ORIGINAL PAPER

MULTIANNUAL DYNAMICS OF MYCOTOXIN RESIDUES IN FOOD AND FEEDSTUFFS

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Abstract. *This paper presents the temporal dynamics of the concentrations for three mycotoxins – Aflatoxin B1 (AFB1), Ochratoxin A (OTA), and Zearalenone (ZEN) – monitored between 2013 and 2022 in Tulcea County, SE Romania, in selected food (wheat, maize) and feed products (combined forage and cereals and fodder products). Among the products intended for human consumption, maize exhibited higher concentrations of mycotoxins than the wheat. AFB1, OTA and ZEN concentrations varied in wheat in the intervals 0.91–1.92 µg/kg, 0.77–4.36 µg/kg, and 1.34–56.94 µg/kg, respectively, and in maize in the intervals 1.1–3.06 µg/kg, 0.91–4.69 µg/kg and 2.11–249 µg/kg, respectively. In the case of the products intended for animal consumption, the cereals and fodder products showed greater mycotoxin concentrations than the combined forage. AFB1, OTA and ZEN concentrations in combined forage were in the ranges 1–16 µg/kg, 0.90–98.30 µg/kg, and 2.1–157 µg/kg, respectively, while in cereals and fodder products their levels varied in the ranges 1–20 µg/kg, 0.80–136 µg/kg, 2–391 µg/kg, respectively. The average mycotoxin concentrations reported in this study do not exceed the legislated guideline levels and present a decreasing order ZEN>OTA>AFB1.*

Keywords: *mycotoxins; Aflatoxin B1; Ochratoxin A; Zearalenone; food and feed; ELISA.*

1. INTRODUCTION

The name mycotoxin is a combination of the Greek word for fungus 'mykes' and the Latin word 'toxicum' meaning poison [1]. Mycotoxins are secondary metabolites produced by different species of filamentous fungi: *Aspergillus* section *Flavi* – Aflatoxins*; Aspergillus* section *Circumdati, Aspergillus* section *Nigri, Penicillium verrucosum, Penicillim nordicum* – Ochratoxin A; *Fusarium graminearum, Fusarium roseum, Fusarium culmorum, Fusarium equiseti, Fusarium cerealis, Fusarium verticillioides, Fusarium incarnatum* – Zearalenone [2–4]. Mycotoxins presence in products intended for human consumption, such as wheat, maize, and from products intended for animal consumption – combined forage and cereals

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and fodder products – are considered to be inevitable due to the widespread occurrence of mycotoxin-producing fungi in the environment [3-9].

Mycotoxins (Aflatoxin B1, Zearalenone, Ochratoxin A) are a threat that we are not able to completely eliminate from the surrounding environment. Their occurrence is favored by factors commonly present in our environment, including humidity. In order to minimize the risk to the human and animal health, the priority should be prevention, systematic testing of the products intended for human and animal consumption, in terms of the content of harmful microorganisms and their metabolites.

Aflatoxins are a group of secondary fungal metabolites (mycotoxins) [10-12]. Aflatoxin B1 (AFB1), the most potent of these compounds, is considered to be the most toxic and many studies have indicated its hepatotoxic, immunosuppressive, mutagenic and teratogenic properties [5, 12-16]. AFB1 impair the function of the liver cells and facilitate liver cancer development [2]. Also, AFB1 is classified as a group 1 human carcinogen by the International Agency for Research on Cancer (IARC) [15].

Ochratoxin A (OTA) is nephrotoxic, hepatotoxic, embryotoxic, teratogenic, neurotoxic, immunotoxic, genotoxic, and carcinogenic in many species with species and sex-related differences [17] and impair human and animal immune system and its ability to cope with an infection OTA are a real threat for human beings and animal health [2,11]. The IARC classified OTA as a possible human carcinogen (group 2B) [16–18].

Due to similarity with natural estrogens, zearalenone (ZEN) is considered as xenoestrogen and enables its binding to the estrogen receptors leading to hormonal misbalance and numerous reproductive diseases [11,19]. ZEN is found in crops (temperate regions), primarily in maize and other cereal crops that form an important part of various food and feed. It has significant adverse effects on human and animal [19] and cause animal hyperestrogenism and degeneration of the reproductive cells [2]. ZEN has been categorized as a Group 3 carcinogen by the IARC due to its unclassifiable carcinogenicity to humans with inadequate evidence [19-21].

The co-occurrence of AFB1, OTA and ZEN in food and feed could pose a risk to consumers' health, as the AFB1 toxicity is amplified in the presence of ZEN and OTA [4]. The European Union (EU) established the maximum residue limits (MRLs), in Commission Regulation (EU) 2023/915 of 25 April 2023 [22] on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006, which is applied in Romania (Table 1).

In general, the basic elements of human and animal nutrition are cereals and cereal-based products that constitute a favorable environment, under certain conditions, for the growth of mold. Thus, cereal crops can be contaminated in the field during harvesting, transportation and storage. This aspect can be enhanced during heavy rains, in which mold contamination and subsequent mycotoxin synthesis can be expected much more frequently [2].

Table 1. Maximum Residue Limits (MRLs) according to the European legislation, in µg/kg.

Product	AFB1	OTA	ZEN
Wheat	2	5	100
Maize	5	5	350
Combined forage	20	50(pigs)/ 100(poultry)	100(piglets and gilts)/250(sows and fattening pigs)/500 (cows and goats)
Cereals and fodder products	20	250	2000(without corn)/3000(corn)

For animal feed, Romania also adopted the maximum residue limits MRLs established at European level: for AFB1- Directive 2002/32/EC of the EUROPEAN Parliament and of the Council, from May 7, 2002, on undesirable substances in feed [23], while for ZEN and OTA it is applied the Commission Recommendation no. 576/2006, concerning in the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2, HT-2 and Fumonisin in food intended for animals [24] (Table 1).

Due to the large amounts of consumed cereals (wheat, maize), combined forage and fodder products and the scarcity of regional data related to mycotoxin levels in these products, it becomes extremely important to monitor them regularly for safety and to detect the harmful substances. The aim of this paper is to provide a mycotoxin temporal occurrence dataset on cereal-based food and various feedstuffs from Tulcea County (SE Romania).

2. MATERIALS AND METHODS

During 2013-2022, the following samples were collected from Tulcea County (SE Romania) and analyzed: for AFB1 - 87 (wheat), 30 (maize), 183 (combined forage) and 142 (cereals and fodder products); for OTA- 87 (wheat), 37 (maize), 90 (combined forage) and 74 (cereals and fodder products) and for ZEN - 169 (wheat), 64 (maize), 88 (combined forage) and 78 (cereals and fodder products).

Analytical methods for the determination of mycotoxins commonly have the following steps: sampling, homogenization, solid phase extraction (SPE) followed by a clean-up, detection and quantitation using enzyme-linked immunosorbent assay (ELISA) [25] (Fig.1).

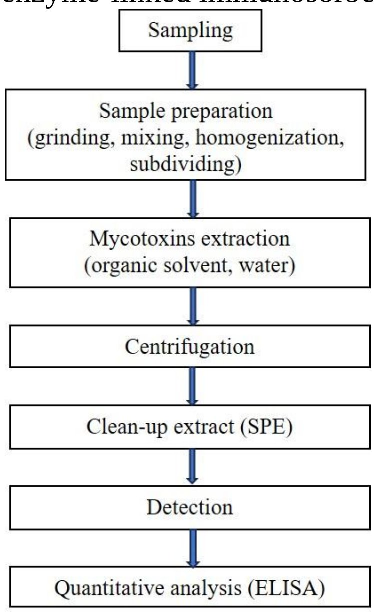

Figure 1. Flow diagram in mycotoxins analysis.

The basic principle of ELISA [6,16,26,27] for mycotoxins screening and quantification is the antigen-antibody reaction. The microtiter wells are coated with capture antibodies against the interest mycotoxin. The standards and sample solutions, enzyme conjugate and anti- mycotoxin antibodies are added. Free mycotoxin and mycotoxin enzyme conjugate compete for the mycotoxin antibody binding sites. At the same time, the anti-mycotoxin antibodies are also bound by the immobilized capture antibodies. Any unbound enzyme conjugate is then removed in a washing step. Substrate chromogen is added to the wells and the bound enzyme conjugate converts the chromogen into a blue product. The addition of the stop solution leads to a color change from blue to yellow. The determination of mycotoxins was carried out at the Tulcea Veterinary Sanitary and Food Safety Laboratory, using the ELISA 96-well absorbance microplate reader (Sunrise, Tecan, Austria). The measurements were performed photometrically at a wavelength of 450 nm.

Detection and quantification capabilities represent fundamental performance characteristics of measurement processes and are defined by the limit of detection and quantification, determined by the blank method [28]. The Detection Limits (DL) and the Quantifications Limits (QL) are presented in the Table 2.

Table 2. Detection and Quantification Limits (DL and QL) for AFB1, OTA and ZEN.

Mycotoxins	AFB1, µg/kg		OTA, µg/kg		ZEN, µg/kg	
Limits	DL	QL	DL	QL	DL	QL
Products intended for human and animal consumption	1.075	1.179	1.054	1.755	2.061	2.457

Quality assurance was ensured by drawing up control charts, fortifying samples at MLRs and calculating the degree of recovery using Quality Control Materials: for AFB1 (4.6 µg/kg, in corn, Trilogy, TQC-M1111-100), OTA (2.1 µg/kg food/feed agricultural product, Trilogy, TQC-M1612-100), and ZEN (102.1 µg/kg, Trilogy, TQC-M1711-100).

3. RESULTS AND DISCUSSION

In the analysis of the selected mycotoxins, respectively AFB1, OTA, ZEN, from both animal and non-animal samples, the recovery degrees were determined for each series of samples, whose values were reported to the reference intervals according to European legislation, respectively EC Regulations 401/2006 [29] and 519/2014 [30] (Table 3). In the selected period, all the values determined for recovery degrees, fall within the optimal domain imposed by the legislation in force. The minimum values obtained for recovery degrees are 84% (AFB1), 94% (OTA) and 91 % (ZEN), and the maximum ones are 110 % (AFB1, ZEN) and 109 % (OTA).

In Figs. 2-7 it is represented the multiannual dynamics of the average concentrations, expressed in µg/kg, with standard deviations, for the three target mycotoxins (AFB1, OTA and ZEN), detected between 2013 and 2022 in products intended for human consumption (wheat, maize) (Figs. 2–FB1, 4–OTA, 6–ZEN) and from products intended for animal consumption (combined forage and cereals and fodder products) (Figs. 3–FB1, 5–OTA, 7–ZEN). It can be noticed that the values of mycotoxin concentrations determined from products intended for animal consumption are higher than those intended for human consumption, as found in similar studies [5].

Table 3. Recovery degrees of three mycotoxins.

Mycotoxin	**AFB1 [%]**	**OTA [%]**	**ZEN [%]**
Recommended recovery degree	70-110	70-110	60-110
2013	107	100	91
2014	108	94	101
2015	110	109	110
2016	109	103	105
2017	103	109	108
2018	98	107	91
2019	89	108	94
2020	84	103	101
2021	90	104	102
2022	105	103	94

The concentration of AFB1 in maize has higher values than the concentrations determined in wheat, with the exception of the years 2015, 2018, 2019 (Fig. 2). Juan et al.

[31] reported much higher average values of OTA concentrations in maize, compared to those determined in wheat, similar to the determinations made from the samples taken and analyzed in this work (Fig. 4).

The values of ZEN concentrations found in this study in maize samples are higher than those determined in wheat samples (except in the years 2018, 2020 when they have comparable values) (Fig. 6), as reported by Curtui et al. [32]. Analyzing the multi-annual average values from the samples intended for animal consumption, it can be seen that 80% of the values determined for AFB1 and OTA from cereals and fodder products have higher values than those determined from combined forage and only 20% of the values determined from combined forage exceed them those from fodder products (Figs. 3-5).

In the case of ZEN, for products intended for animal consumption, 70% of the average values determined from cereals and fodder products are much higher than those determined from combined forage (Fig. 7).

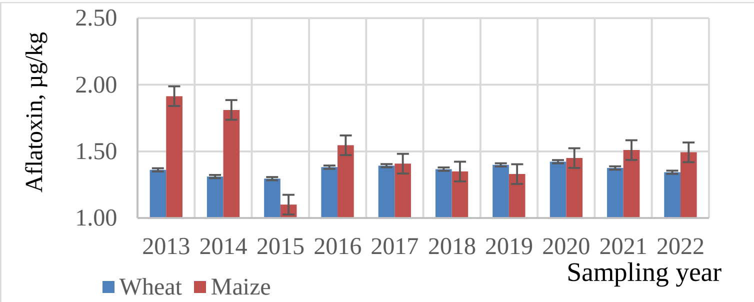

Figure 2. The multi-annual dynamics of the average concentrations of AFB1 in products intended for human consumption.

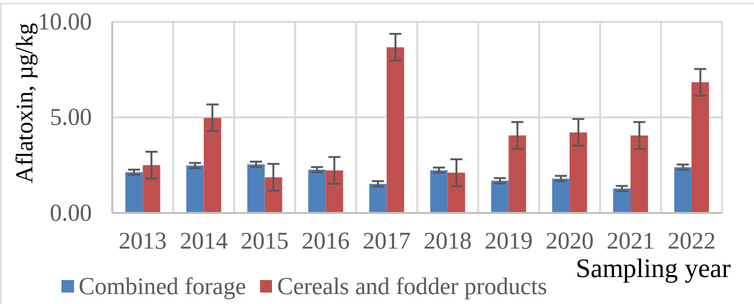

Figure 3. The multi-annual dynamics of the average concentrations of AFB1 in products intended for animal consumption.

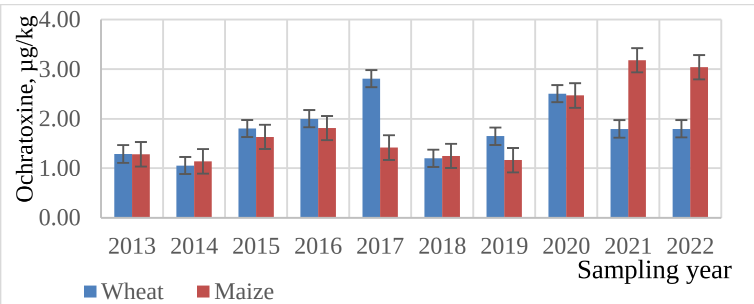

Figure 4. The multi-annual dynamics of the average concentrations of OTA in products intended for human consumption.

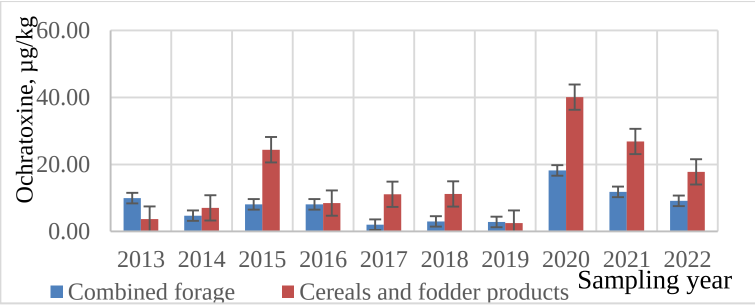

Figure 5. The multi-annual dynamics of the average concentrations of OTA in products intended for animal consumption.

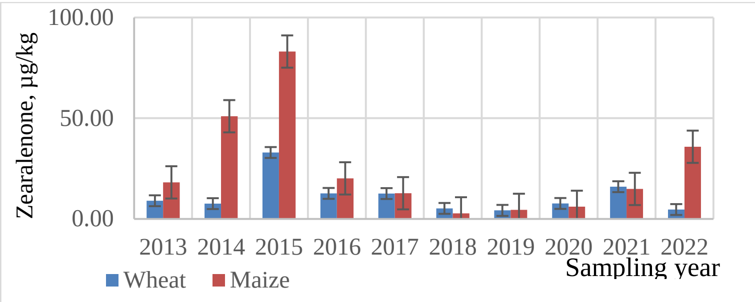

Figure 6. The multi-annual dynamics of the average concentrations of ZEN in products intended for human consumption.

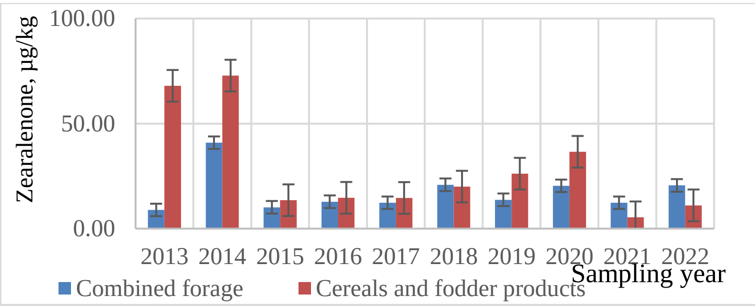

Figure 7. The multi-annual dynamics of the average concentrations of ZEN in products intended for animal consumption.

Table 4. Minimum, maximum, mean and median values (in µg/kg) for AFB1, OTA, ZEN in products intended for human and animal consumption.

Mycotoxin	Product	Count	Max	Min	Mean	Median
ZEN	Maize	64	249.00	2.11	23.64	5.90
	Wheat	169	56.94	1.34	7.19	6.38
	Combined forage	88	157.00	2.10	18.61	11.00
	Cereals and fodder product	78	391.00	2.00	25.49	7.45
AFB1	Maize	30	3.06	1.101	1.60	1.49
	Wheat	87	1.92	0.912	1.36	1.31
	Combined forage	183	15.7	1	2.14	1.70
	Cereals and fodder product	142	20	1	4.53	2.00
OTA	Maize	37	4.69	0.91	1.97	1.59
	Wheat	87	4.36	0.769	1.41	1.14
	Combined forage	90	98.3	0.9	7.62	2.70
	Cereals and fodder product	74	136	0.8	14.93	2.10

In the Table 4 there are presented the minimum, maximum, mean and median concentration values for AFB1, OTA, ZEN in products intended for human and animal consumption collected in Tulcea County, SE Romania. Overall, the minimum value was obtained for OTA in wheat (0.769 µg/kg) and the maximum for ZEN (391 µg/kg) in cereals and fodder products. The mean values are ranging between the minimum value of 1.36 μg/kg (AFB1–wheat) and the maximum of 25.49 μg/kg (ZEN–cereals and fodder products)., presenting a decreasing order ZEN>OTA>AFB1, trend which was reported in other studies [7].

4. CONCLUSIONS

Mycotoxin contamination in food and feed products is a safety issue of growing concern worldwide. During the mycotoxin multiannual (2013-2022) surveys carried out in Tulcea, SE Romania, none of the product samples intended both for human consumption (wheat, maize) and animal consumption (combined forage and cereals and fodder products) exceeded the MRLs. However, residues of AFB1, OTA, and ZEN were quantifiable and, therefore, concerns are raised regarding the functionality of the quality management systems involved in production and storage conditions of products intended for human and animal consumption.

Nowadays, due to the global climate changes and also the Tulcea County neighborhood with Ukraine, it is essential to develop new methods for screening and analysis of mycotoxins in order to manage the cross-border epidemiological risks. Subsequent mycotoxin contamination studies will be extended to different types of food ingredients and by-products intended for human and animal consumption.

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