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Occurrence and Co-Occurrence of Mycotoxins in Cereal-Based Foods Intended for Infants and Children in Albania: A First Report

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Abstract

A survey of multi-mycotoxin contamination in processed-cereal-based foods intended for children and infants/toddlers and in maize flour marketed in Tirana, Albania, was conducted. Fourteen mycotoxins were determined in 17 samples (8 cornflakes, 4 infant/toddler cereals and 5 maize flours) by liquid chromatography–tandem mass spectrometry (LC–MS/MS). Maize flour was the most contaminated matrix: aflatoxin B1 (AFB1) was detected in all five samples (0.33–4.47 µg/kg) and exceeded the European Union (EU) maximum level of 2.0 µg/kg in three, and the sum of aflatoxins exceeded 4.0 µg/kg in one. Fumonisins B1 and B2 were found in three maize flour samples (FB1+FB2 337–11,326 µg/kg), two of which exceeded the EU limit of 1000 µg/kg; zearalenone (ZEA) was found in two, one above the 75 µg/kg limit (97.5 µg/kg), and HT-2 toxin was found in one (42.6 µg/kg). In cornflakes, AFB1 (2/8), deoxynivalenol (DON, 4/8), FB1 (3/8) and ZEA (1/8) were detected at levels well below EU maximum levels. In infant/toddler cereals, only DON was detected—in one sample and below the limit of quantification—and no aflatoxins were detected (limit of detection: 0.2 µg/kg). AFB2, AFG1, AFG2, T-2, DAS, 3-AcDON, 15-AcDON and OTA were not detected in any samples. The findings show a matrix-dependent contamination pattern, with maize flour as the critical commodity, and point to the need for intensified monitoring, stricter raw material controls and improved storage practices within maize supply chains.

Keywords: breakfast cereals; infant/toddler foods; mycotoxins; AFB1; deoxynivalenol; fumonisins; ochratoxin A; zearalenone; LC-MS/MS; Albania

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1. Introduction

Cereal products are a primary dietary component for infants and young children because of their high digestibility and substantial mineral content B [1]. However, cereals and cereal-based products are among the food matrices most susceptible to fungal colonization both in the field and during storage, making them a principal dietary vehicle for the entry of mycotoxins into the human food chain [2,3]. Mycotoxins are naturally occurring fungal metabolites that can cause acute poisoning and long-term health effects, including immune

deficiency and cancer. Children are particularly vulnerable because of their lower body weight, immature metabolic and elimination systems, and restricted diets [4,5].

Molds can grow on various crops and foods, including cereals, nuts, spices, dried fruits, apples, and coffee beans, often under warm, humid conditions [6,7]. The principal mycotoxin-producing genera are Aspergillus, Penicillium, and Fusarium. The most important mycotoxins include aflatoxins (AFs) produced by Aspergillus species; ochratoxin A (OTA) produced by Aspergillus and Penicillium; and trichothecenes—deoxynivalenol (DON), HT-2, and T-2 toxin—as well as zearalenone (ZEA), fumonisin B1 (FB1), and fumonisin B2 (FB2), produced by Fusarium species [8–10].

Four aflatoxins—AFB1, AFB2, AFG1, and AFG2—are the most significant because they are widespread in maize, edible nuts, and legumes [11]. Contamination can occur before, after, or both before and after harvest. Even at very low levels, AFs can cause cancer, liver damage, genetic mutations, and congenital disabilities in humans and animals [12].

Fumonisins, produced primarily by *Fusarium verticillioides* and *F. proliferatum*, are the most prominent FB1 and FB2 [13]. The IARC classifies them as Group 2B, possible human carcinogens [14,15]. ZEA is an estrogenic mycotoxin produced by Fusarium species [4]. It is found mainly in maize and wheat and is able to disrupt endocrine function [16].

DON (deoxynivalenol) or vomitoxin, a stable type B trichothecene, contaminates cereal crops and persists through processing [17,18]. It also occurs in the food matrix as acetylated derivatives, 3-AcDON and 15-AcDON. Its toxicity mainly results from its targeting of the intestinal epithelium [19,20]. Diacetoxyscirpenol (DAS) is a highly toxic Type A trichothecene mycotoxin produced mainly by fungi of the Fusarium genus. Contaminated food or feed can pose dangers to both humans and livestock.

Mycotoxins and Children's Health Implications

Cereals used in infant food are subjected to strict quality assurance procedures before market distribution. Nevertheless, crops remain susceptible to toxigenic fungi during both pre- and post-harvest stages, producing small toxic compounds known as mycotoxins [21]. Corn, barley, wheat, oats, and rice are common ingredients in infant food formulations. Fusarium species infect these major cereal crops, and the resulting toxins are chemically stable, allowing transfer from the raw crop into processed food [4]. Research indicates that children are more vulnerable than adults to toxic food contaminants, such as mycotoxins commonly found in cereals [5]. This increased sensitivity is attributed to their limited diets, higher intake of specific foods relative to their body weights, and immature metabolic systems [6,22–24]. Pregnancy, infancy, and early childhood represent critical periods during which toxicant exposure can result in long-term developmental consequences [22,25].

Hepatocellular carcinoma and stunting in children have been associated with AFB1 chronic exposure [26,27]. FB1, the most toxic fumonisin, exhibits embryotoxicity in animals and degrades poorly at temperatures below 150 °C. The IARC has classified DON as Group 3, while other toxicological activities include intestinal damage and immunotoxic effects at relevant dietary doses [19,28]. ZEA's estrogenic activity may impair reproductive development, particularly in young females [16]. OTA exerts nephrotoxic, teratogenic, and immunotoxic effects and is classified as a Group 2B carcinogen by the IARC [29].

The aim of this study was to identify mycotoxin contamination of infant and children's cereal-based foods in Tirana, Albania. Altogether, 14 mycotoxins were quantified in 17 samples of processed-cereal-based foods intended for children and infants/toddlers in Tirana, Albania (cornflakes, infant/toddler cereals and maize flour).

2. Results and Discussion

A comprehensive survey of regulated mycotoxins—including aflatoxins (AFB1, AFB2, AFG1, AFG2), DON, ZEA, FB1, FB2, OTA, and T-2/HT-2 toxins—across cornflakes, infant/toddler cereals, and maize flour collected in Tirana, Albania, revealed a consistent matrix-driven contamination pattern rather than a toxin-specific distribution. Contamination data for the analyzed samples were compared with the EU maximum levels (MLs) established in Commission Regulation (EU) 2023/915 [30]. Fusarium-associated toxins (DON, ZEA, fumonisins) and Aspergillus-associated aflatoxins were most prevalent in maize flour, which was identified as the most heavily and frequently contaminated matrix. Cornflakes exhibited a high detection incidence but low absolute concentrations, whereas infant/toddler cereals consistently had the lowest concentrations.

2.1. Aflatoxin Occurrence

In cornflakes, AFB1 was the only aflatoxin detected (Table 1), having been found in two of the eight samples (25.0%; 0.223 and 0.469 µg/kg), both below the LOQ (0.6 µg/kg); AFB2, AFG1 and AFG2 were not detected. Cornflakes, as a milling/extrusion product placed on the market for the final consumer, fall under the maximum levels for cereals and cereal products—2.0 µg/kg for AFB1 and 4.0 µg/kg for the sum of aflatoxins—rather than the considerably stricter 0.10 µg/kg AFB1 limit that applies specifically to baby food and processed cereals for infants and young children [30]. Evaluated against the maximum level, none of the corn flakes samples approached non-compliance for aflatoxins. The low detection frequency and the concentrations below the LOQ indicate only sporadic, low-level aflatoxin contamination of this product group.

No aflatoxins were detected in the infant/toddler cereals ($n = 4$; LOD 0.2 µg/kg for each aflatoxin). However, the LOD and LOQ of the method (0.2 and 0.6 µg/kg) are above the EU maximum level of 0.10 µg/kg AFB1 for processed-cereal-based food for infants and young children [30]. Compliance of these products with this limit could therefore not be verified, and a method with a lower LOQ would be required for this purpose.

The maize flour samples had the highest levels, with AFB1 ranging from 0.33–4.47 µg/kg (mean 2.54 ± 1.66 µg/kg), and against the maximum levels of 2.0 µg/kg (AFB1) and 4.0 µg/kg (sum of aflatoxins), three out of five samples (60.0%) exceeded the AFB1 limit, and one out of five (20.0%) exceeded the sum limit. AFB1 was the only aflatoxin detected in maize flour; AFB2, AFG1 and AFG2 were below the LOD in all samples. These results are consistent with European data showing a higher frequency of AF contamination in maize-based products [2]. Compared to previous studies on mycotoxin contamination in cereal grains from Albania [31,32], aflatoxin contamination in this commodity group remains a concern.

These results are consistent with the data reported in neighboring Balkan countries. In Kosovo AFB1 contaminated 62.1% of baby food samples [33], whereas a higher incidence in cereal foods from Serbia was reported, but the concentrations were lower [34], and Croatia [35], reflecting geographic and climatic variation.

Table 1. Occurrence of aflatoxin in cereal-based foods and maize flour from retail chains and drug stores.

	AFB1 (µg/kg)	AFB2 (µg/kg)	AFG1 (µg/kg)	AFG2 (µg/kg)	Sum AFs [a] (µg/kg)
Corn flakes ($n = 8$)					
Incidence (%)	2/8 (25.0)	0/8	0/8	0/8	2/8 (25.0)
Range	0.223–0.469	n.d.	n.d.	n.d.	0.223–0.469
Mean ± SD [b]	0.346 ± 0.174	–	–	–	0.346 ± 0.174
>EU ML [d] (AFB1 2.0/ Sum 4.0 µg/kg)	0/8 (0.0)	–	–	–	0/8 (0.0)
Infant/toddler cereals ($n = 4$)					
Incidence (%)	0/4	0/4	0/4	0/4	0/4
Range	n.d.	n.d.	n.d.	n.d.	n.d.
Mean ± SD	–	–	–	–	–
>EU ML [c] (AFB1 0.10 µg/kg)	n.v.	–	–	–	–
Maize flour ($n = 5$)					
Incidence (%)	5/5 (100)	0/5	0/5	0/5	5/5 (100)
Range	0.329–4.47	n.d.	n.d.	n.d.	0.329–4.47
Mean ± SD	2.54 ± 1.66	–	–	–	2.54 ± 1.66
>EU ML [d] (AFB1 2.0/ Sum 4.0 µg/kg)	3/5 (60.0)	–	–	–	1/5 (20.0)
All cereal samples ($n = 17$)					
Incidence (%)	7/17 (41.2)	0/17	0/17	0/17	7/17 (41.2)
Range	0.223–4.47	n.d.	n.d.	n.d.	0.223–4.47
Mean ± SD (positive samples)	1.91 ± 1.73	–	–	–	1.91 ± 1.73

[a] Sum of AFB1 + AFB2 + AFG1 + AFG2. [b] Mean and standard deviation of positive samples. [c] EU maximum levels for processed-cereal-based food for infants and young children (Commission Regulation (EU) 2023/915) [30]. [d] EU maximum levels for all cereals and cereal-derived products (Commission Regulation (EU) 2023/915) [30]. LOD, limit of detection; n.d., not detected; –, not applicable. LOQ, limit of quantification; n.v., compliance not verifiable, as the LOD of the method is above the maximum level. Results were corrected for recovery. LOD and LOQ were 0.2 and 0.6 µg/kg for each aflatoxin; positive samples are those with values ≥ LOD. Values between the LOD and LOQ (detected, not quantified): AFB1 in two cornflake samples (0.223 and 0.469 µg/kg) and in one maize flour sample (0.329 µg/kg).

2.2. Incidence and Co-Occurrence of Fusarium Toxins and OTA

Among the Fusarium-derived toxins (Table 2), FB1, FB2, DON, HT-2, and ZEA were detected in at least one sample, whereas 3-AcDON, 15-AcDON, DAS, and T-2 remained below the limit of detection—a pattern consistent with the sporadic, environmentally dependent occurrence typical of these compounds [28]. OTA was also not detected in any sample.

2.3. Fumonisin B1 and B2

Fumonisins were found mainly in maize flour: FB1 and FB2 were each detected in three of the five samples (60.0%), with FB1+FB2 concentrations of 337–11,326 µg/kg (mean 6778 µg/kg; FB1 maximum 8557 µg/kg), which confirms that maize flour is a matrix highly susceptible to fumonisin contamination. In cornflakes, FB1 was detected in three of the eight samples (37.5%; 20.4–48.3 µg/kg), all below the LOQ (50 µg/kg), whereas FB2 was not detected; no fumonisins were detected in infant/toddler cereals. Overall, FB1 was detected in 35.3% and FB2 in 17.6% of the samples.

Under Commission Regulation (EU) 2023/915 [30], fumonisins in maize are regulated according to a processing-stage-dependent scheme: 4000 µg/kg for unprocessed maize, 1000 µg/kg for maize and milling products of maize placed on the market for the final consumer, 800 µg/kg for maize-based breakfast cereals and snacks, and 200 µg/kg for processed maize-based foods for infants and young children. Two of the five maize flour samples exceeded the 1000 µg/kg limit (maximum FB1+FB2 11,326 µg/kg), whereas fumonisins in cornflakes remained below the LOQ (50 µg/kg), far below the 800 µg/kg limit, and were not detected in infant cereals. This indicates that, at least for the most contaminated samples, the maize flour analyzed here would not meet EU maximum levels and stresses the importance of raw-material sourcing and post-harvest management methods as the primary control point for fumonisins.

Globally, fumonisin contamination in infant foods varies widely. European studies report FB1 in the range of <LOQ to 8.3 µg/kg in infant formula from Austria and Czechia [36]. Higher levels were found in Germany (up to 955 µg/kg) in some corn-based toddler foods [37] and in Spain, with FB1 up to 449 µg/kg [38]. In a worldwide context, a study from China found a low incidence rate in infant noodles but noted noncompliance with EU maximum levels, with two samples exceeding the EU MRL of 200 µg/kg for FB1 (242.3 µg/kg) and FB2 (252.4 µg/kg) [39]. In this study, fumonisins were below the LOQ in cornflakes and not detected in infant cereals but reached high levels in maize flour. Taken together, these results identify maize flour as the matrix requiring the most critical attention, and point toward pre-harvest drought stress and/or inadequate post-harvest drying and storage as the most likely shared drivers.

2.4. Deoxynivalenol

In contrast to the fumonisin pattern, DON was detected in four of the eight cornflake samples (50.0%; 14.2–70.1 µg/kg, mean 42.5 µg/kg; two values below the LOQ of 40 µg/kg) and in one infant/toddler cereal (35.9 µg/kg, below the LOQ) but not in maize flour. Overall, DON was detected in 29.4% of the samples, and all concentrations were well below the EU maximum levels currently in force (Commission Regulation (EU) 2024/1022) [40], i.e., 400 µg/kg for breakfast cereals and 150 µg/kg for processed-cereal-based food for infants and young children.

Table 2. DON, DAS, HT2, T2, FB1, FB2, OTA, and ZEA toxins in different cereals (in µg/kg).

Commodity	DON	DAS	HT2	T2	Sum HT2+T2	FB1	FB2	Sum FB1+FB2	OTA	ZEA
Maize flour ($n = 5$)										
Incidence (%)	0/5	0/5	1/5 (20.0)	0/5	1/5 (20.0)	3/5 (60.0)	3/5 (60.0)	3/5 (60.0)	0/5	2/5 (40.0)
Range	n.d.	n.d.	42.6	n.d.	42.6	250–8557	86.4–2769	337–11,326	n.d.	72.2–97.5
Mean	–	–	42.6	–	42.6	5266	1512	6778	–	84.9
SD	–	–	–	–	–	4413	1350	5734	–	17.9
EU ML ($n >$ ML)	750 (0/5)	–	–	–	50 (0/5)	–	–	1000 (2/5)	3.0 (0/5)	75 (1/5)
Cornflakes ($n = 8$)										
Incidence (%)	4/8 (50.0)	0/8	0/8	0/8	0/8	3/8 (37.5)	0/8	3/8 (37.5)	0/8	1/8 (12.5)
Range	14.2–70.1	n.d.	n.d.	n.d.	n.d.	20.4–48.3	n.d.	20.4–48.3	n.d.	27.9
Mean	42.5	–	–	–	–	38.8	–	38.8	–	27.9
SD	27.7	–	–	–	–	15.9	–	15.9	–	–
EU ML ($n >$ ML)	400 (0/8)	–	–	–	50 (0/8)	–	–	800 (0/8)	3.0 (0/8)	100 (0/8)
Infant/toddler cereals ($n = 4$)										
Incidence (%)	1/4 (25.0)	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
Range	35.9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Mean	35.9	–	–	–	–	–	–	–	–	–
SD	–	–	–	–	–	–	–	–	–	–
EU ML ($n >$ ML)	150 (0/4)	–	–	–	10 (0/4)	–	–	200 (0/4)	0.50 (0/4)	20 (0/4)
All samples ($n = 17$)										
Incidence (%)	5/17 (29.4)	0/17	1/17 (5.9)	0/17	1/17 (5.9)	6/17 (35.3)	3/17 (17.6)	6/17 (35.3)	0/17	3/17 (17.6)
Range	14.2–70.1	n.d.	42.6	n.d.	42.6	20.4–8557	86.4–2769	20.4–11,326	n.d.	27.9–97.5
Mean	41.2	–	42.6	–	42.6	2652	1512	3408	–	65.9
SD	24.2	–	–	–	–	3998	1350	5175	–	35.2

Results are corrected for recovery; incidence, range, mean and SD refer to positive samples (values ≥ LOD). Values between the LOD and LOQ (detected, not quantified): DON 14.2 and 23.5 µg/kg (cornflakes) and 35.9 µg/kg (infant/toddler cereal); FB1 20.4, 47.7 and 48.3 µg/kg (cornflakes). 3-AcDON and 15-AcDON were not detected in any sample. n.d., not detected (<LOD); –, not applicable. EU ML, maximum level (µg/kg) according to Commission Regulation (EU) 2023/915 [30], as amended by Regulations (EU) 2024/1022 (DON) [40] and 2024/1038 (sum of T-2 and HT-2) [41]; in parentheses, number of samples exceeding the ML. Maize flour: milling products of maize placed on the market for the final consumer; cornflakes: maize-based breakfast cereals; infant/toddler cereals: processed-cereal-based food for infants and young children.

Data across European studies indicate DON is the most prevalent mycotoxin in infant cereals, with occasional exceedances. A study of cereal samples in Poland reported DON incidence of 9.09% and the concentration ranging from 62 to 148 µg/kg [17]. In Portugal, levels reached up to 207.8 µg/kg in breakfast cereals and 270.57 µg/kg in baby food, with some exceeding MLs [42,43]. In Spain, two samples exceeded the ML (range 33–245 µg/kg) [44]. In contrast, Turkey reported a DON incidence of 14.1–21% at generally lower levels [45,46]. The DON levels observed in our study are within the lower range reported in different European studies.

2.5. Zearalenone, OTA, and T-2/HT-2 Occurrence

ZEA was detected in 3 of 17 samples (17.6%)— in two out of five maize flour samples (40.0%) and in one out of eight cornflake samples (12.5%)—whereas it was not detected in infant/toddler cereals. Maize flour showed the highest concentrations (mean 84.9 µg/kg; range 72.2–97.5 µg/kg), and one sample (97.5 µg/kg) exceeded the EU maximum level of 75 µg/kg for maize milling products placed on the market for the final consumer [30]. The single positive cornflake sample contained 27.9 µg/kg, well below the 100 µg/kg limit for maize-based breakfast cereals. This pattern confirms maize-based products as inherently more susceptible to ZEA contamination. Low ZEA signals in two infant/toddler cereals and one cornflake sample were below the LOD (6 µg/kg) and were not considered.

Globally, ZEA incidence in infant foods is approximately 9.3% [47]. European studies from Germany and Austria/Czechia report ZEA below 7 µg/kg and below 1.2 µg/kg, respectively, all within MLs [36,48]. In contrast, Tunisia reported ZEA up to 44 µg/kg in baby mix [49], and one US study found 32.1 µg/kg in a cereal sample, exceeding the EU ML [50]. The elevated ZEA levels in Albanian maize flour point out the need for food safety controls on imported goods and improved raw material sourcing and storage practices for domestic food matrices.

OTA was not detected in any of the analyzed samples, which is consistent with its typically sporadic occurrence [51]. Globally, OTA incidence in infant foods is approximately 14.17%. [47] Moderate OTA levels have been reported in Italy [52,53], Turkey [45,54], Portugal [43,55], and Serbia [34]. A study from Italy found OTA in 20% (15/75) of infant formula and baby food samples [52]; another study, from Portugal, reported 69% OTA-positive breakfast cereals [42], while data from Turkey revealed levels of up to 0.380 µg/kg in baby foods [54]. The absence of OTA in Albanian samples may reflect specific storage and climate conditions for domestic products, whereas imported products mainly originate from EU countries. However, sustained monitoring is still essential, given OTA's nephrotoxic and carcinogenic properties.

T-2/HT-2 toxins were detected in only a single maize flour sample, with HT-2 (42.6 µg/kg), below the maximum level of 50 µg/kg for the sum of T-2 and HT-2 toxins in milling products of maize (Commission Regulation (EU) 2024/1038) [41].

2.6. Regulatory Compliance and Implications for Infants and Young Children

Because no consumption data were collected, dietary exposure and health risk were not quantified in this study; the findings are therefore discussed in terms of occurrence and compliance with EU maximum levels. Aflatoxins, DON, fumonisins, and ZEA were detected in the analyzed samples, with aflatoxins representing the most frequently identified group. DON was detected mainly in cornflakes and, in infant/toddler cereals, only once and below the LOQ. Although most mycotoxin concentrations were below EU regulatory limits, recurrent exceedances highlight constant challenges in raw-material sourcing and cereal processing. Aflatoxins, classified as Group 1 carcinogens, may also exhibit synergistic toxicity with other mycotoxins. Previous studies have documented aflatoxins and their

metabolites in various food commodities [31,56,57]. Cereal-based baby foods and breakfast cereals are frequently contaminated with multiple mycotoxins and fungal species. Enhanced surveillance, more rigorous raw material selection, and improved storage conditions are warranted.

The main concern arises from frequent mycotoxin occurrence and occasional exceedances, particularly AFB1, fumonisins and ZEA in maize flour. Infants and young children are especially susceptible due to their higher intake relative to their body weight and may therefore be repeatedly exposed to low levels of several toxins; quantifying this exposure requires consumption data and was beyond the scope of this occurrence study. While DON and fumonisin concentrations were within EU limits for cornflakes and cereal baby foods, their co-occurrence requires consideration of combined toxicity, which current regulations do not fully address.

Infant/toddler cereals are subject to the most stringent EU maximum levels of any food category, reflecting this population group's lower body weight and narrower margin of exposure [30]. Fusarium toxins in products formulated for the most vulnerable consumers warrant persistent monitoring, particularly because EU policy explicitly sets infant-food limits as low as reasonably achievable rather than based on a fixed safety margin alone [30].

The relatively small number of samples per category (n = 4–8) limits the statistical strength of the incidence and mean values reported here, particularly for maize flour. The results should therefore be regarded as a first indication of the contamination situation in products marketed in Tirana rather than as representative national data. Further work should prioritize an expanded sample set to determine whether the elevated levels observed in maize flour originate from a specific supply source or storage practice that could be targeted for corrective action. In addition, the LOD of the aflatoxin method (0.2 µg/kg) is above the EU maximum level for AFB1 in infant food (0.10 µg/kg), so the absence of detectable aflatoxins in infant/toddler cereals does not demonstrate compliance with that limit.

3. Conclusions

Mycotoxins were detected in all three categories of cereal-based foods analysed in Tirana, Albania, but at markedly different levels. Maize flour was the most contaminated matrix: AFB1 was detected in all five samples and exceeded the EU maximum level of 2.0 µg/kg in three (60.0%). The sum of aflatoxins exceeded 4.0 µg/kg in one, FB1+FB2 exceeded 1000 µg/kg in two, and ZEA exceeded 75 µg/kg in one. In cornflakes, AFB1, DON, FB1 and ZEA were detected in some samples at concentrations well below EU maximum levels, and in infant/toddler cereals, only DON was detected—in one sample, below the LOQ. Overall, AFB1 was the most frequently detected mycotoxin (41.2%), followed by FB1 (35.3%) and DON (29.4%); AFB2, AFG1, AFG2, ochratoxin A, T-2, DAS and the acetylated DON derivatives were not detected, and HT-2 was found in only one maize flour sample. Because the LOD of the aflatoxin method is above the EU limit for infant food, compliance of infant/toddler cereals with the 0.10 µg/kg AFB1 limit could not be verified. The co-occurrence of Aspergillus and Fusarium toxins in maize flour indicates that raw-material handling, pre-harvest field conditions and post-harvest maize storage are critical control points. Sustained monitoring, stricter raw material controls, improved drying and storage practices, and routine multi-mycotoxin surveillance with methods sensitive enough for infant-food limits are needed to protect this vulnerable population.

4. Materials and Methods

4.1. Sample Collection

Three categories of cereal foods—maize flour, breakfast cereals (cornflakes), and multi-grain infant/toddler cereals—were collected from retail markets and pharmacies in Tirana during the spring-summer of 2025. Sampling was conducted in accordance with Commission Implementing Regulation (EU) 2023/2782 [58] on sampling methods for mycotoxins. All products were randomly selected in line with the surveillance plan. Samples of 100–200 g from bulk commodities or complete product units were combined to form 1–2 kg representative samples, which were labeled and transported under cool, dry conditions. In the laboratory, samples were reduced and homogenized prior to analysis. For each collected sample, the batch number, expiration date, and manufacturer details were recorded. A total of 17 samples (8 cornflakes, 5 maize flour, 4 multi-grain cereals) were analyzed for AFB1, AFB2, AFG1, AFG2, DON, 3-AcDON, 15-AcDON, DAS, HT-2, T-2, OTA, FB1, FB2, and ZEA.

4.2. Standards and Chemicals

Standards and chemicals were as described previously [31,59]. Mixed aflatoxin standard solution in acetonitrile (AFB1, AFB2, AFG1, AFG2; Romer Labs, Tulln, Austria) was used with stock concentrations of 2 µg/mL (AFB1 and AFG1) and 0.5 µg/mL (AFB2 and AFG2). Mixed trichothecene standards (DON, 3-AcDON, 15-AcDON, T-2, HT-2, DAS; Trilogy Analytical Laboratory, Washington, MO, USA) and single standards of ZEA, OTA, FB1, and FB2 (Romer Labs) at 100 µg/mL and 50 µg/mL, respectively, were prepared in acetonitrile and stored in amber vials at −20 °C. Acetonitrile, methanol and acetic acid (Sigma-Aldrich, Steinheim, Germany) were of LC–MS grade, and ammonium acetate (Merck, Darmstadt, Germany) was of analytical grade; deionized water was prepared using a Milli-Q system (Millipore, Burlington, MA, USA).

4.3. Sample Extraction

Samples were prepared as described previously [31,59], with minor modifications. Ground cereal samples (particle size ≤ 1 mm; Retsch ZM 100 mill, Retsch GmbH, Haan, Germany) (10 g) were extracted with 100 mL acetonitrile–water (84:16, v/v) by shaking for 1 h on a linear shaker (IKA HS 501; IKA-Werke, Staufen, Germany). An aliquot (4 mL) of filtered extract was evaporated to dryness (Syncore Polyvap, BÜCHI Labortechnik AG, Flawil, Switzerland), reconstituted in 0.5 mL H_2O–MeOH (20:80), and 10 µL was injected into the UPLC–MS/MS system (Acquity UPLC H-Class, Waters, Milford, MA, USA); vials were kept in the autosampler at 15 °C. Matrix-matched calibration was performed by spiking 4 mL aliquots of the extract with standard solutions. Extracts were diluted when concentrations exceeded the calibration range.

4.4. LC–MS/MS Analysis

Chromatographic separation was performed on a Zorbax Eclipse Plus C18 Rapid Resolution HD column (2.1 × 100 mm, 1.8 µm; Agilent Technologies, Santa Clara, CA, USA) coupled to a triple-quadrupole mass spectrometer (Xevo TQ MS; Waters Corporation, Milford, MA, USA) equipped with an electrospray ionization (ESI) source and controlled by MassLynx software (version 4.1, Waters Corporation, Milford, MA, USA). Two chromatographic methods were applied. Aflatoxins (AFB1, AFB2, AFG1 and AFG2) were separated under isocratic conditions with deionized water (A) and methanol (B) (60:40, v/v) [31]. The remaining mycotoxins (DON, 3-AcDON, 15-AcDON, DAS, HT-2, T-2, FB1, FB2, OTA and ZEA) were separated by gradient elution with water (A) and methanol (B), both containing 0.5% acetic acid and 2.5 mM ammonium acetate, starting at 95% A and 5% B.

The proportion of B was increased linearly to 40% within 4 min and to 70% within the next 8 min, held for 4 min, increased to 90% within 1.5 min and held for 2.5 min, then returned to 5% within 1 min and re-equilibrated for 4 min (total run time 25 min) [59]. In both methods, the flow rate was 0.3 mL/min and the column temperature 40 °C. Detection was performed in multiple reaction monitoring (MRM) mode; aflatoxins were acquired in ESI+, whereas the multi-toxin method used polarity switching (ESI+/ESI−). Capillary voltages were 3.4 kV (ESI+) and 3.0 kV (ESI−); the desolvation temperature was 500 °C and the ion source temperature 150 °C. Retention times, ionization modes, monitored transitions, cone voltages and collision energies are given in Table 3.

Table 3. LC–MS/MS parameters for the analyzed mycotoxins.

Mycotoxin	Ionization Mode	RT * (min)	Precursor Ion (m/z)	Quantifier Ion (m/z)	Qualifier Ion (m/z)	Cone Voltage (V)	Collision Energy (V)
AFB1	ESI+	5.25	313.2	213.2	241.1	42/42	48/38
AFB2	ESI+	4.75	315.2	243.2	259.1	42/42	28/28
AFG1	ESI+	2.92	329.1	214.8	199.9	46/46	58/40
AFG2	ESI+	5.33	331.1	189.0	285.1	44/38	44/28
DON	ESI+	3.14	297.3	203.1	249.1	18/18	16/10
3-AcDON	ESI+	5.35	339.2	203.1	231.1	24/24	16/14
15-AcDON	ESI+	5.40	339.2	137.0	261.1	16/16	12/10
DAS	ESI+	7.06	384.3	307.2	247.2	20/20	14/10
HT-2	ESI+	8.91	442.4	215.1	263.2	12/12	16/14
FB1	ESI+	10.20	722.4	334.2	352.2	30/30	40/35
T-2	ESI+	10.27	484.4	185.1	215.2	18/18	24/20
OTA	ESI+	11.10	404.2	221.0	239.0	24/24	36/24
ZEA	ESI−	11.30	317.2	131.0	174.95	35/40	22/24
FB2	ESI+	12.70	706.4	318.2	336.2	35/35	40/40

* Retention time.

5. Method Validation and Quality Control

The multi-mycotoxin method was validated in the same laboratory for cereal grains and compound feed [60]. Matrix-matched calibration curves were prepared by spiking blank samples within the validated concentration range. Recovery, repeatability (RSDr) and within-laboratory reproducibility (RSDwR) were determined in blank samples spiked in duplicate at three levels (LOQ, 2 × LOQ and 20 × LOQ). The LOD and LOQ were estimated as the concentrations giving signal-to-noise ratios of 3:1 and 10:1, respectively, and the lowest successfully validated level was adopted as the LOQ. The LOD and LOQ were 0.2 and 0.6 µg/kg for each aflatoxin; 12 and 40 µg/kg for DON, DAS, HT-2 and T-2; 2 and 5 µg/kg for OTA; 6 and 20 µg/kg for ZEA; and 15 and 50 µg/kg for FB1 and FB2, respectively (Table 4). The average recovery ranged from 90% to 110% for the single Fusarium toxins and OTA and was 65% for the aflatoxins. RSDr ranged from 4.5% to 18%, while RSDwR for the aflatoxins, DON, 3-AcDON, 15-AcDON, DAS, ZEA, OTA, HT-2, T-2, FB1 and FB2 was 11–24% at the LOQ, 8–26% at 2 × LOQ and 2–22% at 20 × LOQ. All results were corrected for recovery. Samples with a mycotoxin concentration equal to or above its LOD were considered positive; values between the LOD and LOQ are reported as detected but not quantified and are identified in the footnotes of Tables 1 and 2.

Table 4. Limits of detection (LOD) and quantification (LOQ) and recoveries of the LC–MS/MS methods.

Mycotoxin	LOD (µg/kg)	LOQ (µg/kg)	Recovery (%)
AFB1, AFB2, AFG1, AFG2	0.2	0.6	65
DON	12	40	95
3-AcDON	12	40	98
15-AcDON	12	40	90
DAS	12	40	97
HT-2	12	40	99
T-2	12	40	101
ZEA	6	20	110
FB1	15	50	97
FB2	15	50	98
OTA	2	5	98

LOD and LOQ apply to each toxin; the aflatoxin recovery refers to AFB1. Recoveries of 3-AcDON, 15-AcDON, DAS, T-2 and OTA are from Babič et al. [60]. Precision data are given in the text. All results were corrected for recovery.

6. Statistical Evaluation

Data for all detected mycotoxins are left-censored; mean and standard deviation were calculated from positive samples only (concentrations ≥ LOD), including values between the LOD and LOQ as measured.

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