

## Assessment of mycotoxins (total aflatoxins and ochratoxin-A) contamination of staple cereals

Obida M. Gwary<sup>1</sup>, Stephen S. Hati<sup>2\*</sup>, Goni A. Dimari<sup>1</sup>, James A. Ameh<sup>3</sup>

<sup>1</sup>Department of Chemistry, University of Maiduguri, Nigeria

<sup>2</sup>Department of Chemistry, IBB University Lapai, Nigeria

<sup>3</sup>Department of Veterinary Medicine, University of Maiduguri, Nigeria

### Abstract

This study presents supportive monitoring information of the levels of mycotoxins in staple cereal grains (Guinea corn, Millet and Maize) under storage conditions at six locations in Borno State, Nigeria. The AgraQuant® (Romer Labs, Inc. USA) enzyme-linked immunosorbent assay (ELISA) was used for the determination of mycotoxins (Total Aflatoxin and Ochratoxin A) in the cereal grains samples. The results generally revealed a wide and markedly significant ( $P < 0.05$ ) variations in the concentrations of the mycotoxins and were mainly dependent on location of storage rather than cereal type. Overall averages of Aflatoxin ( $2.36 \pm 1.68$  ppm) and Ochratoxin ( $0.12 \pm 0.07$  ppb) irrespective of cereal type was recorded. An overall order of concentration in the cereals by Aflatoxin is Guinea corn > Maize > Millet, while by Ochratoxin is Maize > Millet > Guinea corn. This study confirms high levels of Aflatoxins that is capable of posing health hazards, with environmental and poor storage conditions as major cause of mycotoxins.

**Key words:** Cereal grains, Aflatoxin, Ochratoxin-A, ELISA, Health hazards

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\*Corresponding Author, e-mail: [stevehati@yahoo.com](mailto:stevehati@yahoo.com)

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### 1. Introduction

Mycotoxins are chemically and biologically active secondary metabolites produce by fungal (mold) growth in cereals, nuts, fruits and vegetables. They are produced easily by practicing poor grain husbandry [1, 2]. They consist of Aflatoxin, produced by fungus *Aspergillus flavus* and *Aspergillus parasiticus*, Ochratoxins produced by *Aspergillus ochraceus* and *Penicillium verrucosum* fungi [3, 4, 5] and several others (Zearalenone, Trichothecenes, Fumonisin, and the Ergot Alkaloids). Four different aflatoxins, B1, B2, G1 and G2 have been identified, with B1 being the most toxic, carcinogenic and prevalent. Aflatoxins taken in the diet are further converted to the still harmful aflatoxins M1 and M2 that are secreted in dairy products. The complex process of enzymology and molecular biology of aflatoxin biosynthesis has been well documented [5-7]. Ochratoxin is a mycotoxin often referred to as Ochratoxin A – OTA [8]. It is an innately fluorescent compound and detection during analysis is usually based on this property. Conditions supporting the toxin formation in the field are little known. Therefore, Ochratoxin has been regarded as being produced most likely in storage, under conditions that would favor mold growth (adequate moisture/humidity and temperature). Ochratoxin is mainly a kidney toxin and

suspected to be the causative agent of a human disease, Balkan Endemic Nephropathy, but if the concentration is amply high there can be damage to the liver as well. It has also been reported to be a carcinogen in rats and mice [9, 10].

Mycotoxin occurrence in cereal foodstuff is unavoidable [11] as well as the attendant challenges to crop production, yield and quality loss of approximately 25% annually [12], and the aforementioned health hazards are of great concern. We here present supportive monitoring information of the levels of Aflatoxins and OTA in staple cereal grains under storage conditions at six locations in Borno State, Nigeria.

### 2. Materials and methods

#### 2.1. Samples and Sampling

Millet (*Pennisetum glaucum*), Maize (*Zea mays*) and Guinea corn (*Sorghum* spp.) were collected from six local government areas (LGAs), Bama, Biu, Damboa, Gubio, Maiduguri Metropolitan, and Monguno in Borno State. Sampling was conducted six months post-harvest period (June – July, 2009) from public storage and preservation facilities. Ten kilograms composites of each

sample were collected in sterile poly bags on location basis.

**2.2. Determination of Mycotoxins**

The AgraQuant® (Romer Labs, Inc. USA) enzyme-linked immunosorbent assay (ELISA) was used for the determination of mycotoxins (Total Aflatoxin and Ochratoxin A) in the cereal samples. The determinations were carried out according to procedures described by the Romer Labs methods PI-000041-1 for Total Aflatoxin and PI-000073-1 for Ochratoxin A [13]. The methods are similar and direct with high sensitivity, accuracy and reproducibility. It is based on the principle of allowing the toxins in samples and control standards to compete with enzyme-conjugated toxins for the antibody binding sites. After a washing step, an enzyme substrate is added and blue color develops. The intensity of the color is inversely proportional to the concentration of toxins in the sample. A stop solution is then added which changes the color from blue to yellow, which is optically measured using an absorbance filter of a specific wavelength and an interpretive result is determined by comparison.

**2.2.1. Sample Preparation and Extraction**

One kilogram of each cereal sample obtained from composite homogenized bulk samples was ground using a Romer Series II® and sieved through a 20-mesh screen. From which a 20g analysis sample placed in a clean tightly sealed jar was extracted with 100 mL of 70/30 (v/v) methanol/water extraction solution. The mixture was shaken for 3 minutes and allowed to settle. Thereafter the top layer was filtered through a Whatman #1 filter paper and filtrate collected. The filtrate was tested and maintained at pH 6-8.

**2.2.2. Mycotoxins Assay**

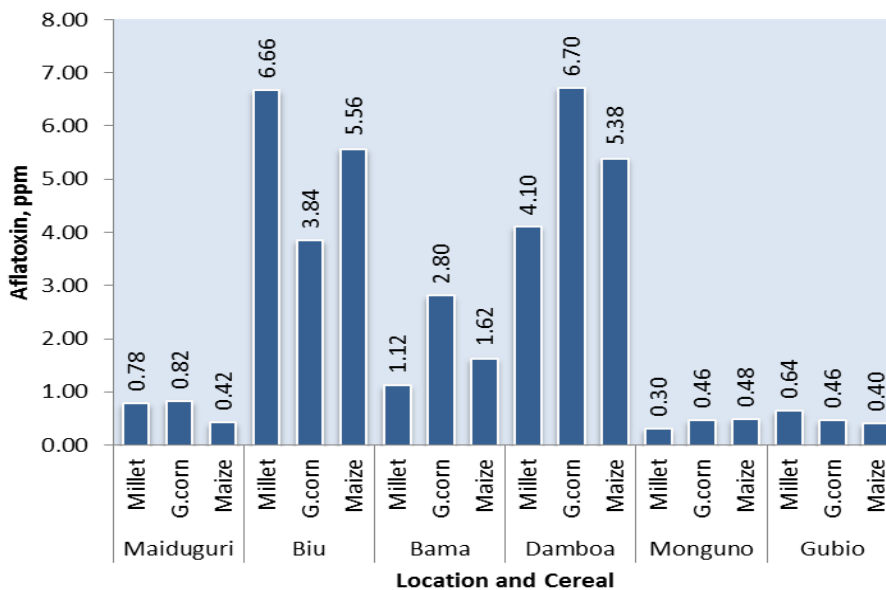
The AgraQuant® kit used for the assay is a complete set with 6 standards, 48 antibody-coated

microwells, 48 color-coded dilution microwells, 8-channel pipette, substrate, conjugate, and stop solution. Using the 8-channel pipette, 200 µl conjugate was placed into each of 48 blue-coded dilution wells. This was followed by 100 µl of samples to the conjugate, mixed and 100 µl of content transferred to antibody coated wells. This was incubated for 15 minutes. Thereafter the content of the wells dispose of, washed with deionized water and tap dried. Into each well was now placed 100 µl substrate and incubated for 5 minutes, to which is then added 100 µl stop solution to the wells. Using the ELISA reader with 450 nm filter results for sample analysis was obtained after a colour change from blue to yellow.

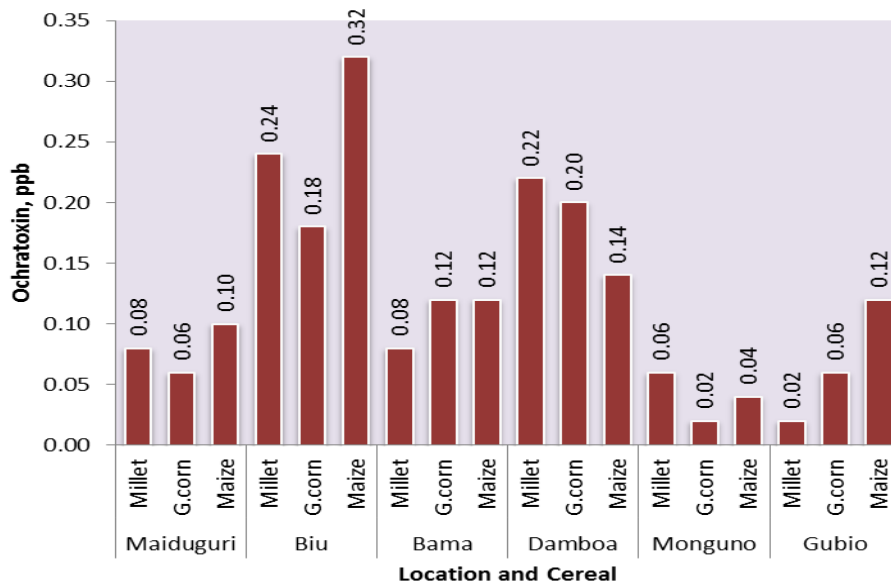
This procedure was run separately for standards at suitable concentrations (0, 10, 20, 30 and 40 ppm). Dilution factors were taken into consideration. Excepting the incubation period for Ochratoxin A assay which is 10 minutes and microwells measured optically by a microplate reader with an absorbance filter of 450nm and a differential filter of 630nm, the determinations are similar for both Total Aflatoxin and Ochratoxin A.

**2.3. Data Analysis**

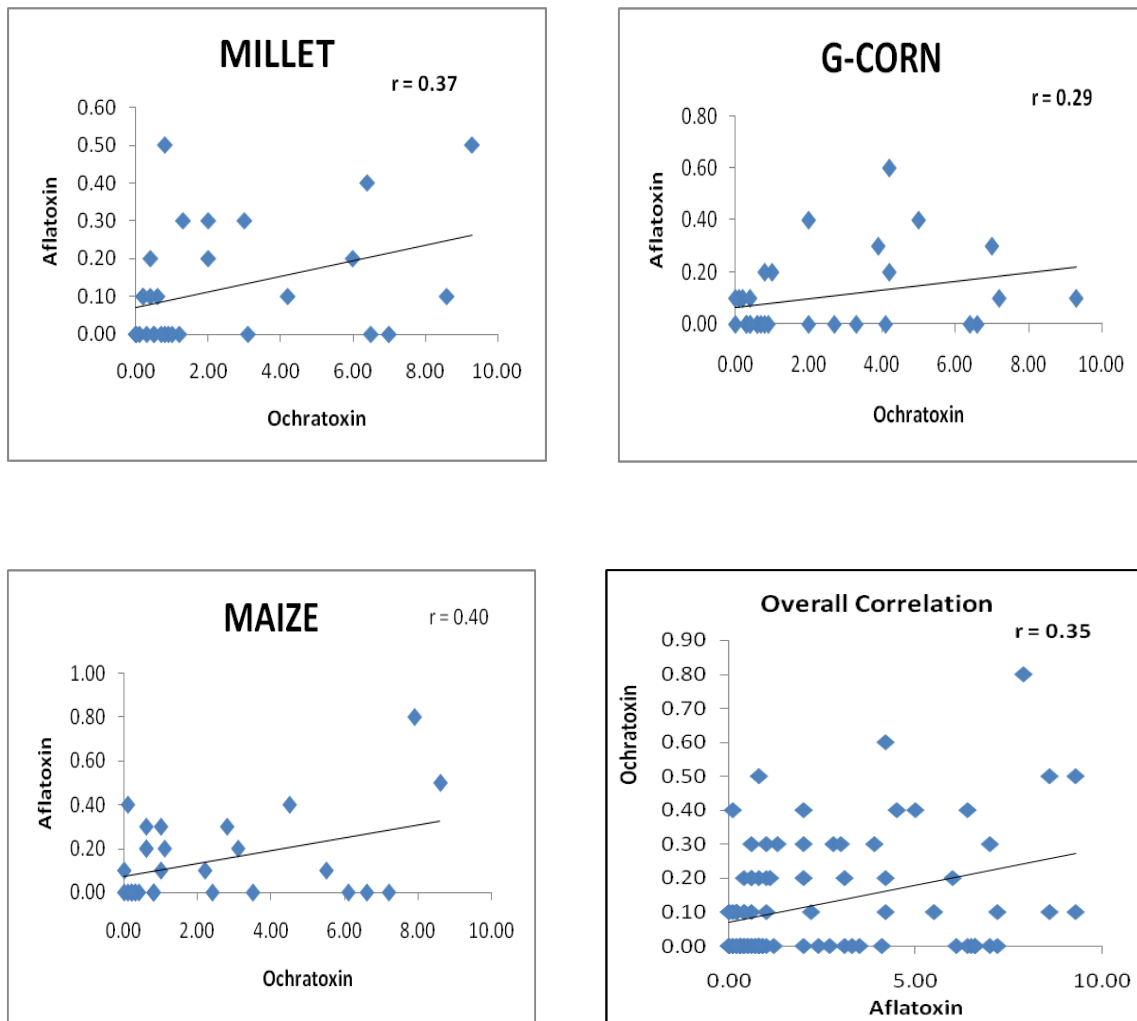
Data obtained from the determinations were subjected to descriptive statistical analysis and results are presented in Mean ± Standard Deviation (SD). Statistical analysis was conducted using Analyse-it® (version 2.20) statistical software for Microsoft Excel 2007. Inferential statistics for variations, analysis of variance - ANOVA - with Tukey post-hoc test between variables were considered significant at 95% confidence interval i.e. P<0.05.



**Figure 1: Concentration of Aflatoxin in Cereal Samples**



**Figure 2:** Concentration of Ochratoxin in Cereal Samples



**Figure 3:** Correlation analysis of Aflatoxin and Ochratoxin in Cereal Samples

### 3. Results and Discussion

The results of Aflatoxin concentration in the different cereals samples as well as at the different locations are presented in Figure 1. The result revealed wide variation of Aflatoxins concentrations that showed dependence on location. Aflatoxins concentration in millet was highest (6.66 ppm) in Biu LGA and lowest (0.30 ppm) in Monguno LGA. Aflatoxins concentration in guinea corn was highest (6.70 ppm) in Damboa LGA and lowest (0.46 ppm) in both Monguno and Gubio LGAs. Similarly, the concentration of Aflatoxins in maize was highest (5.56 ppm) in Biu LGA and lowest (0.40 ppm) in Gubio LGA. On the whole, concentrations of Aflatoxins in the cereals where highest in Biu and Damboa LGAs, moderate in Maiduguri and Bama LGAs, and lowest in Monguno and Gubio LGAs of Borno State. These observed variations were statistically (analysis of variance - ANOVA) significant ( $p < 0.05$ ), markedly between the locations as well as between and within cereal types. Aflatoxin concentrations are higher in this study than in other studies report in Nigeria [11, 14]. Udoh et al. [15] reported that 33% of maize samples from different ecological zones of Nigeria were contaminated with aflatoxins.

Figure 2 shows the results of Ochratoxin concentrations in the cereals samples and at different locations. Similar to the result of Aflatoxins, there was wide variation of concentrations that also showed dependence on location rather than on the cereal type. Ochratoxin concentration in millet was highest (0.24 ppb) in Biu LGA and lowest (0.02 ppb) in Gubio LGA. Ochratoxin concentration in guinea corn was highest (0.20 ppb) was highest in Damboa LGA and lowest (0.02 ppb) in Monguno. The concentration of Ochratoxin in maize was highest (0.32 ppb) in Biu LGA and lowest (0.02 ppb) in Monguno LGA. In general, similar to Aflatoxins, the concentrations of Ochratoxin in the cereals where highest in Biu and Damboa LGAs, moderate in Maiduguri and Bama LGAs, and lowest in Monguno and Gubio LGAs of Borno State. These observed variations were statistically (analysis of variance, ANOVA) significant ( $p < 0.05$ ), markedly between the locations as well as between and within cereal types.

Mycotoxigenic fungi are notable to inhabit a good number of food grains and build up mycotoxins when conditions of the environment are complimentary for their development in the field and at storage locations [16]. The results of this study revealed that the concentrations of mycotoxins (Total Aflatoxin and Ochratoxins A) assayed in the cereal samples were generally dependent on the locations of storage rather than on the cereal type. The locations (Biu and Damboa LGAs) with higher concentrations of mycotoxins are in the southern part of Borno State, while the locations (Monguno and Gubio LGAs) with the lowest concentrations of mycotoxins are in the northern part of the state. The wide variations in the environmental conditions of these two sets of locations tend to reflect in the results obtained. High temperatures are recorded in Borno State almost all the year round, with an average hot season temperatures at  $40 \pm 2^\circ\text{C}$  in northern part. In the southern part of the state, the weather condition is comparatively mild. The difference in the mean annual rainfall between these two regions is over 300mm [17]. Cereals stored without adequate drying as well as weather

conditions, moisture/humidity ( $>14\%$ ) and warm temperatures ( $>20^\circ\text{C}$ ) can potentially become contaminated. Damaged and insect infested grains can cause mold "hot spots." The early development of fungi in grains can form adequate moisture from metabolism to allow for further growth and mycotoxin formation [18, 3].

Table 1 presents an overall summary descriptive result of mycotoxins concentrations in the cereals samples analysed. Aflatoxin ( $2.36 \pm 1.68$  ppm) and Ochratoxin ( $0.12 \pm 0.07$  ppb) are the overall averages of concentrations irrespective of cereal type. An overall order of concentration in the cereals by Aflatoxin is Guinea corn  $>$  Maize  $>$  Millet, while by Ochratoxin is Maize  $>$  Millet  $>$  Guinea corn. The FDA Aflatoxins action Levels-- 300 ppb---for feeder cattle, 200 ppb---for finishing swine 100 ppb---for breeding beef cattle, swine and mature poultry, 20 ppb---for humans, and for immature animals (including poultry) and all dairy animals, 0.5 ppb---for milk (Romer, 2004a). Ochratoxin A was highest in Maize in relation to other grains. The toxicological properties of Ochratoxin A include nephrotoxicity, teratogenicity, citrotoxicity and genotoxicity [19]. The EU has recently introduced maximum permissible limits for Ochratoxin A to reduce risk to the consumer. In cereals, these limits are  $5\mu\text{g}/\text{kg}$  for whole grain and  $3\mu\text{g}/\text{kg}$  for processed products [20].

**Table 1:** Summary descriptive statistics for overall concentrations of mycotoxins in cereals samples

| Statistics         | Aflatoxin (ppm) | Ochratoxin (ppb) |
|--------------------|-----------------|------------------|
| Mean               | 2.36            | 0.12             |
| Standard Deviation | 1.68            | 0.07             |
| Standard Error     | 0.28            | 0.02             |
| Sample Variance    | 7.19            | 0.03             |
| Kurtosis           | 0.02            | 2.57             |
| Skewness           | 1.14            | 1.61             |
| Range              | 9.30            | 0.80             |
| Minimum            | 0.00            | 0.00             |
| Maximum            | 9.30            | 0.80             |

Figure 3 further buttress the disordered trend in concentrations by the cereals. It shows the correlation analysis results of the mycotoxins in the cereals. The result generally shows positively weak correlations between Aflatoxins and Ochratoxins in the cereals. Though an appreciably high positive correlation ( $r = 0.4$ ) was recorded in Maize. There are studies on the simultaneous relationship linking more than a few pairs of mycotoxins, which are known to coexist in foodstuffs. These include aflatoxin B1 and Ochratoxin A [21], ochratoxin A and penicillic acid [22]. Comparative analysis revealed close relationship to mitochondrial genomes of *Penicillium* and *Aspergillus* species, both in gene content and in arrangement [23]. In this study, a positive correlation of up to 40% level of

association from concentration results was observed between Aflatoxin and Ochratoxins in the Maize samples.

#### 4. Conclusion

Mycotoxin concentrations are greatly influenced by storage conditions as observed at the different locations rather than by cereal type. The study also confirms high levels of Aflatoxins that is capable of posing health hazards, but Ochratoxins A was found to be below regulation limits. However regular monitoring of mycotoxins in the food stuff is highly recommended including those not covered in this study. Significantly also is the improvement of storage conditions that will forestall the growth of Mycotoxigenic fungi, which will consequently reduce the production of mycotoxins.

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