

### **Original Article**

Dietary Aflatoxin B1 Exposure and its Association with Growth and Learning Outcomes among School Children: Evidence from Osara, Nigeria

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#### **ABSTRACT**

Aflatoxin B1 (AfB1) is a toxic secondary metabolite produced by Aspergillus species. Aflatoxin B1 poses a major food safety concern in sub-Saharan Africa due to its prevalence in dietary staples. This study investigated the relationship between chronic dietary exposure to AfB1, nutritional status, and academic performance in school-aged children in Osara, a rural community in Kogi State, Nigeria. A cross-sectional study was conducted among 100 primary school pupils aged 3 to 7 years. Anthropometric assessments were carried out to determine weight-for-age (WAZ), height-for-age (HAZ), mid-upper arm circumference (MUAC), head-circumference, and body mass index (BMI)-for-age using WHO growth standards. Cognitive performance was evaluated through a multi-subject academic test. Dietary AfB1 exposure was estimated from the analysis of 52 maize and rice samples using ELISA, and the estimated daily intake (EDI) was calculated based on individual body weight and reported cereal consumption. High levels of AfB1 contamination were observed in cereal samples, with 87.10% of maize and 93.55% of rice samples exceeding the 2 µg/kg safety limit. Estimated daily AfB1 intake among children ranged from 56.01 to 66.14 ng/kg bw/day. Nutritional assessment revealed that 16% of children were underweight, 10% were stunted, and 10% had low BMI-for-age, indicating malnutrition. Academic performance was generally poor, with high failure rates across all tested subjects. A strong negative correlation was observed between AfB1 exposure and weight (r = -0.96), and BMI was shown to mediate the relationship between AfB1 exposure and cognitive performance partially. This study establishes a link between high aflatoxin B1 exposure, impaired nutritional status, and poor academic outcomes in children. The findings underscore the urgent need for integrated food safety, nutrition, and educational interventions to protect vulnerable populations in rural Nigeria from the multifaceted harms of aflatoxin exposure.

**Keywords:** Aflatoxin B1 exposure, malnutrition, Osara Nigeria, academic performance, food safety

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#### INTRODUCTION

Aflatoxins are a group of highly toxic and carcinogenic secondary metabolites produced primarily by Aspergillus flavus and Aspergillus parasiticus. Among them, aflatoxin B1 (AfB1) is the most toxicologically potent and prevalent, with significant implications for food safety and public health Aflatoxin [1]. contamination commonly occurs in staple foods such as maize, rice, groundnuts, and other cereals, which constitute key dietary components in sub-Saharan Africa [1,2]. The warm, humid climatic conditions of many tropical and subtropical countries, including Nigeria, provide an ideal environment for fungal proliferation and mycotoxin production during both preand post-harvest stages [3].

Exposure to aflatoxins occurs predominantly through the ingestion of contaminated foods. While acute exposure can result in aflatoxicosis, chronic lowlevel exposure is equally of great public health concern. In children, chronic aflatoxin exposure has been associated with growth impairment, particularly stunting, underweight, and wasting [1,4]. Mechanistically, AfB1 impairs the integrity of the intestinal barrier, disrupts immune function, and interferes with protein synthesis and insulin-like growth factor 1 (IGF-1) pathways, which are critical for growth linear [5]. Numerous epidemiological studies support the

association between aflatoxin exposure and adverse growth outcomes. For instance, researchers [1,4]found significant inverse relationships between serum AfB1-albumin adduct levels and height-for-age z-scores (HAZ) in West African children. A recent meta-analysis [6] confirmed the robustness of this association, showing that AfB1 exposure significantly increases the risk of stunting and other forms of malnutrition in infants and young children. Moreover, studies from Asia [7] corroborate these findings, indicating a global health burden that is often underappreciated. Data from United Nations Children's Fund (UNICEF) [8] has it that in Nigeria, approximately 6.5% of children under the age of five suffer from wasting, 31.5% of children experience stunting. These distressing statistics led to Nigeria's ranking at the 109th position out of 125 countries on the global hunger index in 2023, scoring 28.3%. The index considers four indicators: undernourishment, child wasting, child stunting, and child mortality [8].

Mahfuz *et al.* [9] also established a relationship between aflatoxin and cognitive development in young children in Bangladesh. Studies on animals revealed that exposure to AfB1 disrupts cognitive function, impairs spatial memory, and induces anxious and depressive behaviors in mice [10]. Additionally, prenatal exposure to AfB1 in rats' results in deficits in motor

coordination and learning ability in their offspring, indicating the impact of AfB1 on neuronal development and function [11,12]. Interestingly, AfB1 was identified in brain samples from children in Nigeria who had been exposed to contaminated food [13].

In Nigeria, where maize and rice are dietary staples consumed daily by most households. exposure aflatoxins to remains a persistent threat. Widespread contamination of foodstuffs with AfB1 levels exceeding regulatory thresholds has been reported in Nigeria [14,15]. Makun et al. [16] and Onvedum et al. [17] have reported contamination of Nigerian rice with aflatoxins, while Egbuta et al. [18] and Onyedum et al. [17] also reported contamination of Nigerian maize with aflatoxins. Despite growing awareness, surveillance aflatoxin and control measures remain inadequate in many parts of the country. The Osara community in Kogi State, central Nigeria, exemplifies this challenge. As a predominantly agrarian settlement, the local diet relies heavily on self-produced cassava, maize, and rice, often managed under suboptimal conditions. Coupled with limited awareness about mycotoxin risks and poor access to healthcare, children in Osara may face heightened vulnerability to both dietary toxins and malnutrition.

This study investigates the exposure of school children to AfB1 from local cereals and its potential relationship with the nutritional status and learning outcome of children aged 3 to 7 years in Osara. By integrating field-level contamination data with anthropometric and subject-based assessments, this research aims to provide context-specific evidence that can inform food safety interventions, public health strategies, and policy recommendations

targeting rural Nigerian young populations. The study is also significant as being the pioneer assessment of aflatoxin exposure and its relationship with nutritional and learning outcome among this population.

#### **MATERIALS AND METHODS**

#### Study Area and Population

The study was conducted in Osara, a rural agrarian community in Kogi State, central Nigeria. The target population comprised children aged 3 to 7 years enrolled in two private primary schools. One hundred (100) pupils participated in the study, and their anthropometric measurements and learning outcome were assessed.

#### **Anthropometric Assessment**

Anthropometric measurements were performed according to standardized World Health Organization (WHO) and UNICEF guidelines. Parameters assessed included weight, height, body mass index (BMI), mid-upper arm circumference (MUAC), and head circumference. These taken measurements were calibrated instruments (O'divine weighing balance, SECA213 Stadiometer) procedures. appropriate with BMI calculated as weight divided by height squared (kg/m<sup>2</sup>). For children and teens, BMI is interpreted using sex-specific BMIfor-age percentiles. BMI percentiles show how a child's measurements compare with others of the same gender and age. All 100 children were assessed in a school environment by trained research personnel. For further emphasis, body weight measurement was achieved using a weighing scale in light clothing with no jackets or coats, shoes, and additional clothing to the nearest 0.1 kg. For height, a portable stadiometer with no shoes;

shoulders, buttocks, and heels touching the vertical stand; and the head in Frankfurt position was recorded to the nearest 0.1 cm. Mid-upper arm circumference (MUAC) was measured by marking midway between shoulder tip and the elbow tip on the vertical axis of the upper arm with the arm bent at right angle and between the lateral and medial surface of the left arm.

The WHO child growth standard and the percentile approach were both used to determine underweight, stunting, and malnutrition status. Stunting is if the height-for-age Z score is found to be below −2 SD or 5<sup>th</sup> percentile for the population (WHO, 2007; 2009). Underweight is if the weight-for-age Z score is found to be below -2 SD or  $5^{th}$  percentile for the population (WHO, 2007; 2009). Malnourishment is if the BMI-for-age Z score < -2 SD or 5<sup>th</sup> percentile for the population (WHO, 2007; 2009). Mid upper arm circumference (MUAC) < 13.5 cm is classified as severe acute malnutrition, Head circumference less than 46.5 is recorded as microcephaly.

#### Sampling Method and Sample Size

A total of sixty-two (62) cereal samples consisting of maize (31) and rice (31) were collected for aflatoxin B1 analysis. These samples were obtained through a dual approach: some children brought in grains from their homes and additional samples were purchased from the Osara community market. At each sampling point, approximately 500 grams of milled cereal were collected into sterile Ziploc bags. The samples were homogenized using Excella Grinder an Mixer (KHANCHAN INTERNATIONAL LIMITED), labeled appropriately, and stored at room temperature pending analysis.

#### **Ethical Consideration**

Informed written consent was received from parents/ guardians prior to the inclusion of their children/wards in the study. The study methodology was approved by the Ethics committee of Confluence University of Technology, Osara.

# Exclusion criteria encompass the following

Students with chronic medical conditions impacting growth and/or necessitating specialized care, identified at baseline or subsequently diagnosed during the study period, including congenital heart disease, genetic disorders, kidney ailments, neurological impairments, and cleft lip or palate issues. Students whose caregivers posed cognitive impairments or other limitations hindering their ability to provide informed consent or accurate information.

# Assessment of Academic Performance of Pupils

The assessment was an Ex post facto study which involved 100 pupils from two primary schools in Osara Community. The study involved children aged 3-7 years from two private schools. A specially prepared test. the English Mathematics Competency Test (EMCT), basic science test, general knowledge test and logical reasoning tests were used to gather information about the academic performance of the pupil. The results of the test were compiled and used for the analysis [19]. The test questions were standardized and validated at the Faculty of Science and Technology Education of the Confluence University of Science and Technology Osara.

#### Aflatoxin B1 Extraction

The extraction of aflatoxin B1 from cereal samples was carried out according to the procedure outlined by Onyedum et al. [18]. Precisely 5 grams of homogenized sample was weighed into a sterile extraction container. To this, 25 milliliters of 70% ELISA-grade methanol was added. The mixture was subjected to vigorous agitation using a mechanical shaker for a duration of three minutes. This was followed by centrifugation at  $3500 \times g$  for minutes under ambient room conditions (20-25 °C). From the resulting clear supernatant, a 1 mL aliquot was drawn and diluted with 1 mL of deionized water, achieving a 1:1 dilution ratio. This diluted extract was subsequently utilized for ELISA-based aflatoxin detection.

#### Quantification of Aflatoxin B1

The quantification of aflatoxin B1 levels in the prepared extracts was performed using the RIDASCREEN® Aflatoxin B1 30/15 ELISA kit (R-Biopharm Darmstadt, Germany), following the manufacturer's specified protocol. For each assay, 50 uL of aflatoxin standards (0, 1, 5, 10, 20, and 50  $\mu$ g/kg) and 50  $\mu$ L of the diluted sample extract were pipetted into wells of a microtiter plate pre-coated with specific capture antibodies. This was followed by the addition of 50 µL each of enzyme conjugate and anti-AfB1 antibody. The plate was gently mixed and incubated in the dark at room temperature (20-25 °C) for 30 minutes. After incubation, wells were emptied, tapped dry, and washed three times using 250 µL of phosphatebuffered saline (PBS) with Tween. Subsequently, 100 µL of chromogenic substrate solution was added to each well, and the plate was further incubated in the dark for 15 minutes. The enzymatic reaction was halted by adding 100  $\mu$ L of stop solution per well, and absorbance was immediately measured at 450 nm using a STAT FAX ELISA Reader (Model: 303 PLUS).

All samples and standards were analyzed in duplicate to ensure reproducibility. The assay exhibited a detection threshold of 1  $\mu g/kg$ , with an estimated recovery efficiency of 93% and a specificity of 100% for AfB1. Aflatoxin concentrations were extrapolated from a standard calibration curve, and the final values were adjusted to account for the dilution factor. The percentage absorbance was calculated relative to the absorbance value of the zero standard.

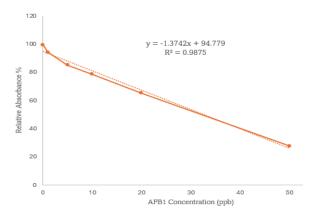


Figure 1: Standard curve for Aflatoxin B1 by competitive ELISA

### Determination of Aflatoxin exposure

To estimate Aflatoxin B1 (AfB1) exposure from rice and maize for children aged 3–7 years in Osara, we use the standard Estimated Daily Intake (EDI) formula:

$$EDI = \frac{C \times IR}{BW}$$

Where:

**EDI** = Estimated Daily Intake (ng/kg body weight/day)

C = Aflatoxin concentration in food (ng/g)

IR = Daily intake rate of the food (g/day)

BW = Body weight (kg)

Note:  $1 \mu g/kg = 1 ng/g$ 

#### **Data Analysis**

All analyses were conducted using Python (pandas, seaborn, statsmodels) and MS 2019. Descriptive Excel statistics summarize central tendencies and dispersion. Visualizations included scatter plots, boxplots, and a heatmap to show variable associations. Linear regression and Baron & Kenny mediation tested relationships between EDI, BMI, and academic scores. Multivariate models were applied to predict academic scores using anthropometric and exposure variables. WHO reference thresholds were used to classify malnutrition (BMI categories), stunting (height), and wasting (weight) by age. Duncan's Multiple Range Test (DMRT) was employed to evaluate the statistical significance of differences between sample groups at a confidence level of 95% (p < 0.05).

#### **RESULTS**

The dataset comprises anthropometric data (head circumference, mid-upper arm circumference, weight, height, and BMI) of one hundred (100) pupils aged 3 to 7 years attending private primary school in Osara, aflatoxin contamination of rice and maize and exposure data, as well as subject-based test scores. The result section is divided into four: the first speaks about the nutritional status of the children by age, the next speaks about the academic performance of the children, the third section speaks about the aflatoxin b1 incidence in cereal and exposure by school children, and the fourth section considers

the interrelationship among these parameters.

# Nutritional Status of Primary school pupil in Osara, Nigeria

### Weight-by-Age

The WHO child growth standards stipulate a weight range considered normal for children at certain age, outside which the child could be classified into various stages of underweight or overweight.

Figure compares weight (kg) distribution across ages 3 to 7. It was observed that median weight generally increased with age, as expected. However, some children at older ages still weigh less than WHO recommended thresholds, indicating possible growth faltering or chronic undernutrition, more pronounced with age 7. The weight-for-age Z score (WAZ) (table 1a) showed an increasing incidence of underweight with increasing age while age 3 revealed no underweight child, age 4 (6.2%), age 5 (7.1%), age 6 (13%) and age 7 (33.4%) indicated underweight. Summarily, 16% of the population are underweight based on WHO WAZ.

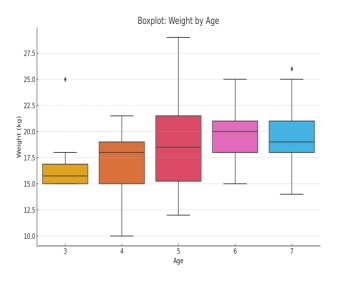


Figure 2: Average Weight by Age

Table 1b shows the percentile distribution of weight (kg) for age by sex. It confers underweight status on the population that has weight (kg) below the 5<sup>th</sup> percentile. This status is based on the median weight for same age in the population. while no

child at age 3 was underweight, female age 4 (11.1%), 6 (7.1%) and 7 (4.8%) and male age 5 (25%) were seen to have weight far low compared to their age median.

Table 1a: Weight-for-Age Z-Score (WAZ) Distribution (%)

Age	<-3SD Severely Underweight	-3 to -2SD Moderately Underweight	-2 to -1SD (%)	-1 to 0SD (%)	0 to +1SD (%)	+1 to +2SD (%)	+2 to +3SD (%)	>+3SD (%)	Underweight (<-2SD) n/N
3	0.0	0.0	0.0	0.0	42.9	35.7	14.3	7.1	0/14
4	6.2	0.0	0.0	25.0	6.2	31.2	12.5	18.8	1/16
5	7.1	0.0	21.4	14.3	14.3	14.3	0.0	28.6	1/14
6	8.7	4.3	21.7	17.4	17.4	13.0	8.7	8.7	3/23
7	18.2	15.2	24.2	27.3	6.1	3.0	6.1	0.0	11/33

Table 1b. Weight-for-Age Percentiles (%)

Age	Sex	N	Mean Weight (kg)	SD	Weight p-value	5 <sup>th</sup> (%)	15 <sup>th</sup> (%)	50 <sup>th</sup> (%)	85 <sup>th</sup> (%)	95 <sup>th</sup> (%)	Underweight (%) (< 5th)
3	F	5	15.8	0.91	0.3648	15.0	15.0	15.5	16.7	16.9	0.0%
3	M	9	16.9	3.22		15.0	15.0	16.0	17.8	22.2	0.0%
4	F	9	16.8	3.07	0.4303	12.0	15.0	18.0	19.0	19.6	11.1%
4	M	7	17.9	2.59		15.0	15.0	18.0	21.1	21.4	0.0%
5	F	10	19.3	4.27	0.4890	15.0	15.4	18.5	22.0	25.9	0.0%
5	M	4	17.3	4.79		12.5	13.4	17.0	21.2	22.4	25.0%
6	F	14	20.0	2.66	0.3289	16.3	17.0	20.0	23.0	23.5	7.1%
6	M	9	18.8	3.07		15.0	15.6	18.0	20.8	23.4	0.0%
7	F	21	19.5	2.97	0.5398	16.0	16.0	20.0	22.0	25.0	4.8%
7	M	12	18.9	2.35		15.0	17.0	19.0	21.0	21.9	0.0%

All p-values are greater than 0.05, indicating that sex is not a significant factor for anthropometric differences in this cohort

### Height-for-Age

This boxplot (figure 3) compares Height (m) by age. The distribution shows an upward trend with age, but interquartile ranges are tight in some groups, and outliers are present below the WHO height thresholds, especially at ages 4–6. These deviations contribute to the stunting diagnosis.

Table 2a compares the percentage of children with heights below WHO age-specific thresholds based on HAZ. Stunting is most prominent at ages 3 (14.3%) and 4 (18.8%) and persists across all age groups. Summarily, 10% of the population were

stunted based on WHO HAZ. Table 2b shows the percentile distribution of height (m)-for-age by sex. It confers stunting on the population that has weight (kg) below the 5<sup>th</sup> percentile

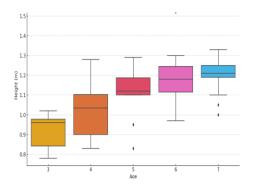


Figure 3: Height-for-Age

Table 2a: Height-for-Age Z-Score (HAZ) Distribution (%)

Age	<-3SD Severely Stunted (%)	-3 to -2SD Moderately Stunted (%)	-2 to -1SD (%)	-1 to 0SD (%)	0 to +1SD (%)	+1 to +2SD (%)	+2 to +3SD (%)	>+3SD (%)	Stunted (<-2SD) n/N
3	0.0	14.3	21.4	14.3	50.0	0.0	0.0	0.0	2/14
4	0.0	18.8	12.5	18.8	18.8	18.8	6.2	6.2	3/16
5	7.1	0.0	14.3	0.0	35.7	28.6	14.3	0.0	1/14
6	0.0	8.7	0.0	30.4	17.4	30.4	8.7	4.3	2/23
7	0.0	6.1	6.1	42.4	42.4	3.0	0.0	0.0	2/33

Table 2b: Height-for-Age Percentiles

Age	Sex	N	Mean Height	SD	Height p-	5 <sup>th</sup>	15 <sup>th</sup>	50 <sup>th</sup>	85 <sup>th</sup>	95 <sup>th</sup>	Stunted
			(m)		value	(%)	(%)	(%)	(%)	(%)	(< 5th)
3	F	5	0.89	0.10	0.4980	0.784	0.792	0.910	0.988	0.996	20.0%
3	M	9	0.93	0.08		0.812	0.840	0.970	0.986	1.008	11.1%
4	F	9	1.05	0.13	0.4519	0.858	0.912	1.080	1.150	1.202	11.1%
4	M	7	1.00	0.15		0.859	0.877	0.990	1.073	1.211	14.3%
5	F	10	1.13	0.08	0.5838	1.018	1.100	1.120	1.193	1.238	10.0%
5	M	4	1.06	0.21		0.848	0.884	1.070	1.245	1.275	25.0%
6	F	14	1.21	0.13	0.2144	1.048	1.080	1.215	1.300	1.377	7.1%
6	M	9	1.15	0.09		1.022	1.104	1.150	1.228	1.242	11.1%
7	F	21	1.20	0.08	0.4676	1.050	1.100	1.220	1.270	1.270	4.8%
7	M	12	1.21	0.03		1.181	1.190	1.205	1.244	1.254	8.3%

All p-values are greater than 0.05, indicating that sex is not a significant factor for anthropometric differences in this cohort

### BMI by Age

Figure 4 illustrates the distribution of Body Mass Index (BMI) across age groups (3–7 years). The figure shows that median BMI appears to decrease slightly with age, it also indicates that outliers are present at the upper end (possible overweight cases), and most values remain below the expected healthy BMI range, indicating malnutrition risk. A gradual decrease in normal BMI with age suggests nutritional interventions are needed.

Table 3 shows there is incidence of malnutrition both sexes for all ages 3 through 7. This was determined by the percentage of children who fall below the

5<sup>th</sup> percentile for their age and sex. Summarily 10% of the population have BMI that suggests malnurishment.

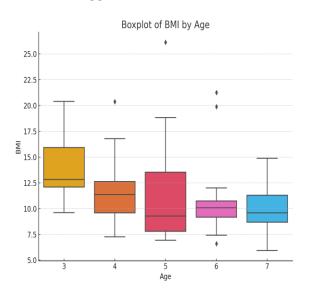


Figure 4: BMI for age

Table 3. BMI Classification by Age (%)

Age	Sex	N	Mean BMI	SD	p-value	5 <sup>th</sup> (%)	15 <sup>th</sup> (%)	50 <sup>th</sup> (%)	85 <sup>th</sup> (%)	95 <sup>th</sup> (%)	Malnourished (<5 <sup>th</sup> percentile) n/N	Malnourishme nt (BMI < 5 <sup>th</sup> ) (%)
3	F	5	13.98	3.60	0.7745	10.4	11.3	12.5	17.4	18.4	1/5	20.0%
3	M	9	13.78	3.31		10.0	10.9	12.9	16.6	19.1	1/9	11.1%
4	F	9	10.58	2.05	0.1439	7.6	8.2	11.2	12.3	12.8	1/9	11.1%
4	M	7	13.61	4.34		9.3	9.7	12.5	17.2	19.3	1/7	14.3%
5	F	10	11.46	4.12	0.7747	7.2	7.6	10.5	16.1	18.1	1/10	10.0%
5	M	4	12.34	9.21		7.2	7.4	8.1	18.2	23.5	1/4	25.0%
6	F	14	10.57	3.00	0.7792	7.7	8.8	9.9	11.9	14.8	1/14	7.1%
6	M	9	10.79	4.18		7.1	8.1	10.4	10.8	17.1	1/9	11.1%
7	F	21	10.17	2.31	0.2845	7.8	8.1	9.8	12.4	14.0	1/21	4.8%
7	M	12	9.47	1.68		6.8	8.0	9.5	11.1	11.8	1/12	8.3%

All p-values are greater than 0.05, indicating that sex is not a significant factor for anthropometric differences in this cohort

### Mid-Upper Arm Circumference

For children 6-59 months, MUAC is a quick way to assess acute malnutrition. From Table 4, a significant portion of children at ages 3 and 4 fall below WHO-recommended MUAC values. This suggests widespread protein-energy malnutrition

Table 4: Mid-Upper Arm Circumference Status

among preschool aged children in this setting.

#### **Head Circumference (Ages 3–5)**

As seen in Table 5, most children fell below the WHO thresholds, indicating possible early-life undernutrition and risks for impaired cognitive development.

Age	Number children (N)	of	Below WHO reference (n)	D Below %	WHO Referenc (cm)
3	14		3	21.4	13.5
4	16		4	25.0	14.0
_ 5	14		4	28.6	14.5

Table 5: Head Circumference Status

Age	Number	of Belov	v WHO	Below %	WHO Reference (cm)
	children (N)	refere	ence (n)		
3	14	7		50.0	49.5
4	16	10		62.5	50.5
5	14	8		57.1	51.0

# Academic performance of 100 pupils studying in primary schools within Osara community

This bar chart illustrates the distribution of academic grades across five core subjects English, Mathematics, Science, General Knowledge, and Logical Thinking among primary school children aged 3 to 7 years in Osara. Grades were assigned based on percentage scores, categorized as follows: A  $(\geq 70)$ , B (60-69), C (50-59), D (45-49), E (40-44), and F (<40). Each bar

represents the number of students who attained a specific grade in each subject.

Each subject's grade distribution reveals significant trends in academic achievement and areas requiring attention. In English, pupil performance is notably polarized. Many students scored within the C (36 students) and F (35 students) grade bands, while 29 students excelled with an A. Interestingly, no pupils fell into the B, D, or E categories. This suggests a sharp divide in competency levels, possibly pointing to disparities in language exposure, teaching efficacy, or learning support. In Mathematics, the performance is concerning. Many students (53) scored E, with another 24 students failing (F). Only a very small number; two students achieved an A, reflecting significant challenges in understanding or engagement with numeracy. The narrow spread of high scores indicates that foundational mathematical concepts may not be adequately grasped at early stage.

General Knowledge also shows a bimodal distribution. While 41 students failed (F), another 30 achieved an A, and 29 received a C. This pattern mirrors that of English, reflecting a possible gap between learners who are exposed to diverse information and those who are not. Science follows a

similar trend, with equal numbers of students scoring A (24), C (38), and F (38). The absence of students in the B, D, and E bands suggests that pupils are either grasping scientific concepts well or struggling entirely, possibly depending on the mode of delivery or access to practical examples. Logical Thinking presents a concentrated distribution. dominant 64 students scored C. while the remaining 36 fell into the F grade. There were no students in the A through E categories. This suggests that while a substantial number of pupils demonstrate average reasoning ability, a significant portion is still unable to meet basic logical benchmarks. The absence of higher scores may reflect curriculum limitations at the foundational level.

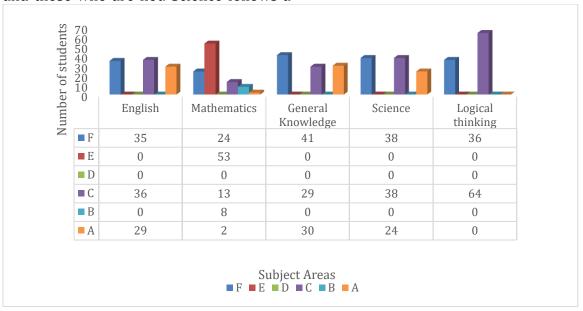


Figure 5: Grade distribution by Subject

## Aflatoxin Level in cereals and Exposure to aflatoxin

Table 6 summarizes the incidence and level of AfB1 in rice and maize consumed in Osara community, while table 7 estimates the exposure to aflatoxin. The exposure estimate was derived based on

the weight (kg) of each pupil. The average of 160g/day of rice/maize (based on estimated daily consumption by FAO/WHO for children 3–7 in Nigeria consuming 120g of rice and 200g of maize per day), mean aflatoxin level of  $7.15 \mu g/kg$  and average body weight of each child was used.

Table 6: Aflatoxin level in rice and maize samples from Osara, Kogi State

Sample type	n/N	Mean (μg/kg)	Min (µg/kg)	Max (μg/kg)	n/N%	
Maize	27/31	5.748±5.008	2.400	16.394	87.10	
Rice	29/31	7.885 <u>+</u> 7.445	2.436	29.211	93.55	
Average		7.1500				

**Table 7**: Aflatoxin B1 exposure of children in Osara Community

Age	N	Mean (ng/kg bw/day)	SD	Min (ng/kg bw/day)	Max ng/kg bw/day)
3	14	66.14	7.78	42.90	71.50
4	16	64.08	13.57	49.88	107.25
5	14	60.09	13.43	36.98	89.38
6	23	56.01	8.31	42.90	71.50
7	33	56.71	8.17	41.25	76.61

## Interrelationship of parameters and variables

# Correlation Heatmap for Anthropometry, Academic Scores, and EDI

This heatmap shows the Pearson correlation coefficients among aflatoxin exposure (EDI), anthropometric indices (Age, BMI, height, weight, MUAC, Head circumference), and academic performance variables (e.g., Average Score, English, Mathematics, science, general knowledge, logical reasoning). Generally, a weak to moderate negative

correlation was observed between EDI and academic performance, while positive correlations among different test scores. suggested internal consistency academic performance. A strong negative correlation existed between dietary intake of aflatoxin (EDI) and weight (-0.96), suggesting that higher exposure to aflatoxins is associated with reduced weight. The effect of this is also seen as there was also negative correlation between EDI and BMI. Also, very subtle negetive correlation (-0.20 to -0.29) existed between EDI and all subjects tested except mathematics.

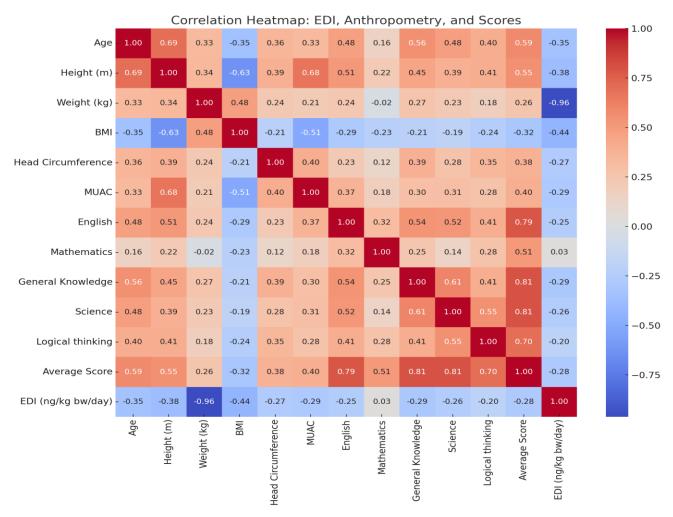


Figure 6. Correlation Heatmap

### Relationship among test parameters

Scatter plots was also used to further illustrate several relationships. In figure 7 where the relationship between **EDI** (ng/kg bw/day) and BMI was considered (with point colour denoting age). The negative trend line indicates that higher aflatoxin exposure is generally associated with lower BMI, supporting the hypothesis that chronic aflatoxin exposure contributes to undernutrition. This agrees with result from the heatmap earlier described.

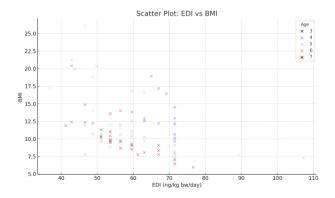


Figure 7. Scatter BMI vs Average score

In figure 8, the plot shows the association between BMI and Average Score. The negative slope implies that lower BMI may be associated with poorer cognitive performance. The clustering of points at low BMI and low score regions may reflect overlapping risks of malnutrition and poor academic achievement.

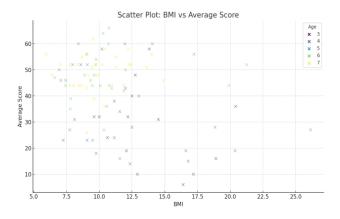


Figure 8. Scatter: EDI vs Average Score

In figure 9, the plot shows that as **aflatoxin exposure increases**, **academic performance decreases**, although the association is not highly linear. This supports the proposed pathway where aflatoxin exposure negatively affects cognitive outcomes, possibly through nutritional or neurotoxic effects.

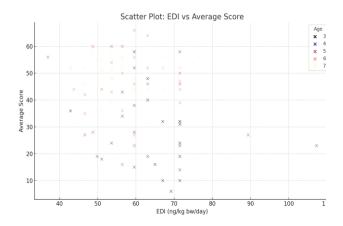


Figure 9. Scatter: EDI vs Average Score

### 3.4.3. Mediation Analysis

Baron & Kenny mediation results indicate that BMI partially mediates the effect of aflatoxin exposure (EDI) on academic performance:

Path a (EDI  $\rightarrow$  BMI): -0.1501

Path b (BMI → Score | EDI): -2.099 Indirect effect (a\*b): 0.315 Direct effect (c'): -0.69 Total effect (c): -0.3751

Thus, signifying that higher EDI significantly reduces BMI, Lower BMI is associated with lower academic scores, and BMI suppresses part of EDI's impact on academic performance (revealed from positive indirect effect i.e. partial mediation).

#### DISCUSSION

#### 4.1 Nutritional Status of Pupils

The findings of this study indicate a high malnutrition prevalence of among children aged 3-7 years in Osara, Nigeria. The anthropometric indices WAZ, HAZ, BMI-for-age reveal significant and deviations from WHO growth standards. The incidence of underweight increased with age, with children aged 6 and 7 years recording the highest rates (13% and 33.4%, respectively). Although age 3 recorded no underweight cases, the prevalence increased sharply by age 7. suggesting progressive nutritional deterioration as children grow older. The percentile-based analysis reinforced this, showing that a notable proportion of children, particularly males at age 5 (25%), were significantly below median weight.

Stunting was also prominent, particularly at younger ages, with the highest rates observed at ages 3 and 4 (14.3% and 18.8% severely stunted, respectively), suggesting early-life chronic malnutrition. These findings align with earlier epidemiological reports showing that linear growth faltering often begins in the first 1,000 days of life and persists without timely intervention [5,6].

BMI analysis further highlighted malnutrition, with consistent underweight classifications across all age groups. Median BMI was below the healthy range of 14–18 kg/m² for most children, with 25% of 5-year-old males classified as underweight. These findings suggest widespread protein-energy malnutrition, possibly exacerbated by poor dietary quality and environmental conditions.

Additional indices such as MUAC and head circumference further emphasized this trend. Over 20% of children at ages 3-5 fell below WHO MUAC references, indicating acute malnutrition. Moreover, over half of the children in this age group had head circumferences below WHO thresholds, potentially reflecting impaired neurodevelopment linked to chronic nutritional deficiencies [20]. together, these anthropometric indicators highlight a multidimensional nutritional crisis affecting young children in Osara. The overlapping prevalence underweight, stunting, low BMI, and poor MUAC readings indicates both chronic and acute forms of malnutrition, reflective of food insecurity, poor dietary diversity, and potentially compromised absorption due to environmental enteropathy or toxin exposure such as AfB1. Given the role of early nutrition in shaping not only physical growth but also cognitive development and lifelong health, these findings underscore an urgent need for targeted nutrition programs, improved food safety measures, and sustained community health interventions in the region.

### 4.2 Academic Performance of Pupils

Academic performance across core subjects English, Mathematics, Science, Logical Thinking, and General Knowledge

was generally poor. A high frequency of D, E, and F grades were recorded. Overall, the data reveals key insights into the academic strengths and weaknesses of early-grade pupils in Osara. There is a consistent pattern of polarized performance in most subjects, with significant numbers either excelling or failing, and few students occupying the middle-performance range. The results call for focused intervention in Mathematics and Logical Thinking, as well as differentiated instruction strategies to bridge learning gaps in English and Science. Enhancing teacher training, curriculum delivery. and learning resources could play a pivotal role in addressing these disparities improving overall educational outcomes in the community.

This underperformance may reflect both cognitive deficits and external factors such poor learning environments. as socioeconomic limitations. inadequate early childhood education. Cognitive performance is closely tied to both nutritional status and environmental stimuli. Malnutrition, particularly during critical periods of brain development, has been associated with impaired learning, reduced attention span, and diminished memory [21]. These effects are not only physiological but also behavioural, as undernourished children may be less engaged and more prone to fatigue, impacting academic achievement.

Furthermore, the clustering of low BMI and low academic scores especially in Mathematics and Logical Reasoning suggests an interplay between physical health and cognitive outcomes, a relationship explored further in section 4.4.

#### 4.3 Exposure to Aflatoxin B1

The cereal samples analyzed in this study showed alarmingly high levels of aflatoxin B1 contamination. Average AfB1 concentrations were 5.748  $\mu$ g/kg for maize and 7.885  $\mu$ g/kg for rice both significantly above the WHO/EU safety threshold of 2  $\mu$ g/kg. Over 88% of maize and 94% of rice samples exceeded this limit. Consequently, children consuming these staples were chronically exposed to unsafe levels of aflatoxin, with average estimated daily intakes (EDI) ranging from 56.01 to 66.14 ng/kg bw/day.

This exposure range falls within, or above levels previously linked with growth impairments. For example, Makori et al. [22] found EDI values between 0.1 and 23,172 ng/kg bw/day among Tanzanian infants, with a significant proportion showing stunted growth. Similarly, Ezekiel et al. [23] reported widespread urinary AFM1 detection in Nigerian children, confirming systemic exposure.

Given the dietary reliance on maize and rice in Osara, the pervasive contamination is deeply concerning. The results support prior conclusions that food safety lapses in rural settings significantly increase exposure risks aflatoxin [24,25]. Importantly, there is currently tolerable daily intake for AfB1 due to its carcinogenic genotoxic and highlighting the urgency of intervention.

# 4.4 Relationship Between Nutritional Status, Academic Performance, and Aflatoxin B1 Exposure

A clear pattern emerged linking high aflatoxin exposure, poor nutritional status, and low academic performance. The strong negative correlation between EDI and weight (-0.96), and between EDI and

BMI, suggests that chronic dietary exposure to AfB1 contributes directly to undernutrition. These findings consistent with the mechanism proposed by Rasheed et al. [5], where AfB1 disrupts intestinal integrity, immune function, and nutrient absorption. Moreover, academic performance negatively was also correlated with both EDI and BMI. The mediation analysis confirmed that BMI partially mediates the impact of EDI on academic outcomes. Specifically, higher aflatoxin intake led to lower BMI, which in turn contributed to reduced academic scores. This implies that aflatoxin exerts both direct neurotoxic effects and indirect effects through malnutrition, a dual pathway supported by earlier animal studies and human observational data [10,11].

These results underscore a vicious cycle where contaminated diets impair both physical and cognitive development. The clustering of low BMI and low cognitive scores in scatterplots further validates this relationship. While environmental and educational factors likely contribute to poor academic outcomes, the role of aflatoxin as both a nutritional and neurocognitive hazard is significant. In alignment with findings from Andrews-Trevino et al. [7] and Kiarie et al. [26], this study provides new evidence from Osara supporting the global hypothesis that aflatoxin exposure is a critical determinant of both poor growth outcome and learning deficits in children. It also supports calls for integrated strategies that combine food safety, nutrition, and education interventions to break the cycle of underdevelopment in vulnerable communities.

#### CONCLUSION AND RECOMMENDATIONS

This study reveals a troubling intersection of food contamination, malnutrition, and academic underperformance school-aged children (3-7 years) in Osara, Nigeria. Dietary exposure to aflatoxin B1 was alarmingly high, with 93.55% of rice and 87.10% of maize samples exceeding the safety limit of 2 µg/kg, and children's estimated daily intake (EDI) ranging from 56.01 to 66.14 ng/kg bw/day. Nutritional assessment revealed that 16% of children were underweight, 10% were stunted, and 10% had low BMI-for-age indicating malnutrition. Α strong negative correlation (r = -0.96) was found between aflatoxin exposure and weight, and mediation analysis confirmed that BMI partially mediates the effect of aflatoxin on academic performance. These findings underscore the urgent need for food safety regulations, community-based nutrition programs, and school feeding initiatives to mitigate the impact of aflatoxin exposure on child growth and learning in vulnerable rural communities.

# 6. DECLARATIONS Authors' Contribution

ADO conceptualized the study. ADO, BAE, AKA and SY designed the study. YT, AVI and AMA participated in fieldwork and data collection. ADO, OSO, KJ performed the data analysis; ADO, OSO, KJ and EY interpreted the data. ADO prepared the first draft of the manuscript, reviewed by BAE, AKA, OSO, AMA and AKA. All authors contributed to the development of the final manuscript and approved its submission.

# **Disclosure of Conflict of Interest** *None*

#### **Ethics Approval and Informed Consent**

Ethical approval for this study was granted by the Research Ethics Committee of Confluence University of Technology, Osara. All participants were duly informed of the objectives of the study and the protocol for sample collection. All participants signed an informed consent form. Participation was voluntary.

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