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Geospatial distribution of mycotoxins in wheat collected in summer and winter seasons from a selected area

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Abstract

Mycotoxins are toxic metabolites generated by various fungal species. Mycotoxins in wheat may pose dangers for the health of consumers as most of them are potent carcinogens. The objective of this study was to check the level of contamination by multiple mycotoxins in wheat samples obtained from local stores in Lahore, during the summer and winter seasons. HPLC coupled with the diode array detector (DAD) was utilized for the quantification of multiple mycotoxins. In total, 50% of wheat samples were contaminated with multiple mycotoxins. This exceeds the allowable limit set by the European Union. The highest levels of total aflatoxins (AFs), ochratoxins (OTAs), deoxynivalenol (DON), and fumonisins (FUMs) were found to be 115.19 µg/kg, 24.13 µg/kg, 112.00 mg/kg, and 2.81 mg/kg, respectively. The contamination levels in wheat samples were as follows: AF (0.56–115.19 µg/kg), OTA (0.12–24.13 µg/kg), DON (0.01–112.00 mg/kg), and FUMs (0.02–2.81 mg/kg). The presence of multiple mycotoxins was observed to be more rampant in wheat gathered during summer compared to winter. Nonetheless, a statistically significant difference ($p<0.05$) was observed among AF, OTA, and DON in both seasons, whereas no significant difference ($p>0.05$) was detected for FUM. This research highlighted the geospatial distribution of contamination by multiple mycotoxins in wheat, and the results are quite alarming. It is essential to actively monitor contamination by multiple mycotoxins in wheat crops to ensure safety and safeguard consumer health.

Keywords: Aflatoxin, ochratoxin, deoxynivalenol, fumonisins, HPLC

Introduction

Pakistan ranks sixth among wheat-producing countries, with approximately 24 million tons cultivated in 2010 (Iqbal *et al.*, 2013). Although wheat is grown throughout the country, maximum yields are achieved in Punjab and Sindh. Because of outdated agricultural methods and the leakage of toxins from unsuitable storage, wheat becomes infected and contaminated with mycotoxins, rendering it unfit for human consumption (Nada *et al.*, 2022).

According to Asif I. (2024), the average annual wheat production in Pakistan, which is a staple food, amounts to 24 million tons, which is sufficient to satisfy the domestic demand. The contamination of wheat and other food products with mycotoxins is a major public health issue because of their high toxicity (Gozzi *et al.*, 2024). Research has been carried out globally on mycotoxins to assess their occurrence in various food items and the extent of their severity (Bouelet *et al.*, 2023). Food entities can be severely contaminated by mycotoxins, as some of these toxins are classified as highly carcinogenic by the International Agency of Research on Cancer (IARC). Mycotoxins can have immunosuppressive, teratogenic, and neurotoxic effects on the human body (Latham *et al.*, 2023). Well-known mycotoxins are aflatoxins (AFs), ochratoxins (OTAs), trichothecene or deoxynivalenol (DON), and fumonisins (FUMs) (Agriopoulou *et al.*, 2020; Aloui *et al.*, 2023; Milani, 2013).

In many developing nations, molds grow and spread as a result of a combination of humid environmental conditions and inadequate drying, that is, because of poor pre-harvest and post-harvest conditions (Feldman and Levy, 2023). This leads to unacceptable concentrations of different mycotoxins, especially AFs, in corn, wheat, rice, red chili, tree nuts, turmeric, groundnuts, and various other agricultural products. The presence of mycotoxins in diverse food items has been linked to prolonged health effects, such as instances of cancers and immune deficiency. Currently, numerous mycotoxins have been recognized, which have attracted the attention of scientists because of their severe impacts on human health and presence in food (Patriarca and Pinto, 2017; Sadef *et al.*, 2023).

Fungal species such as *Aspergillus* and *Fusarium*, which produce mycotoxins, can develop on various crops and food items (Nazir *et al.*, 2021). They have the ability to penetrate deep into food, rather than being limited to growth on the outer surface. Depending on their types and quantities, mycotoxin contamination can manifest as acute or chronic venoms, even at very low to high doses. Food-borne mycotoxins have adverse effects, which lead to serious illnesses that manifest as acute symptoms shortly after the ingestion of contaminated food. The allowable concentration of AFs in wheat is 4 µg/kg (Nisa *et al.*, 2016). However, according to the European Commission Regulation (EC, 2010) and Codex Alimentarius Commission (2012), the permissible levels for DON, FUMs, and OTAs are, respectively, 1000 µg/kg, 1000 µg/kg, and 5 µg/kg.

It is of utmost importance to create sensitive, rapid, and reproducible methods for identifying the occurrence of considerable mycotoxins in staple crops. The thin layer chromatography (TLC) method can be used for the qualitative analysis of mycotoxins in different food products. It is necessary to validate the method and develop a rapid detection method for mycotoxins. Reliable methods like HPLC and ELISA are extremely useful for quantifying mycotoxins. These tools will undeniably assist the wheat, rice, and corn sectors in identifying mycotoxins for the quality assurance of food commodities (Jaimez *et al.*, 2000; Turner *et al.*, 2015). The present study involves identifying different mycotoxins in wheat collected from local stores in Lahore in the summer and winter seasons. The purpose of this study is to create an awareness about mycotoxin contamination in wheat in local shops that have improper storage. This could help in preventing health hazards.

Materials and Methods

Wheat varieties were chosen for mycotoxin assessment based on their nutritional and economic significance. The following four district types of mycotoxins were chosen for investigation:

a. Total AFs (B1+B2+G1+G2)
b. OTAs (A+B+C)
c. DON (Vomitoxin)
d. FUMs (FB_1+FB_2+FB_3)

Study of seasonal variations

The production of mycotoxins relies on climate, which is considered a primary driving factor in the development of fungi and mycotoxin contamination. From this perspective, nations such as Pakistan are more vulnerable to climatic influences such as temperature changes, drought, flooding, and humidity (Magan *et al.*, 2011). Soil fertilizers, various pesticides, contamination by harmful heavy metals, and numerous potential causes for concern in developing nations are also accountable for mycotoxin contamination. The research was categorized into the following two primary seasonal groups for sample collection:

- **Summer/humid/rainy seasons**: When temperature may rise from 25°C to as high as 45°C.
- **Winter/foggy/meek weather**: Temperature may go down from 20°C to 4°C.

Lahore is situated on the western side of the flat floodplain along the River Ravi. Lahore is located at a longitude range of 74°-01' to 74°-39' east and a latitude range of 31°-15' to 31°-42' north. It is the second largest and the second most densely populated district in Pakistan (Rana and Bhatti, 2018). The average range of the lowest and highest temperatures throughout the year were

from 5.9°C to 22°C. The summer season has lower relative humidity compared to the winter season. The months of April, May, and June are extremely hot and arid in Lahore, often accompanied by dust storms. The monsoon season begins in late June or early July, bringing humid and muggy conditions along with intense rainstorms. In Lahore, July, August, and September are primarily hot and humid. Cold and dry conditions persist from October to March (Khan *et al.*, 2021).

Collection of samples

The Lahore district has been chosen for wheat collection because of its high consumption in this region of Punjab, Pakistan. Twenty wheat samples (n=20) were obtained from local stores in Lahore, where the storage conditions were observed to be extremely inadequate and unhygienic. Ten samples were collected during summer and ten during winter. All samples were collected by assessing their physical characteristics, storage environments, hygiene, and contamination. The temperature recorded during sample collection ranged from 25 to 40°C in summer and 13 to 22°C in winter, with humidity levels at 45–69% in summer and 40–55% in winter. Since mycotoxins are not evenly distributed and wheat grains can contain areas with elevated levels of mycotoxins, an appropriate sampling strategy was implemented. The sampling method outlined in AOAC no. 977.16 was adhered to (Trucksess, 2005). A 1 kg sample was obtained using a probe from two to three different diagonal locations within the container. Subsequently, the gathered wheat samples were processed through a sample divider to 200 g after thoroughly homogenizing the sample. Each sample was once more thoroughly mixed and ground into a fine powder prior to analysis (Nisa *et al.*, 2016; Yi *et al.*, 2022).

Storage conditions

The wheat samples were stored in airtight polyethylene bags and kept at 4°C prior to analysis (Cheng *et al.*, 2022).

Chemicals used in the study

All chemicals utilized in the current study were of analytical grade. Acetonitrile (sourced from Merck, Darmstadt, Germany) was used for the extraction of mycotoxins and also as a mobile phase.

Preparation of standard solutions

Various stock solutions for AFs (50 µg/kg), OTA (25 µg/kg), DON (6 mg/kg), and FUMs (6 mg/kg) were freshly prepared. The corresponding standards were diluted in a mixture of acetonitrile and water (20:80, v/v) and kept at −20°C (Irakli *et al.*, 2017).

Specifications of equipment

The Agilent 1200 system (Agilent Technologies, Urdorf, Switzerland) employed reversed phase-HPLC for quantifying various mycotoxins. The HPLC was outfitted with a Rheodyne injector valve (Irakli *et al.*, 2017) featuring a 20 µL loop and a pump. The diode array and fluorescent detectors were linked in series within the HPLC setup. Agilent Chemstation Software of Agilent Technologies, version B.04.01 was utilized for the capture and assessment of various chromatograms.

Protocol followed

Multiple mycotoxins were analyzed in wheat samples using HPLC equipped with a diode array detector (DAD). One kilogram of every wheat sample was collected and mixed thoroughly (Santos *et al.*, 2022) for homogenizing the sample. The sample was crushed in a grinding mill (Romer Labs). A conical flask received 5 g of the homogenized ground wheat sample, to which 50 mL of 70% acetonitrile was added. The sample solution was agitated on a shaker for 30 min. The sample solution underwent filtration, and the collected extract was filtered again using filter paper (Whatman 4). The sample extracts were subsequently filtered one more time using 0.5-micron syringe filters prior to injection into the HPLC C18 column. Twenty microliters of the extract was injected into the HPLC system. The mobile phase employed consisted of acetonitrile: water (45:55 v/v). The flow rate was 1 mL/min with a duration of 15 min. The excitation and emission wavelengths were 254 nm and 460 nm, respectively.

Statistical analysis

IBM Statistics Version 22.0 was utilized for the statistical evaluation of mycotoxins in wheat (Zahra *et al.*, 2017). Mean and standard deviation were also calculated through descriptive analysis. A one-way analysis of variance (ANOVA) at $\alpha = 0.05$ was conducted to assess the impact of seasons on levels of mycotoxin in wheat samples collected from local stores in Lahore, Pakistan.

Results and Discussion

Wheat is widely used as a key ingredient in various food items such as cakes, biscuits, and breads. Various wheat-based food items can become contaminated with

mycotoxins, compromising the quality of these products (Hedayati *et al.*, 2016). Various mycotoxins in wheat were measured to assess the highest level of contamination. Mycotoxins (AFs, OTAs, DON, and FUMs) were measured through HPLC. The chromatograms for various mycotoxins are presented in Figures 1–4.

Contamination levels of total AFs, OTA, DON, and FUMs in wheat samples collected from Lahore were recorded at 0.56–115.19 µg/Kg, 0.12–24.13 µg/kg, 0.01–112.00 mg/kg, and 0.02–2.81mg/kg, respectively (Table 1).

The concentration of mycotoxins satisfied the assumptions of one-way ANOVA. Levene's test confirmed that the homogeneity of variances was satisfied (Table 2) and indicated that the data are significant for AFs across two seasons. For OTA and DON, it is again notable, whereas for FUM it is not noteworthy.

The outcomes of the ANOVA are presented in Table 3. ANOVA indicated a significant difference among groups with p = 0.046, whereas for OTA, DON, and FUM, the results are nonsignificant. While elevated temperature and humidity are crucial for mycotoxin production, some samples were found to be contaminated during winter at levels exceeding permissible limits because of inadequate storage conditions (Taheri *et al.*, 2012).

Descriptive analysis showed that in summer 115.19 µg/kg AF, 24.13 µg/kg OTA, 112.00 mg/kg DON, and 1.19 mg/kg

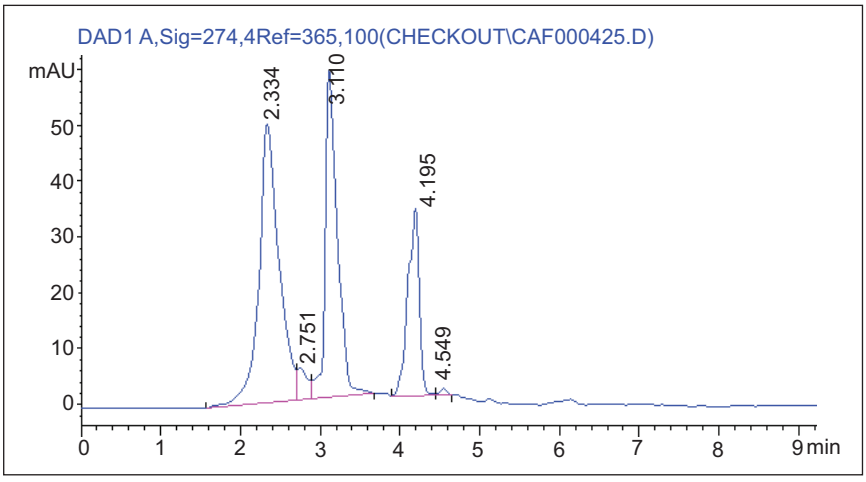


Figure 1. Aflatoxins HPLC chromatogram.

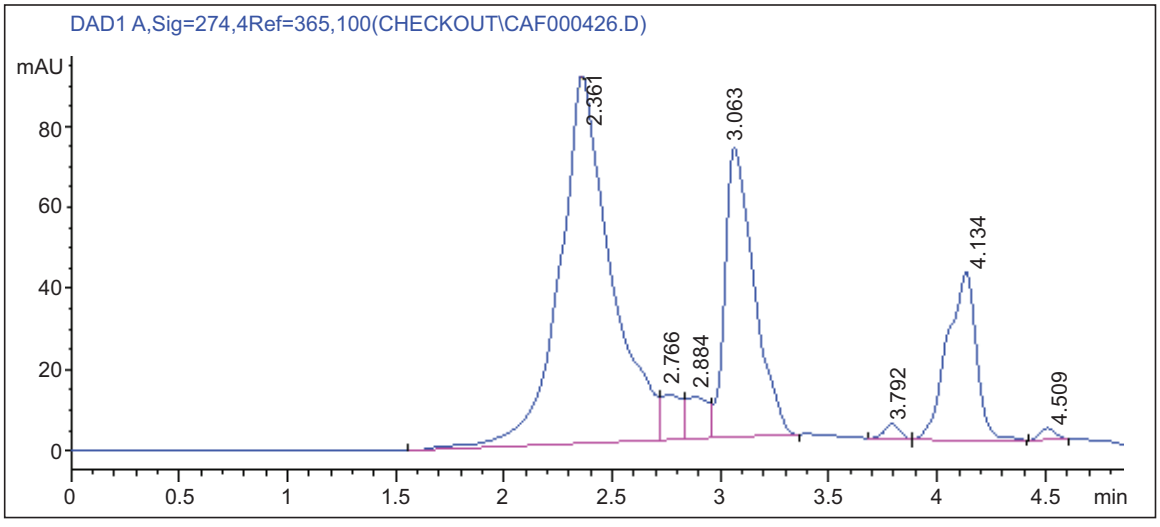


Figure 2. Ochratoxins HPLC chromatogram.

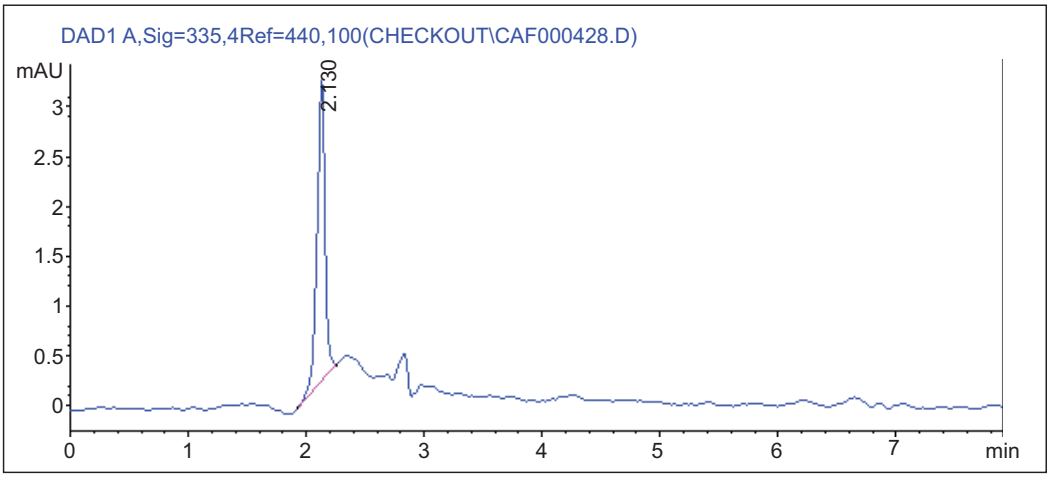


Figure 3. Deoxynivalenol HPLC chromatogram.

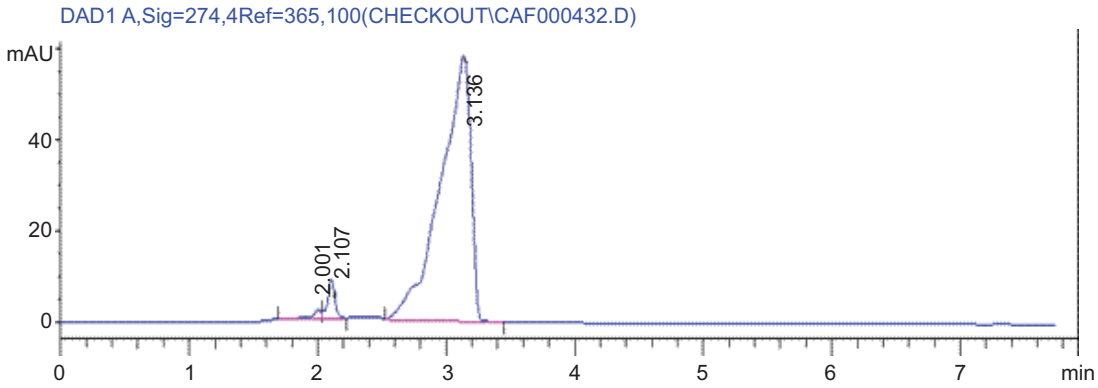


Figure 4. Fumonisins HPLC chromatogram.

FUM were present in wheat as maximum concentration while in winter 4.43 µg/kg AF, 6.77 µg/kg OTA, 33.69 mg/kg DON, and 2.81 mg/kg FUM were present in wheat as maximum concentration.

Lutfullah and Hussain (2012) examined the levels of AF contamination in various cereals in Pakistan. Maize samples exhibited the highest contamination at 18.5µg/kg, while wheat, barley, rice, sorghum, and broken rice showed contamination levels of 15.5µg/kg, 12.6µg/kg, 10.8µg/kg, 9.4µg/kg, and 8µg/kg, respectively. Various studies globally have reported the determination of multiple mycotoxins in wheat. In two different studies performed in China, researchers discovered DON, Afs, and FUMs in concentrations of 221.4 µg/kg, 0.1–0.8 µg/kg, and 0–400 µg/kg, respectively (Sun *et al.*, 2011; Sun and Wu, 2016). In similar research carried out in Nigeria by Ezekiel and Sombie (2014), the levels of AFs in analyzed snacks were found. The levels of AF contamination ranged from 0.8 to 3.5 ppb in all selected food samples. In wheat-based pastas, 1.2 ppb of mean AFs were found.

A study in Mazandaran Province, Iran, analyzed 118 wheat samples for the quantification of various *Aspergillus* species and discovered a 64.6% contamination rate of *Aspergillus flavus*. According to FAO/UNICEF/WHO and the Standard Administration of Iran, the allowable limit for AFs in wheat is 30 µg/kg (Feyzi *et al.*, 2017). AF B1 and G1 were detected at approximately 2.54% and 3.39%, with averages of 1.53 and 2.75 µg/kg, respectively. The highest level of contamination detected in wheat was 7.10 µg/kg.

The overall findings indicated that the samples gathered in the summer season exhibited higher levels of contamination compared to those collected in winter (Figures 5 and 6). Six samples of summer wheat and four from winter were found to have mycotoxin contamination

Table 1. Contamination of mycotoxins in wheat samples collected from Lahore.

Sr. No.	Sample No.	AFs (µg/kg) + SD	OTA (µg/kg) + SD	DON (mg/kg) + SD	FUM (mg/kg) + SD
Summer Samples					
1.	Whe-S-51	115.19±8.67	4.55±1.51	ND	ND
2.	Whe-S-52	ND	ND	ND	ND
3.	Whe-S-53	ND	ND	0.06±0.03	ND
4.	Whe-S-54	ND	5.21±1.54	0.03±0.01	1.06±0.04
5.	Whe-S-55	7.52±0.27	24.13±4.61	112.00±2.64	ND
6.	Whe-S-56	6.27±0.36	ND	ND	ND
7.	Whe-S-57	1.16±0.11	5.03±1.11	ND	1.19±0.51
8.	Whe-S-58	1.89±0.83	0.90±0.21	28.16±2.48	ND
9.	Whe-S-59	2.45±0.65	0.15±0.06	ND	0.02±0.01
10.	Whe-S-60	2.46±0.14	0.23±0.07	0.01±0.01	0.11±0.01
Winter Samples					
11.	Whe-W-61	ND	ND	ND	ND
12.	Whe-W-62	0.56±0.26	5.77±0.33	33.69±5.68	ND
13.	Whe-W-63	ND	ND	ND	ND
14.	Whe-W-64	ND	ND	ND	ND
15.	Whe-W-65	ND	0.12±0.08	ND	ND
16.	Whe-W-66	ND	ND	ND	0.06±0.1
17.	Whe-W-67	2.81±0.12	6.77±0.14	ND	ND
18.	Whe-W-68	ND	ND	ND	2.81±0.44
19.	Whe-W-69	ND	ND	0.56±0.10	0.03±0.02
20.	Whe-W-70	4.43±0.50	ND	1.16±0.31	0.02±0.02

ND= Not detected.

Table 2. Test of homogeneity of variances.

	Levene statistic	df1	df2	Sig.
AF	14.296	1	58	0.000
OTA	6.359	1	58	0.014
DON	11.099	1	58	0.002
FUM	1.149	1	58	0.288

Table 3. ANOVA results of mycotoxins.

		Sum of squares	Df	Mean square	F	Sig.
AF	Between Groups	2501.829	1	2501.829	4.177	0.046
	Within Groups	34738.008	58	598.931		
	Total	37239.837	59			
OTA	Between Groups	113.823	1	113.823	3.811	0.056
	Within Groups	1732.061	58	29.863		
	Total	1845.884	59			
DON	Between Groups	1648.965	1	1648.965	2.569	0.114
	Within Groups	37234.147	58	641.968		
	Total	38883.112	59			
FUM	Between Groups	0.042	1	0.042	0.088	0.768
	Within Groups	28.068	58	0.484		
	Total	28.110	59			

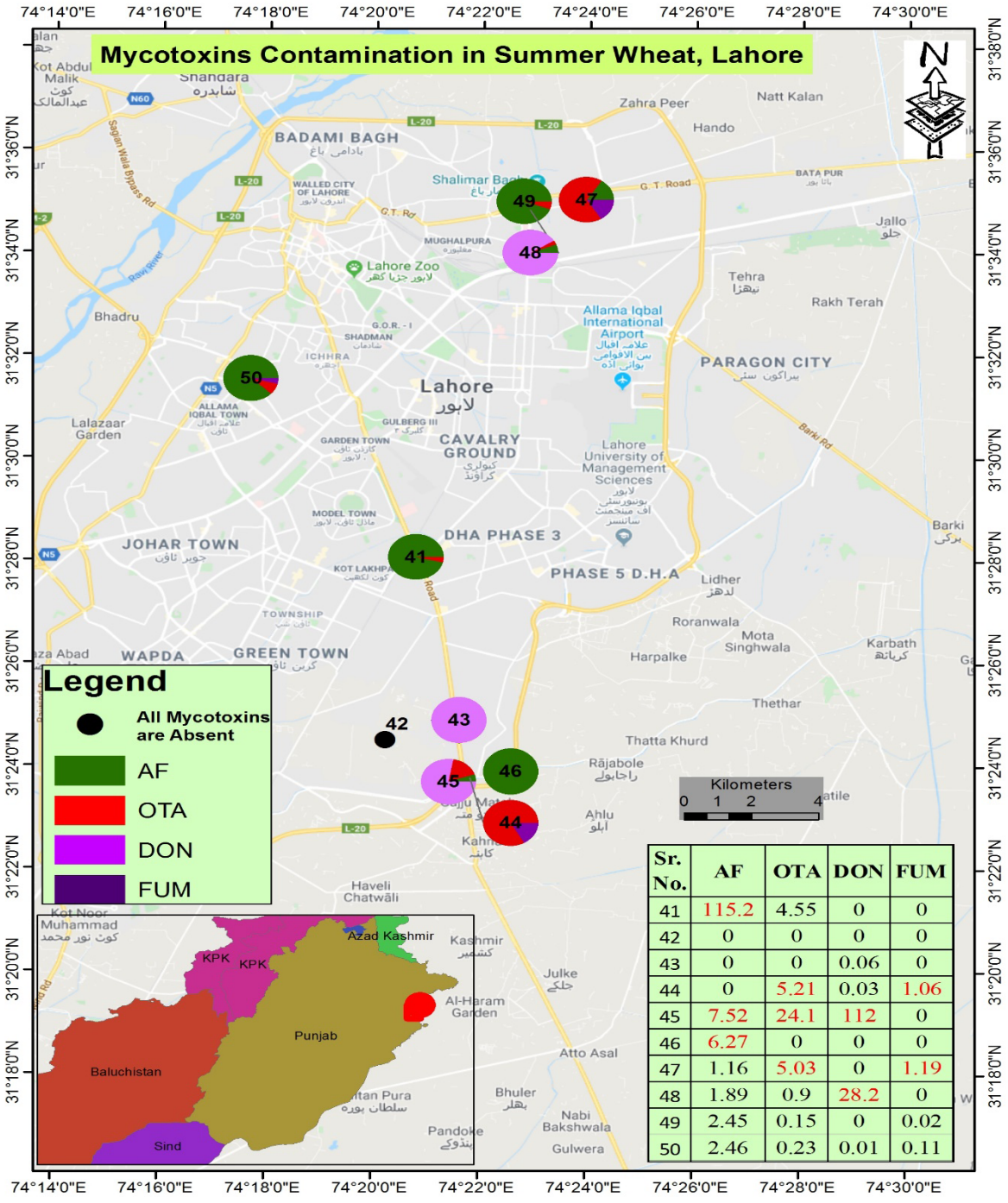
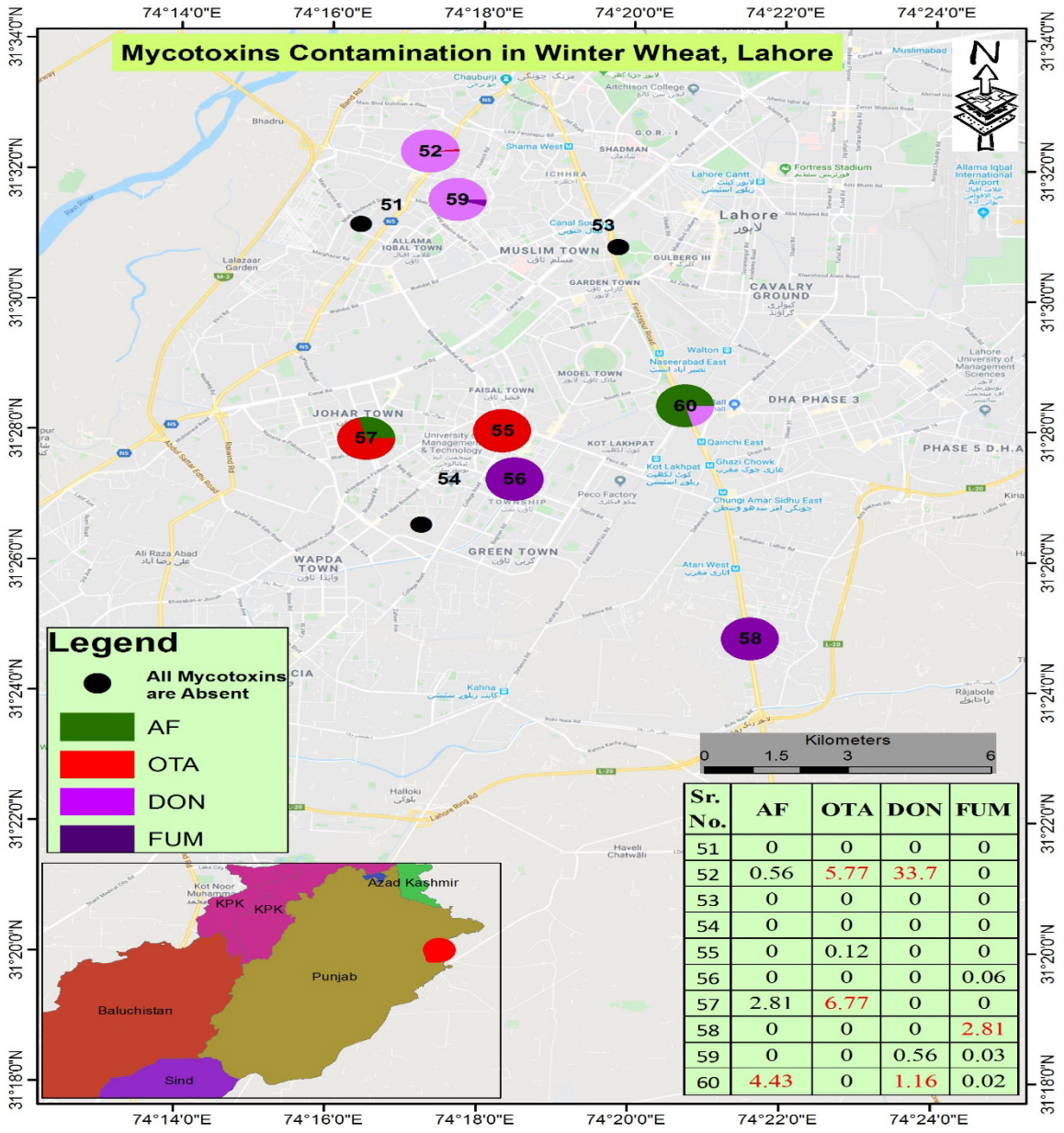


Figure 5. Mycotoxin contamination in wheat samples collected from the Lahore district during the summer season.

exceeding acceptable limits. In wheat from the Lahore district, contamination levels of AFs, OTA, DON, and FUMs were found to be 55%, 45%, 40%, and 40%, respectively, while 20%, 25%, 20%, and 15% of the samples exceeded permissible limits for AFs, OTA, DON, and FUMs, respectively (Table 4).

In Pakistan, a comparable study was carried out by Majeed (2018), in which n=124 wheat samples were gathered from the Punjab Province. Wheat samples contained multiple mycotoxins. The contamination percentages were determined to be 43% AFB1, 41% AFB2, 11% OTA, 21% DON, along with 29% FB1, 18% FB2, and



Sr. No.	AF	OTA	DON	FUM
51	0	0	0	0
52	0.56	5.77	33.7	0
53	0	0	0	0
54	0	0	0	0
55	0	0.12	0	0
56	0	0	0	0.06
57	2.81	6.77	0	0
58	0	0	0	2.81
59	0	0	0.56	0.03
60	4.43	0	1.16	0.02

Figure 6. Mycotoxin contamination in wheat samples collected from the Lahore district during the winter season.

12% FB3. AF was identified as the most common toxin in wheat, a finding that aligns with the current study. Likewise, a study on AF contamination in wheat kernels was conducted (Asghar *et al.*, 2016), revealing 26% AFB1 and 7% AFB2 contamination in wheat samples. In total, just 2.7% of AFs in wheat exceeded the allowable levels. The contamination percentage of wheat with mycotoxins in the current study is elevated compared to both investigations, potentially because the samples were taken from shops with very inadequate storage conditions.

The season may greatly affect fungal growth as it is clear from the study that wheat samples collected in summer are more contaminated with mycotoxins than the wheat samples collected in winter (Figure 7). In a study, it was found that higher temperatures from 20 to 40°C are favorable for toxin production (Sousa *et al.*, 2019) while toxin production was minimum at 5°C. The present study is in accordance with the reported work as contagion of mycotoxins was more in summer than in winter. In another study on feed, the effect of temperature on *Aspergillus flavus* production

Table 4. Number of samples beyond the highest levels for mycotoxins in wheat as established by the European Union.

Area	Aflatoxins Contaminated Samples (%)	Aflatoxins Contaminated Samples beyond permissible levels (%) in total samples	Ochratoxin Contaminated Samples (%)	Ochratoxins Contaminated Samples beyond permissible levels (%) in total samples	Deoxynivalenol Contaminated Samples (%)	Deoxynivalenol Contaminated Samples beyond permissible levels (%) in total samples	Fumonisins Contaminated Samples (%)	Fumonisins Contaminated Samples beyond permissible levels (%) in total samples
District Lahore	55	20	45	25	40	20	40	15

was studied, and it was found that in high temperature months, the incidence ranged between 66 and 93% while in cold temperature months, it reduced to 40%. High temperature and moisture may increase fungal contamination (Asif M, 2024; Iram *et al.*, 2019; Tittlemier, *et al.*, 2025).

Wheat consumption is increasing day by day because of the increase in population. This research was basically conducted to check the presence of multiple mycotoxins in wheat available in local shops. The results were discouarging as 10 out of 20 samples were contaminated beyond permissible levels and were declared as unfit on the basis of one toxin beyond acceptable range.

Conclusion

The presence of mycotoxins in different food commodities needs attention as the incidence of liver cancer, allergies, stomach ulcers, etc., is on the rise. Improper handling, poor storage facilities, and adverse environmental conditions may contaminate food entities with various mycotoxins. The dire necessity to protect food commodities has prompted all countries to pay attention to contamination by mycotoxins in different food entities. Wheat and wheat products are among the major crops of Punjab, Pakistan. Special attention is required to avoid mycotoxin contamination in these products before exporting them. Overall, 20%, 25%, 20%, and 15% of wheat samples were contaminated with AFs, OTA, DON, and FUM, respectively, and were unfit according to EU permissible levels for human consumption because of the presence of multiple mycotoxins in wheat samples. Six samples in the summer season and four samples in the winter season were infected with mycotoxins beyond acceptable limits. This is because warm temperature may increase fungal growth. High temperature and high humidity are favorable conditions for the growth of mycotoxins. Five out of 20 (25%) samples were contaminated with multiple mycotoxins. The presence of high levels of mycotoxins in wheat samples is highly alarming. Proper handling, suitable environmental conditions, and careful storage can reduce the chances of mycotoxin contamination in wheat and wheat products. Inspection on a constant basis is needed to detect any hazardous and dangerous health circumstances. The awareness must be given to farmers and people to inspect the quality of wheat and wheat products in order to avoid mycotoxin contamination.

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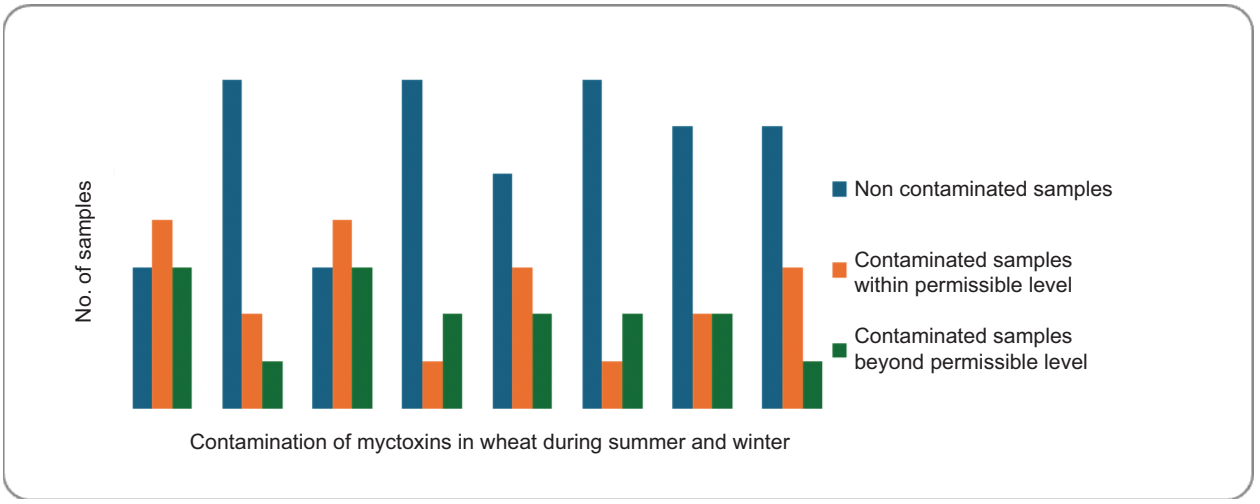

Figure 7. Presence of mycotoxins in wheat during different seasons.

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Authors Contributions

Conceptualization, Naseem Zahra; methodology, Nadia Jamil; software, Muhammad Khalid Saeed; validation, Rania Ali El Hadi Mohamed and Fakhria A. Al-Joufi; formal analysis, Asif Javed; investigation, Najeeb Ullah; resources, Tariq Aziz; data curation, Nadeem Ullah.; writing—original draft preparation, Fahad Al-Asmari.; writing—review and editing, Tariq Aziz; visualization, Abid Sarwar; supervision, Tariq Aziz.; project administration, Naseem Zahra; funding acquisition, Tariq Aziz.

Conflict of Interest

All authors declare no conflict of interest.

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