Kashf Journal of Multidisciplinary Research
Vol: 03 - Issue 03 (2026)
P-ISSN: 3007-1992 E-ISSN: 3007-200X
https://kjmr.com.pk

ASSESSMENT OF MYCOTOXINS IN MAIZE AND ASSOCIATED DIETARY RISK IN SINDH PAKISTAN

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DOI: (*https://doi.org/10.71146/kjmr872*)

Article Info

Abstract

A study was conducted to determine the natural co-occurrence of mycotoxins (aflatoxins and ochratoxins) in maize grown in Sindh, Pakistan. Mycotoxins were determined by using competitive enzyme linked immunosorbent assay. Aflatoxins were detected in 47.4% of samples (1.4–67.7 µg/kg), with 24.1% exceeding the maximum residue limit (MRL, 20 µg/kg). ochratoxin was detected in 19.0% of samples (1–35.5 µg/kg), with 3.4 % exceeding the MRL (5 µg/kg). Co-occurrence was found in 11.2 % and observed no significant association statistically ($p>0.05$). Furthermore, a dietary risk assessment was calculated with mean and worst-case exposure and observed that aflatoxins posed a significant health concern yield a low margin of exposure (MOE: 17.39 and 3.55, respectively). Similarly, ochratoxins exposure through the maize exceeded 3.48-fold tolerable daily intake at worst case scenario. These findings highlight substantial contamination and associated health risks in maize from Sindh.

Keywords: *Mycotoxins, Maize, Aflatoxins, Ochratoxins, Food toxicity, Pakistan.*

1. Introduction

The third most important cereal crop in Pakistan, after rice and wheat, is maize (Zea mays L.), which significantly contributes to agricultural income and food security. Every year, the country cultivates over 1.6 million hectares of maize, producing roughly 10.6 million tons. (Zaid, 2025). In Sindh, maize is grown in a variety of agroclimatic conditions, from the hot, desert higher zone to the humid coastal lower zone, throughout two distinct seasons: the Kharif (fall, sown in July) and Rabi (spring, sown in February) (Group, 2025). Despite its significance, maize is particularly vulnerable to fungal contamination and the associated buildup of mycotoxins during pre-harvest stress events (drought, insect damage) and post-harvest under circumstances of high humidity and warmth (Bereziartua et al., 2025; Zheng et al., 2024). When filamentous fungus, mainly Aspergillus, Fusarium, and Penicillium species, colonize agricultural commodities in the field or during storage, they produce mycotoxins, which are secondary hazardous compounds (Bräse et al., 2009). According to estimates from the Food and Agriculture Organization (FAO) of the United Nations, mycotoxin contamination affects about 25% of global food supplies each year (FAO, 2013).

Aspergillus flavus and Aspergillus parasiticus are the main producers of aflatoxins, a class of structurally similar Di furanocoumarin chemicals (Zheng et al., 2024). The four main aflatoxins, AFB1, AFB2, AFG1, and AFG2, have strong hepatotoxic and carcinogenic effects; the International Agency for Research on Cancer has designated AFB1 as a Group 1 human carcinogen (IARC, 2012). Aspergillus ochraceus and Penicillium verrucosum are the primary producers of OTA, a chlorinated is coumarin–phenylalanine derivative. It has teratogenic, immunosuppressive, and nephrotoxic properties and is categorized as a Group 2B potential human carcinogen (Lyon, 1994). The European Union ((EC)No1881, 2006) established maximum regulatory limits (MRLs) of 2 μg/kg for AFB1 and 4 μg/kg for total aflatoxins in maize, and 5 μg/kg for OTA in cereals meant for direct human consumption.

There is ample evidence that the co-occurrence of several mycotoxins in a single commodity poses a synergistic or additive danger to the health of humans and animals (Ficheux et al., 2012). Aspergillus and Penicillium/Aspergillus ochraceus species thrive in Sindh because to the region's high temperatures (28–45°C), minimal annual rainfall (5–7 inches), and seasonal variations in humidity (30–54%)(Rao et al., 2009; Shafique et al., 2020). Maize is particularly susceptible to mycotoxin accumulation due to post-harvest conditions, such as insufficient drying facilities, subpar storage infrastructure, and a lack of hermetic storage technologies (Javed et al., 2024; Khatoon et al., 2012; Shafique et al., 2020)

Despite the significant public health risks associated with mycotoxins, Pakistan faces critical gaps in its regulatory framework (Qazi & Fayyaz, 2006). The government has not yet set national Maximum Residue Limits (MRLs) for Ochratoxin A (OTA) in maize, however, Pakistan Standards and Quality Control Authority (PSQCA) adopting a general limit of 20 μg/kg for total aflatoxins in food and feed(Ashraf et al., 2023; PSQCA, 2022). Additionally, these requirements are rarely implemented across the domestic supply chain due to the lack of a coordinated national monitoring program, leaving local consumers at risk of contaminated product that does not satisfy international safety standards(Ashraf et al., 2023). The majority of Pakistani research to date has either concentrated on Punjab or only examined samples of chicken feed, leaving the larger human food supply chain inadequately described (Ahmada et al., 2025). Furthermore, no study has thoroughly investigated the natural co-occurrence of OTA and aflatoxins in all three of Sindh's agro-ecological zones.

This study aimed to determine the co-occurrence of aflatoxins and ochratoxins in maize samples collected from representative districts throughout all three climatic zones of Sindh and to evaluate the dietary risk linked to maize consumption.

2. Materials and Methods

2.1 Chemicals and Reagents

Neogen Corporation (Lansing, MI, USA) provided the OTA test kits (Veratox® Ochratoxin, Product No. 8610) and aflatoxin test kits (Veratox® Aflatoxin, Product No. 8030). We bought analytical-grade methanol from Sigma-Aldrich (Steinheim, Germany). For the extraction and washing processes, deionized water was utilized. Before being used, all calibration standards were kept at -20°C in 70:30 (v/v) methanol–water stock solutions. Calibration curve for aflatoxins and ochratoxins was generated by using standards provided with kits. All ELISA absorbance measurements were performed using a Biochrom Anthos 2010 microplate reader (Biochrom Ltd., Cambridge, UK) fitted with a 650 nm filter.

2.2 Study Area and Sample Collection

Sindh province is divided into three distinct agro-ecological zones based on topography and climate: (i) Upper Sindh (Siro) — comprising the districts of Khairpur, Ghotki, Sukkur, Naushahro Feroze, Nawabshah, and Shikarpur; (ii) Middle Sindh (Wicholo) — comprising Hyderabad, Sanghar, , Mirpurkhas, Umerkot, Dadu and (iii) Lower Sindh (Lar) — comprising Sujjawal, Badin, Thatta and Karachi. Sindh experiences 125–200 mm of annual rainfall, which is concentrated during the Kharif monsoon season (July–September). The region also experiences typical annual temperatures of 28–45°C and relative humidity of 30–54%.

One hundred and sixteen samples of maize grain were taken during the spring (Rabi) and fall (Kharif) harvest seasons. Samples were obtained from local grain markets, storage facilities, and farms in each district: Khairpur (n = 10), Ghotki (n = 4), Sukkur (n = 9), Naushahro Feroze (n = 7), Nawabshah (n = 9), Shikarpur (n = 8), Hyderabad (n = 7), Sanghar (n = 9), Badin (n = 4), Mirpurkhas (n = 8), Umerkot (n = 7), Dadu (n = 8), Thatta (n = 9), Lar (n = 7), and Karachi (n = 10). In accordance with European Commission sampling requirements, each sample comprised roughly 2 kg of whole maize grains that were randomly stratified sampled from several bags or heaps at each location ((EC)No1881, 2006). Samples were brought to the lab in sealed polyethylene bags, where they were kept at 4°C until analysis.

2.3 Sample Preparation

Each sample (1 kg) was ground to a particle size of less than 0.75 mm using a Christy hammer mill (Christy Turner Ltd., Chelmsford, UK). Ground samples were thoroughly mixed and a representative sub-sample of 50 g was obtained by coning and quartering. Mycotoxin extraction was performed using a methanol-water solvent system in accordance with the manufacturer's instructions (Neogen Corp., Lansing, MI). For total aflatoxins, 50 g of ground maize was extracted with 250 mL of 70% methanol (v/v) at a 1:5 (w/v) ratio. For ochratoxin A (OTA), 10 g of sample was mixed with 40 mL of 70% methanol (v/v) at a 1:4 (w/v) ratio. All mixtures were shaken vigorously for three minutes using a mechanical shaker at 250 rpm. To generate a clean filtrate for further ELISA measurement, the resultant extracts were filtered using Whatman No. 1 filter paper after being allowed to settle for three minutes. Mycotoxins were measured using a competitive direct enzyme-linked immunosorbent assay (CD-ELISA) in accordance with the kit's usual procedure.

2.4 ELISA Analysis

The optical densities (OD) of the resulting chromogenic reactions were measured within 15 minutes using a Biochrom Anthos 2010 microplate reader (Biochrom Ltd., Cambridge, UK) equipped with a 650 nm filter. A four-point calibration curve was generated for aflatoxins (0, 5, 15, and 50 µg/kg) and a five-point curve for OTA (0, 2, 5, 10, and 25 µg/kg). Sample concentrations were calculated by using calibration equation obtained from the standards. Samples exceeding the upper limit of quantitation (50 µg/kg for aflatoxins; 25 µg/kg for OTA) were appropriately diluted with 70% methanol and re-assayed to ensure results fell within the linear range of the calibration curve.

2.5 Method Validation

The ELISA method was validated in-house for the maize matrix using the standard addition approach, in accordance with Commission Directive 2002/27/EC (Directive, 2002/27/EC). Blank maize samples (confirmed mycotoxin-free by preliminary screening) were spiked at 5 and 50 µg/kg, 10, 25 µg/kg for aflatoxins and ochratoxins respectively. The LOD and LOQ were as specified by the kit manufacturer (aflatoxins: LOD 1.4 µg/kg, ochratoxins LOD 1 µg/kg) and are consistent with values reported in the literature for this method (Majeed et al., 2013; Shar et al., 2014)

2.6 Statistical Analysis

Data were expressed as mean ± standard deviation (SD). The coefficient of variation (CV%) was calculated as (SD/mean) × 100. The chi-square test (χ^2) was used to assess significant differences in contamination incidence among districts and zones. Pearson correlation analysis was performed to examine the relationship between aflatoxin and OTA concentrations in co-contaminated samples. All statistical analyses were conducted using SPSS Version 20.0 (IBM Corp., Armonk, NY, USA). Statistical significance was set at $p < 0.05$.

3. Results

3.1 Method Validation

Mean recoveries of total aflatoxins from spiked maize samples ranged from 85.3 to 92.4% (CV: 10.1–12.4%), and mean recoveries of OTA ranged from 83 to 93.6% (CV: 9.5–13.2%), all within the acceptability criteria of 70–110% specified by Commission Directive 2002/27/EC (Directive, 2002/27/EC). These recovery values are consistent with those reported by comparable Pakistani studies using the same ELISA methodology; Majeed et al. (2013) reported aflatoxin recoveries of 80–92% and OTA recoveries of 78–88% from maize and rice matrices, while Shar et al. (2013) reported aflatoxin recoveries of 83–91% from poultry feed. Khatoon et al. (2012) similarly reported recoveries of 78–89% for aflatoxins in maize from Pakistan, confirming that the performance of the Neogen Veratox® kit is consistent across Pakistani cereal matrices.

The LOD and LOQ were as stated in the manufacturer's specification (Neogen Corporation, Lansing, MI, USA): for total aflatoxins, LOD 1.4 µg/kg with a working range of 5–50 µg/kg; for OTA, LOD 1 µg/kg with a working range of 2–25 µg/kg. Samples containing aflatoxin or OTA concentrations exceeding the upper limit of the working range were diluted appropriately with PBS-Tween buffer supplied with the kit and re-analyzed. Calibration curves were obtained for aflatoxins over the working range of 5–50 µg/kg ($R^2 = 0.992$) and for OTA over 2–25 µg/kg ($R^2 = 0.988$), confirming satisfactory analytical performance

3.2 surveillance results

Fifty-five (47.4%) of the 116 maize samples tested positive for total aflatoxins over the limit of detection (1.4 µg/kg). The range of contamination levels was less than 1.4 to 67.7 µg/kg. The distribution of aflatoxin contamination by district and overall incidence are displayed in Table 1. Karachi had the lowest occurrence, while Sukkur, Sanghar, and Thatta had the highest rates, followed by Ghotki and Badin. The districts' mean aflatoxin levels varied, with Khairpur, Sukkur, and Sanghar showing the highest amounts. The maximum residue limit (MRL) of 20 µg/kg was significantly exceeded by 28 samples (24.1%), raising serious concerns about food safety. A number of samples showed values greater than 50 µg/kg, indicating a higher risk of consumer exposure.

Table 1. Incidence, mean concentration, range and MRL violations of total aflatoxins and Ochratoxin A (OTA) in maize samples

District	n	Aflatoxins				OTA			
		Incidence (%)	Mean (µg/kg)	Maximum (µg/kg)	>*MRL	Incidence (%)	Mean (µg/kg)	Maximum (µg/kg)	>**MRL
Khairpur	10	40.0	15.8	67.7	2	50.0	6.02	30	2
Ghotki	4	50.0	17.2	55	1	25.0	1.7	4.8	0
Sukkur	9	55.6	15.5	65	2	33.3	5.7	35.5	2
N. Feroze	7	42.9	13.2	62.5	1	14.2	1.2	4.8	0
S. Benazir Abad	9	44.4	14.2	50	2	11.1	1.04	5.0	0

Shikarpur	8	37.5	11.3	46	2	12.5	1.01	4.8	0
Hyderabad	7	42.9	15.8	45	3	14.3	1.3	5.0	0
Sanghar	9	55.6	17.56	61	2	22.2	1.2	4.5	0
Badin	4	50.0	8.13	20	0	0.0	ND	ND	0
Mirpurkhas	8	37.5	11.1	46	2	12.5	1.21	5.0	0
Umerkot	7	42.9	13.2	54.4	2	14.3	1.20	4.5	0
Dadu	8	37.5	12.2	50	2	0.0	ND	ND	0
Thatta	9	55.6	16.7	46	3	22.2	1.40	4.3	0
Sujjawal	7	42.9	15.1	47.3	2	14.3	0.99	4.8	0
Karachi	10	30.0	9.3	44.2	2	10.0	0.88	4.2	0
Total	116	47.4	13.8	67.7	28	18.9	1.9	35.5	4

*MRL violations for aflatoxins based on (FDA, 2021) limit of 20 µg/kg. **MRL violations for OTA based on EU limit of 5 µg/kg for cereals (EuropeanCommission, 2023). ND: not detected.

OTA was detected in 22 of 116 samples (19.0%), at concentrations ranging from 1 to 35.5 µg/kg (Table 1). The highest incidence of OTA contamination was observed in upper Sindh districts, particularly Khairpur and Sukkur. A total of 4 samples (3.4%) exceeded the MRL of 5 µg/kg for OTA in cereals. Lower Sindh districts (Badin, Dadu, Karachi) recorded comparatively lower OTA contamination frequencies. The mean OTA concentration in positive samples was highest in Sukkur and Hyderabad, with the maximum individual level of 35.5 µg/kg recorded in a Sukkur sample. Unlike previous assumptions, not all OTA-positive samples were co-contaminated with aflatoxins, indicating partial rather than complete co-occurrence.

Co-occurrence analysis revealed that 13 samples (11.2%) were simultaneously contaminated with both aflatoxins and OTA. Chi-square analysis showed no statistically significant association between the occurrence of the two mycotoxins ($\chi^2 = 2.31$, $p > 0.05$). However, correlation analysis using Spearman's rank test demonstrated a weak but statistically significant positive relationship ($\rho = 0.21$, $p < 0.05$), suggesting a limited tendency for co-contamination under shared environmental conditions. Co-contamination was more frequently observed in upper Sindh districts, where higher temperatures and suboptimal storage conditions favor fungal proliferation.

Table 2: Co-occurrence of Aflatoxin and Ochratoxin in Maize Samples

Zone	**n**	**AF Positive (%)**	**OTA Positive (%)**	**Co-occurrence (%)**	χ^2	**p-value**
Siro	47	61.7% (29)	25.5% (12)	17.0% (8)	2.84	0.092
Wicholo	43	44.2% (19)	11.6% (5)	4.7% (2)	1.76	0.184
Lar	26	26.9% (7)	19.2% (5)	11.5% (3)	0.88	0.348
Total	116	47.4%	18.9%	11.2%	2.31	0.05

4. Discussion

The present study provides the first comprehensive survey of natural co-occurrence of aflatoxins and OTA across all three agro-ecological zones of Sindh province, Pakistan. The overall aflatoxin incidence of 47.4% and OTA incidence of 18.9% are comparable to, but generally lower than, contamination levels reported from other cereal surveys in Pakistan and South Asia (Table 3).

Table 3. Global comparison of aflatoxin and OTA occurrence in maize.

Study	Country	Sample	Aflatoxins		Ochratoxins	
			%	Maximum (µg/kg)	%	Maximum (µg/kg)
Present study	Pakistan	Maize	47.4	67.7	18.9	35.5
(Gillani et al., 2022)	Pakistan	Maize	69	92	61	54
(ul Hassan et al., 2020)	Pakistan	Maize	100	362	71	214
(Iram et al., 2014)	Pakistan	Maize	97	399	ND	ND*
(Majeed et al., 2013)	Pakistan	Maize	36	100	27	90
(Shah et al., 2010)	Pakistan	Maize	89	31	77	7
(Khatoon et al., 2012)	Pakistan	Maize	28	850	NA	NA**
(Lewis et al., 2005)	Kenya	Maize	55	1000	NA	NA
(Zinedine & Mañes, 2009)	Morocco	Maize	80	11	40	7
(Ghiasian et al., 2011)	Iran	Maize	80	317	NA	NA
(Giray et al., 2009)	Turkey	Maize	47	120	57	9
(Tangendjaja et al., 2008)	Indonesia	Maize	100	134	1	2

*Not detected, **Not analyzed

Within Pakistan, Majeed et al. (2013) identified aflatoxin contamination in 56% of rice and 35% of corn samples from Punjab, with average total aflatoxin levels of 19.54 µg/kg and 12.08 µg/kg respectively, and reported that 28% of rice and 14% of corn samples exceeded EU limits. Shah et al. (2010) detected AFB1 in 77.78–88.89% of maize samples from Swat Valley at concentrations up to 30.92 µg/kg. (Niaz & Dawar, 2012) identified mycotoxin contamination in 84.7% of Pakistani maize seed samples. More recently, (ul Hassan et al., 2020) reported that all seven commercially grown maize varieties surveyed in Pakistan contained aflatoxins exceeding the maximum tolerable limit at concentrations up to 362.8 µg/kg(ul Hassan et al., 2020), while Gillani et al. (2022) detected aflatoxins in 69% of maize samples from multiple Pakistani regions(Gillani et al., 2022). According to these comparisons, Sindh maize appears to be slightly less contaminated than maize from Punjab and Khyber Pakhtunkhwa. This is probably due to Sindh's drier climate, which lessens Aspergillus colonization prior to harvest. Nevertheless, a substantial proportion of samples exceeded EU MRLs, indicating a serious food safety concern.

The present study's aflatoxin incidence is consistent with contamination rates in Mediterranean countries sharing a semi-arid climate with Sindh, such as Morocco (55%) (Zinedine & Mañes, 2009), Turkey (47%)(Giray et al., 2009), and Iran (80%) (Ghiasian et al., 2011), but considerably lower than sub-Saharan Africa, where contamination frequencies of 55–83% have been reported in Kenya and West Africa (Hell et al., 2000; Lewis et al., 2005), and South-East Asia, where 74% of maize samples were contaminated in Indonesia(B Tangendjaja et al., 2008), reflecting hotter and more humid conditions in those regions. The maximum aflatoxin concentration of 67.7 µg/kg observed in this study exceeds values from Vietnam (up to 34.8 µg/kg) but remains lower than extreme concentrations in Africa (up to 770 µg/kg) and India (up to 890 µg/kg).

The OTA incidence of 18.9% and concentration range of 1–35.5 µg/kg observed in the present study is lower than those reported in the previous study from Sindh by (Zafar et al., 2001), who detected OTA in poultry feed ingredients. Majeed et al. (2013) reported OTA contamination in 42% of maize samples from Punjab, while Hassan et al. (2020) and Gillani et al. (2022) documented OTA incidences of 71% and 61%, respectively, in Pakistani maize. Globally, OTA contamination rates range from below 10% in Europe (Zinedine et al., 2007; Castillo et al., 2008) to over 50% in tropical African settings, with the 18.9% incidence in Sindh consistent with the intermediate pattern expected from a semi-arid subtropical region.

4.1 Co-occurrence

Co-occurrence analysis demonstrated that 11.2% of maize samples were contaminated with both aflatoxins and OTA. In contrast to earlier assumptions of complete co-occurrence, the present data indicate that the two mycotoxins occur largely independently (Table 1). Chi-square analysis confirmed no statistically significant association between their occurrence ($p > 0.05$), while Spearman correlation revealed only a weak positive relationship ($\rho = 0.21$, $p < 0.05$). These findings suggest that although similar environmental conditions may support the production of both toxins, their biosynthesis is influenced by distinct fungal species and ecological factors.

This observation is consistent with global reports indicating variable co-occurrence patterns depending on climatic and storage conditions (Table 4) (Eskola et al., 2020). Higher temperatures, more grain moisture, and conventional storage methods like clay silos (kothis), which encourage fungal development, are probably the causes of the higher incidence of co-contamination in upper Sindh districts (Khairpur, Sukkur) (Asghar et al., 2020). The toxicological implications remain significant, as combined exposure to aflatoxins and OTA may result in additive or synergistic hepatotoxic, nephrotoxic, and immunosuppressive effects (Ficheux et al., 2012; Eskola et al., 2020). OTA is classified by IARC as a Group 2B possible human carcinogen(Malir et al., 2016)

Table 4. Global studies reporting co-occurrence of aflatoxins and Ochratoxin A in maize and cereals.

Study	Country/Region	AF (%)	OTA (%)	Co-occur (%)	Key Findings
Present study	Sindh, Pak.	44.8	18.1	11.2	Strong AF–OTA correlation
(ul Hassan et al., 2020)	Pakistan	100	71	Common*	Multi-toxins common

(Gillani et al., 2022)	Pakistan	69	61	Common	Multi-toxins common
(Pleadin et al., 2017)	Croatia	10.8	16.2	Common	Maize most contaminated; Fusarium toxins dominant
(Villa & Markaki, 2009)	Greece	56.3	60	Common	AF in corn-based cereals; OTA/ZEA co-occurrence
(Alshannaq & Yu, 2017)	Global review	Var.	Var.	Common	Climate strongly influences AF/OTA co-contamination
(Eskola et al., 2020)	Global survey	Var.	Var.	Common	Most samples contain ≥2 mycotoxins simultaneously
(Kimanya et al., 2008)	Tanzania	35	12	Common	Poor storage increased co-occurrence risk

[a] found co-occurrence, Var: variable

4.3 Dietary Risk Assessment

To evaluate potential health risks, dietary exposure was assessed using updated mean (AF: 13.8 µg/kg; OTA: 1.9 µg/kg) and worst-case concentrations (AF: 67.7 µg/kg; OTA: 35.5 µg/kg). Results are presented in Table 5. Under the mean exposure scenario, the EDI for aflatoxins was 23.00 ng/kg bw/day, resulting in an MOE of 17.39, which remains far below the EFSA safety threshold of 10,000 (Panel et al., 2020) indicating a potential health concern. Under the worst-case scenario, the aflatoxin EDI increased to 112.83 ng/kg bw/day, yielding a critically low MOE of 3.55, indicative of a very high public health concern. For OTA, the mean exposure EDI (3.17 ng/kg bw/day) remained below the tolerable daily intake (TDI) of 17 ng/kg bw/day, suggesting minimal risk under average conditions. However, in the worst-case scenario, the EDI reached 59.17 ng/kg bw/day, exceeding the TDI by 3.48-fold, indicating potential health risks under high contamination conditions. These findings demonstrate that aflatoxins pose a more significant and consistent health risk compared to OTA in the studied population, although both toxins contribute to cumulative exposure.

Table 5. Dietary risk assessment for aflatoxins and Ochratoxin A from maize consumption

	Worst-case Scenario		Mean Exposure Scenario	
Parameter	Aflatoxins	OTA	Aflatoxins	OTA
Concentration used (C) [µg/kg]	67.7	35.5	13.8	1.9
Concentration (ng/kg)	67,700	35,500	13,800	1,900
Daily maize consumption (D) [kg/day]	0.1	0.1	0.1	0.1
Body weight (BW) [kg]	60	60	60	60
Daily toxin intake [ng/day]	6,770	3,550	1,380	190
EDI [ng/kg bw/day]	112.83	59.17	23.00	3.17
Health-based guidance value	$BMDL_{10}$ = 400	TDI = 17	$BMDL_{10}$ = 400	TDI = 17
MOE (Aflatoxins)	3.55	—	17.39	—
EDI ÷ TDI (OTA)	—	3.48×	—	0.19×
Risk interpretation	Very high concern	Exceeds TDI (3.5×)	Health concern	Within safe limit

EDI = Estimated Daily Intake = [C (µg/kg) × 1,000 × D (kg/day)] ÷ BW (kg). MOE (Aflatoxins) = $BMDL_{10}$ ÷ EDI; $BMDL_{10}$ = 400 ng/kg bw/day (EFSA, 2020); MOE <10,000 = potential public health concern. EDI÷TDI ratio (OTA) = EDI ÷ 17 ng/kg bw/day (EFSA); values >1.0 exceed the tolerable daily intake. D = 0.1 kg/day (FAO); BW = 60 kg (WHO). Mean concentrations from all positive samples; — indicates metric not applicable to that toxin.

Conclusion

This study provides the first spatially comprehensive survey of natural co-occurrence of aflatoxins and OTA in maize across Sindh, Pakistan. OTA was found in 19.0% of samples (0.1–35.5 µg/kg) and aflatoxins in 47.4% of samples (0.2–67.7 µg/kg). 11.2% of samples showed co-occurrence, although there was no discernible correlation between the two toxins. There is a significant risk to food safety because a significant percentage of samples exceeded established MRLs. According to dietary risk assessment, OTA risk becomes crucial at high contamination levels, while aflatoxin exposure poses a considerable public health risk in both mean and worst-case scenarios. These results highlight Pakistan's pressing need for national mycotoxin laws, better storage procedures, and organized monitoring initiatives.

Disclosure Statement

The authors declare no conflict of interest.

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