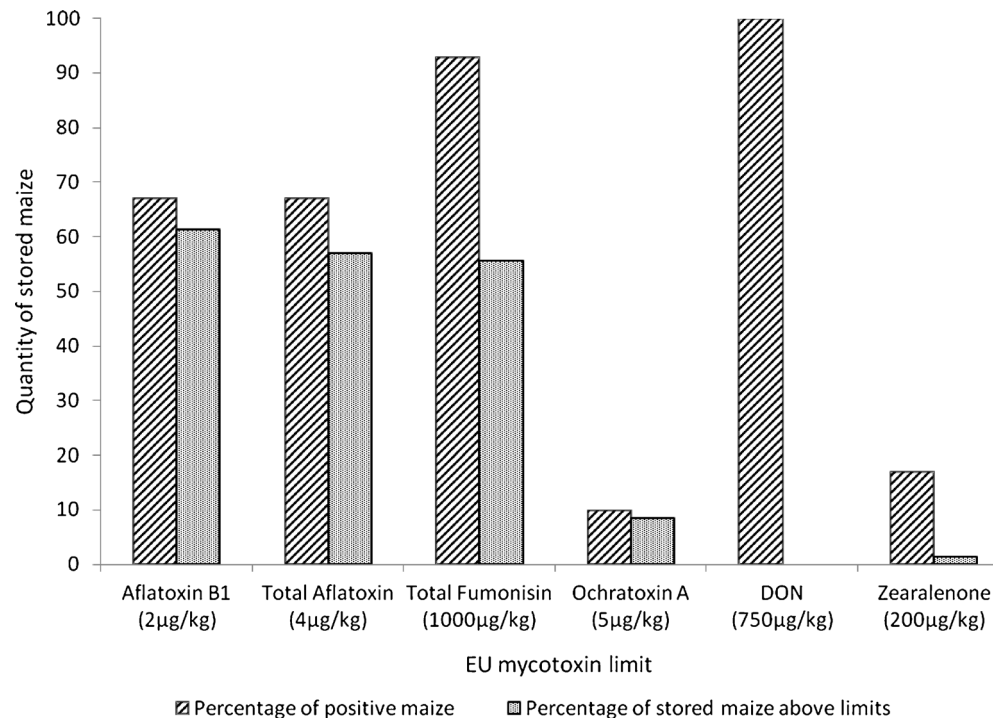
^b Quantity of contaminated grains

^c Percentage of contaminated grains

^d Qualitative determination

^e Bacterial metabolite

Fig. 2 Percentage of contaminated grains above the maximum acceptable limits



and Drug Administration and Control in Nigeria (Atanda et al. 2011). Thus, chronic exposure to aflatoxins through maize consumption is evident in Nigeria, as with other parts of SSA (Bandyopadhyay et al. 2007; Atehnkeng et al. 2008; Diedhiou et al. 2011; Warth et al. 2012a; Abia et al. 2013).

Fumonisin B₁, the most toxic of the fumonisins, has been implicated as the causative agent of equine leukoencephalomalacia, a fatal neurological disease of horses, characterized by liquefactive necrosis of the white matter of the brain (Chilaka et al. 2012). Exposure to this mycotoxin has also been associated with the incidence of neural tube defects (Missmer et al. 2006). The mycotoxin was detected in 92.9 % of the maize grains and the range of contamination was between 1.8 and 10,447 µg/kg (Table 3), while fumonisin B₂ and fumonisin B₃ were found in the ranges of 12.8–3,455 and 6.4–720 µg/kg, respectively. The levels reported in this work are higher than the previous levels of fumonisin contamination in Nigerian maize (Afolabi et al. 2006; Adejumo et al. 2007b; El-Imam et al. 2011) and maize from Cameroun, Democratic Republic of the Congo and Kwazulu Natal province of South Africa (Manjula et al. 2009; Chilaka et al. 2012; Abia et al. 2013). However, some stored maize grains intended for human consumption in Zambia (Kankolongo et al. 2009) and South Africa (Ncube et al. 2011) had fumonisin levels as high as 21,440 and 21,800 µg/kg, respectively, while in a recent study by Shephard et al. (2013), fumonisin B₁ levels in good quality and moldy home-grown maize grains were also found to be as high as 17,120 and 190,100 µg/kg, respectively. The high levels of fumonisins in

the stored maize point to lapses in implementing good on-field agricultural practices during planting and harvesting of maize grains, thus favouring the invasion of grains by fumonisin-producing *Fusarium* species and elaboration of toxins. About 55.7 % of the samples exceeded the MAL of 1,000 µg/kg for total fumonisins in maize (Fig. 2) set by EU (2006).

Deoxynivalenol, a trichothecene that causes nausea, vomiting and diarrhoea in agricultural animals when ingested in low and high doses, induces weight loss and food refusal in pigs and other farm animals (Pestka 2010a, b). This *Fusarium* toxin recorded a 100 % occurrence in the stored maize at a concentration range of 11–479 µg/kg. This is similar to the level of deoxynivalenol contamination (9.6–745.1 µg/kg) of maize previously reported in Nigeria (Adejumo et al. 2007a), Burkina Faso (31.4 µg/kg) and Mozambique (116–124 µg/kg) (Warth et al. 2012a), and for maize-based products such as cornflakes and popcorn (max = 63 µg/kg) from Belgium (De Boevre et al. 2012). In addition, the range of contamination of its masked form, deoxynivalenol glucoside, which is also reported for the first time in Nigeria maize was between 0.1 and 76 µg/kg, but similar to the levels (LOQ–82 µg/kg) found in Camerounian maize (Abia et al. 2013). Furthermore, deoxynivalenol and its glucoside were detected in human urine in Cameroun (Warth et al. 2012b). The mean level of contamination of nivalenol, another type B immunosuppressive, protein inhibitor trichothecene was 14 µg/kg, which was less than those recently reported for maize (34.1 µg/kg) from Mozambique (Warth et al. 2012a) and by Abia et al. (2013) for Cameroun (161 µg/kg). Zearalenone, an oestrogenic toxin

which causes infertility in animals, has been mentioned in relation to human cervical cancer and outbreaks of precocious pubertal changes in children (CAST 2003). As reported by previous workers (Soleimany et al. 2012; Warth et al. 2012a; Abia et al. 2013), this *Fusarium* mycotoxin was also found in the stored maize grains (17.1 %) at concentration ranges of 0.4 to 2,044 $\mu\text{g}/\text{kg}$. In addition, a composite sample of maize grains had a high zearalenone concentration of 2,044 $\mu\text{g}/\text{kg}$, which was far above the MAL limit of 200 $\mu\text{g}/\text{kg}$.

Other emerging *Fusarium* mycotoxins found (Table 3) included enniatins, beauvericin, fusaproliferin and moniliformin (Jestoi 2008). These mycotoxins have not been documented for Nigerian maize except for enniatins which were found in maize from Southwestern Nigeria (Adejumo et al. 2007b). The co-occurrence of these emergent mycotoxins with other mycotoxins of known toxicology and many other metabolites of unknown toxicology is a source of concern. Beauvericin like enniatin is a cyclodepsipeptide with antibiotic, insecticidal, and cytotoxic effects most likely related to their ionophoric properties (Juan et al. 2013). Furthermore, beauvericin is genotoxic to human lymphocytes (Celik et al. 2010), while fusaproliferin is toxic to human non-neoplastic B-lymphocyte cell line IARC/LCL 171 (Logrieco et al. 1996) and moniliformin is cytotoxic to many mammalian systems (Jestoi 2008).

Ochratoxin A, a potent nephrotoxic metabolite from *Penicillium* species, *Aspergillus ochraceus* and *A. carbonarius* was detected in only 10.0 % of the maize grains and the range of contamination was between 4 and 580 $\mu\text{g}/\text{kg}$. The kidneys are the most susceptible organs to ochratoxin A where it can cause both acute and chronic kidney lesions (O'Brien and Dietrich 2005) in sufficiently high

concentrations. However, only 8.6 % of the maize grains had ochratoxin A levels above the MAL of 5 $\mu\text{g}/\text{kg}$ for maize grains (Fig. 2).

Non-regulated metabolites of stored maize grains

Thirty-seven fungal and four bacterial metabolites of the stored maize were non-regulated (Table 4). Metabolites of *Aspergillus* origin (averufin, averufanin, averantin, nidurufin, norsolorinic acid, versicolorins A and C) which are known aflatoxin precursors were found in about half of maize grains at low-to-moderate concentrations (Table 4), with the exception of physcion which was found in only 20.0 % of the stored grains, and kojic acid which had a maximum concentration of 132,909 $\mu\text{g}/\text{kg}$. The *Fusarium* metabolites found in the maize grains included aurofusarin, culmorin and its derivative (15-hydroxyculmorin), diacetoxyscirpenol, equisetin, fusaric acid, monocetoxyscirpenol and monocerin with equisetin having the highest percentage occurrence of 98.6 %. *Penicillium* metabolites occurred less frequently in the stored maize at moderate concentrations except for emodin, pestalotin and skyrin, which were found in more than two-thirds of the stored maize, and citreoviridin which had a mean concentration of 575 $\mu\text{g}/\text{kg}$. The presence of these non-regulated fungal metabolites corroborates previous studies where they were incriminated in the contamination of maize and maize beer (Abia et al. 2013). The bacterial metabolites, chloramphenicol, geldanamycin, monactin and nonactin (Table 4), had low concentrations in the maize grains and were therefore not a source of concern, although chloramphenicol (chlornitromycin) recorded a high incidence of 98.6 %.

Fig. 3 Storage structures of maize in Nigeria

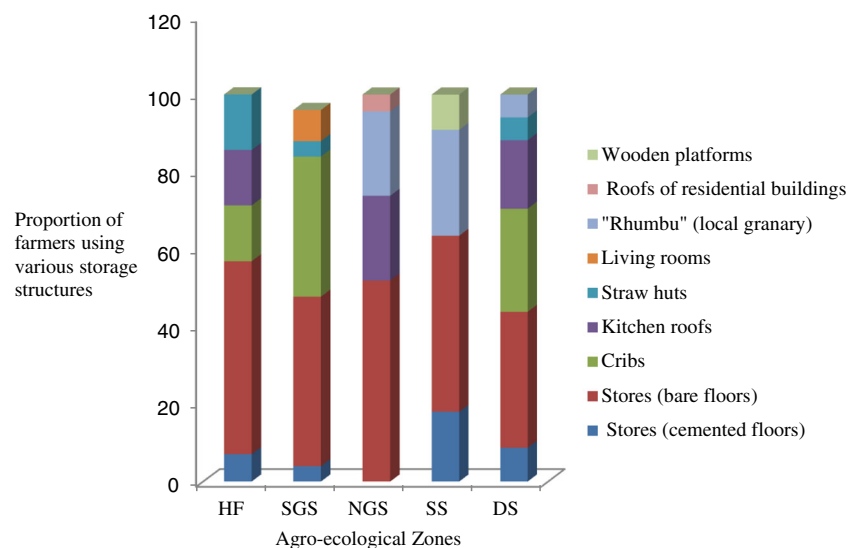


Table 5 Mycotoxin contamination of storage structures across agro-ecological zones of Nigeria

AEZ ^a	Storage structure ^b	Mean mycotoxin concentration ($\mu\text{g}/\text{kg}$)			
		Aflatoxin	Fumonisin	Ochratoxin A	Deoxynivalenol
DS	Stores (bare floor)	164.65±74.85 ab	2,526±1,159.2 a	15.23±2.34 b	49.74±8.72 ab
	Stores (cemented)	20.34±19.11 b	3,038±2,775 a	<LOD	31.15±16.91 b
	Wooden platforms	<LOD	448.06±254.81 a	<LOD	51.58±10.87 a
	Cribs	437.90±178.76 a	2,067.30±383.82 a	78.61±63.35 a	59.13±5.20 a
	Kitchen roofs	1.00±0.76	1,062±458.75 a	<LOD	43.36±3.96 b
SGS	Stores (bare floor)	656.24±370.88 a	2,925.28±1,014 a	<LOD	33.26±7.64 b
	Stores (cemented)	58.35±1.65 b	2,997.36±2.64 a	<LOD	173.2±1.80 a
	Cribs	0.08±0.02 b	116.06±3.94 a	35±5.02 a	47.97±2.03 b
	“Rhumbu”	41.84±41.55 b	1,919.16±1,500 a	<LOD	43.95±3.62 b
NGS	Stores (bare floor)	137.04±108.40 a	4,175.72±1,196 a	<LOD	67.18±15.89 b
	“Rhumbu”	0.67±0.00 b	1,155.33±8.92 ab	<LOD	212.23±134.5 a
	Kitchen roofs	<LOD	78.96±1.03 b	<LOD	35.27±4.73 b
SS	Stores (bare floor)	122.44±29.20	936.53±303.36	4.20±3.00	53.95±6.70
	Wooden platforms	38.88±1.12	791.99±8.01	<LOD	49.21±0.795
	Stores (cemented)	318.45±209.5	435.36±125.44	<LOD	42.34±13.54
	“Rhumbu”	70.75±40.67	272.77±264.56	<LOD	52.36±23.1
HF	Stores (bare floor)	13.25±13.25 b	67.67±67 b	<LOD	91.06±43.65 a
	Stores (cemented)	<LOD	696±38.47 a	<LOD	66.15±3.85 a
	Straw huts	584.96±15.03 a	186.52±3.48 b	<LOD	28.23±1.77 b

Mean mycotoxin concentrations with different lowercase letters in a column are significantly different ($p < 0.05$)

LOD Limit of Detection

^a Agro-ecological zones: DS Derived Savanna, HF Humid Forest, SGS Southern Guinea Savanna, NGS Northern Guinea Savanna, SS Sudan Savanna

^b Dominant storage structures

Correlation between storage structures and levels of mycotoxin contamination of maize grains

Nine storage structures were identified across the AEZs and the storage structures depended on the local materials available for construction of the storage structures (Fig. 3). Storage of shelled maize grains kept in polyethylene bags on bare

Table 6 Correlation between storage structures and levels of mycotoxin contamination of maize grains across the agro-ecological zones of Nigeria

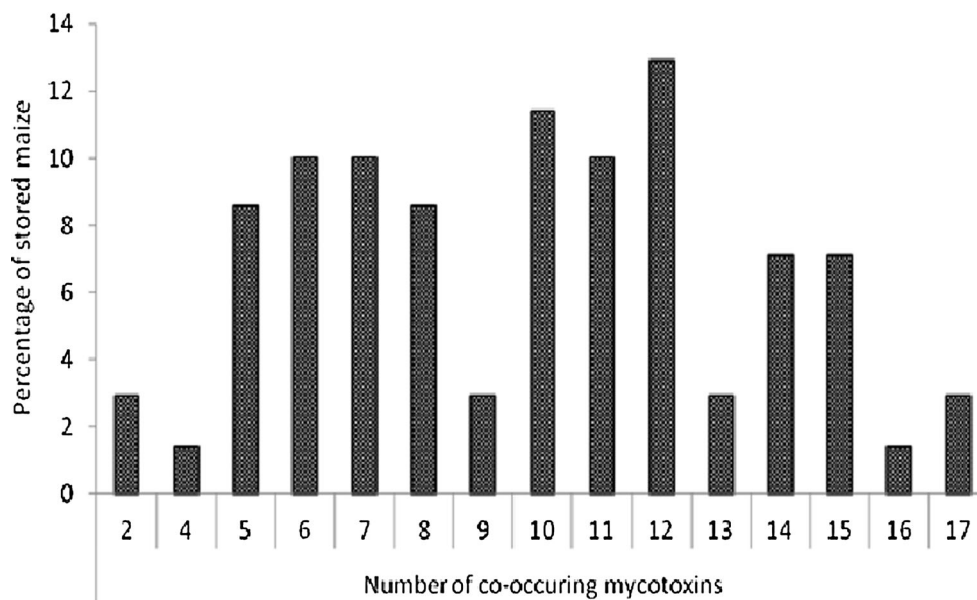
AEZ ^a	Aflatoxin	Fumonisin	Ochratoxin	Deoxynivalenol
SS	0.28	0.105	-0.507**	0.218
NGS	-0.43**	-0.789*	0.369**	0.05
SGS	-0.734**	0.131	0.864*	0.025
DS	0.79*	0.41**	0.205	0.020
HF	0.156	0.873*	0.864*	0.369

*Correlation is significant at $p < 0.05$, **correlation is significant at $p < 0.01$

^a Agro-ecological zones: DS Derived Savanna, HF Humid Forest, SGS Southern Guinea Savanna, NGS Northern Guinea Savanna, SS Sudan Savanna

floors in store rooms was the most common method of storage as the farmers believe that it is easier, cheaper and more suitable for storing large quantities of grains, and this was followed by storage in “rhumbu” (local granary), while storage on roofs of residential buildings was the least common. The range of aflatoxin contamination of grains kept on bare floors was high and was between 13.25 and 656.24 $\mu\text{g}/\text{kg}$, with the least contamination in the HF and the highest in the SGS zones, respectively (Table 5). Furthermore, aflatoxin correlated positively ($r = 0.79$, $p < 0.05$) with storage structures in the DS zone and negatively with SGS and NGS zones ($r = -0.734$, $r = -0.43$; $p < 0.01$) respectively (Table 6). Fumonisin concentration was also high in all the storage structures across the AEZs. The highest contamination of fumonisin (4,175.72 $\mu\text{g}/\text{kg}$) was observed in maize stored in huts in the NGS zone and the least in maize stored in the HF zone (Table 5). Furthermore, a negative correlation ($r = -0.79$, $p < 0.05$) was observed between the storage structures and fumonisin contamination in the NGS zone, while a positive correlation was observed in the DS and HF zones ($r = 0.41$, $p < 0.01$; $r = 0.873$, $p < 0.05$). OTA was found only in storage structures in the DS (2.34–78.61 $\mu\text{g}/\text{kg}$), SS (3.51 $\mu\text{g}/\text{kg}$) and

Fig. 4 Co-occurrence of mycotoxins in Nigerian maize grains



SGS (28.12–41.87 $\mu\text{g}/\text{kg}$) zones. The storage structures and OTA correlated positively with the NGS ($r=0.37$, $p<0.01$), SGS ($r=0.864$, $p<0.05$) and HF ($r=0.86$, $p<0.05$) zones respectively and negatively with the SS zone ($r=-0.507$, $p<0.01$). DON was also present in all the storage structures across the AEZs and there was a positive correlation ($r=0.827$, $p<0.005$) between the storage structures and DON concentration in the HF zone (Table 6).

Co-occurrence of mycotoxins in stored maize grains

Occurrence of mycotoxin cocktails is an important aspect in the assessment of food safety because of the possible synergistic or additive effects produced in humans and animals by co-occurring toxins as a result of the interactions between the biological and toxicological properties of the toxins. In this study, several regulated mycotoxin combinations (2–17) were observed in the stored grains (Fig. 4). Aflatoxins and fumonisins co-occurred in about 65 % of the maize grains with repeated additions of ochratoxin A, deoxynivalenol, zearalenone and the emerging toxins. The co-occurrence of deoxynivalenol and/or its conjugate (deoxynivalenol glucoside) with fusaric acid are additional risks for consumers of the grains because fusaric acid is known to increase deoxynivalenol toxicity several folds, and the conjugate is capable of hydrolysing to its parent compound. The distribution of the mycotoxin mixtures were 2, 16 and 17 in the HF, DS and SGS zones, respectively.

Chronic exposure to mycotoxins, especially deoxynivalenol, aflatoxins and fumonisins through maize consumption is evident in Nigeria. This calls for concern due to chronic mycotoxicoses such as aflatoxicosis that may arise and

possible additive and/or synergistic effects from constant daily exposure to the contaminated grains in addition to the possible large economic losses due to commodity rejection in international markets.

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Conflict of interest The Institute of Food Security, Environmental Resources and Agricultural Research (IFSERAR), Federal University of Agriculture, Abeokuta, Nigeria has no interest in the publication of the results and thus there is no conflict of interest. The authors are in full control of all the primary data reported in this study.

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