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# Mycotoxins in Cereal and Soybean-Based Food and Feed

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Additional information is available at the end of the chapter

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## 1. Introduction

### 1.1. Toxigenic fungi and mycotoxins in cereal and soybean products

Cereals and soybean are plants used extensively in food and feed manufacturing as a source of proteins, carbohydrates and oils. These materials, due to their chemical composition, are particularly susceptible to microbial contamination, especially by filamentous fungi. Cereals, soybean, and other raw materials can be contaminated with fungi, either during vegetation in the field or during storage, as well as during the processing.

Fungi contaminating grains have been conventionally divided into two groups – field fungi and storage fungi. Field fungi are those that infect the crops throughout the vegetation phase of plants and they include plant pathogens such as *Alternaria*, *Fusarium*, *Cladosporium*, and *Botrytis* species. Their numbers gradually decrease during storage. They are replaced by storage fungi of *Aspergillus*, *Penicillium*, *Rhizopus* and *Mucor* genera that infect grains after harvesting, during storage [1]. Both groups of fungi include toxigenic species. Currently, this division is not so strict.

Therefore, according to [2], four types of toxigenic fungi can be distinguished:

- Plant pathogens as *Fusarium graminearum* and *Alternaria alternata*;
- Fungi that grow and produce mycotoxins on senescent or stressed plants, e.g. *F. moniliforme* and *Aspergillus flavus*;
- Fungi that initially colonize the plant and increase the feedstock's susceptibility to contamination after harvesting, e.g. *Aspergillus flavus*.

- Fungi that are found on the soil or decaying plant material that occur on the developing kernels in the field and later proliferate in storage if conditions permit, e.g. *Penicillium verrucosum* and *Aspergillus ochraceus*.

Fungal growth is influenced by complex interaction of different environmental factors such as temperature, pH, humidity, water activity, aeration, availability of nutrients, mechanical damage, microbial interaction or the presence of antimicrobial compounds. Poor hygiene, inappropriate temperature and moisture during harvesting, storage, processing and handling may contribute to increased contamination extent.

Fungal contamination can cause damage in cereal grains and oilseeds, including low germination, low baking quality, discoloration, off-flavours, softening and rotting, and formation of pathogenic or allergenic propagules.

It may also decrease the kernel size and thus affect the flour yield. Moulds growing on stored cereals produce a range of volatile odour compounds, including 3-octanone, 1-octen-3-ol, geosmin, 2-methoxy-3-isopropylpyrazine, and 2-methyl-1-propanol which are responsible for an earthy-musty off-odour and affect the quality of raw materials even when present in very small amounts [3]. Moulds produce a vast number of enzymes: lipases, proteases, amylases, which are able to break down food into components leading to its spoilage. Fungi growing on stored grains can reduce the germination rate and decrease the content of carbohydrate, protein and oils. During storage of soybean seed lasting 12 months, the moisture content was at the level of 10-11%. It was observed that the germination rate decreased from initial 75% to 4% prior to the lapse of a 9-month period. In prolonged storage under natural conditions, the total carbohydrate content decreased from 21% to 16.8%, and protein and the total oil contents became slightly reduced [4]. Moulds as food and feed spoilage microorganisms have been characterized in several review articles [2, 5].

The largest producers of soybean in the World are the United States of America, Brazil, Argentina, China, and India. The climatic conditions in soybean-growing regions (moderate mean temperature and relative humidity between 50 and 80%) provide optimal conditions for fungal growth. Soybean (*Glycine max* L.Merr.) is often attacked by fungi during cultivation, which significantly decreases its productivity and quality in most production areas. Fungi associated with cereal grains and oilseeds are important in assessing the potential risk of mycotoxin contamination. Mycotoxins are fungal secondary metabolites which are toxic to vertebrate animals even in small amounts when introduced orally or by inhalation.

Table 1 summarises the occurrence of contamination of different raw materials in various countries. Some of them are of mycotoxicological interest.

Soybean matrix has been rarely studied compared to cereals in relation to fungal and mycotoxin contamination. The fungi associated with soybean seeds, pods and flowers in North America were reviewed by [20]. The most common species belong to *Aspergillus*, *Fusarium*, *Chaetomium*, *Penicillium*, *Alternaria* and *Colletotrichum* genera. Most of these fungi were recorded in mature seeds prior to storage. About 10% of them are commonly referred to as storage moulds. Most of the isolated fungi are facultative parasites or saprophytes.

Commodities	Country	Fungal species	Ref
Soybean	Ecuador	<i>Aspergillus flavus</i> , <i>A.niger</i> , <i>A.ochraceus</i> , <i>A.parasiticus</i> , <i>Fusarium verticillioides</i> , <i>F.semitectum</i> , <i>Penicillium janthinellum</i> , <i>P.simplicissimum</i> , <i>Nigrospora oryzae</i> , <i>Cladosporium cladosporioides</i> , <i>Arthrinium phaeospermum</i>	[6]
	Romania	<i>Aspergillus flavus</i> , <i>A.parasiticus</i> , <i>A.candidus</i> , <i>A.niger</i> , <i>Penicillium griseofulvum</i> , <i>P.variabile</i> , <i>Fusarium culmorum</i> , <i>F.graminearum</i> , <i>F.oxysporum</i>	[7]
	India	<i>Aspergillus flavus</i> , <i>A.candidus</i> , <i>A.versicolor</i> , <i>Eurotium repens</i> , <i>A.sulphureus</i> , <i>Fusarium sp.</i> , <i>Alternaria sp.</i> , <i>Curvularia sp.</i>	[4]
	USA	<i>Diaporthe phaseolorum var. sojae</i> , <i>Fusarium sp.</i> , <i>Alternaria alternata</i> , <i>Alternaria sp.</i> , <i>Fusarium sp.</i> , <i>Curvularia sp.</i> , <i>Cladosporium sp.</i> , <i>Fusarium equiseti</i> , <i>F.oxysporum</i> , <i>F.solani</i>	[8-10]
	Croatia	<i>Fusarium sporotrichides</i> , <i>F.verticillioides</i> , <i>F.equiseti</i> , <i>F.semitectum</i> , <i>F.pseudograminearum</i> , <i>F.chlamyosporum</i> , <i>F.sambucinum</i>	[11]
	Argentina	<i>Aspergillus flavus</i> , <i>A.niger</i> , <i>A.candidus</i> , <i>A.fumigatus</i> , <i>Fusarium verticillioides</i> , <i>F.equiseti</i> , <i>F.semitectum</i> , <i>F.graminearum</i> , <i>Penicillium funiculosum</i> , <i>P.griseofulvum</i> , <i>P.canescens</i> , <i>Erotium sp.</i> , <i>Cladosporium sp.</i> , <i>Alternaria alternata</i> , <i>A.infectoria</i> , <i>A.oregonensis</i>	[12, 13]
Rice	Ecuador	<i>Aspergillus flavus</i> , <i>A.ochraceus</i> , <i>Fusarium verticillioides</i> , <i>F.oxysporum</i> , <i>F.proliferatum</i> , <i>F.semitectum</i> , <i>F.solani</i> , <i>Penicillium janthinellum</i> , <i>Epicoccum nigrum</i> , <i>Curvularia lunata</i> , <i>Nigrospora oryzae</i> , <i>Rhizopus stolonifer</i> , <i>Bipolaris oryzae</i>	[6]
Wheat	Argentina	<i>Aspergillus flavus</i> , <i>A.niger</i> , <i>A.oryzae</i> , <i>Fusarium verticillioides</i> , <i>Penicillium funiculosum</i> , <i>P.oxalicum</i>	[12]
	Germany	<i>Aspergillus candidus</i> , <i>A.flavus</i> , <i>A.versicolor</i> , <i>Eurotium sp.</i> , <i>Penicillium auriantogriseum</i> , <i>P.verrucosum</i> , <i>P.viridicatum</i> , <i>Alternaria sp.</i>	[14]
	Poland	<i>Alternaria tenuis</i> , <i>Aspergillus aculeatus</i> , <i>A.parasiticus</i> , <i>Fusarium moniliforme</i> , <i>F.verticillioides</i> , <i>Penicillium verrucosum</i> , <i>P.viridicatum</i> , <i>P.crustosum</i>	[15]
	Croatia	<i>Fusarium graminearum</i> , <i>F.poeae</i> , <i>F.avenaceum</i> , <i>F.verticillioides</i>	[11]
Maize	Ecuador	<i>Aspergillus flavus</i> , <i>A.parasiticus</i> , <i>Fusarium graminearum</i> , <i>F.verticillioides</i> , <i>Mucor racemosus</i> , <i>Rhizopus stolonifer</i> , <i>Acremonium strictum</i> , <i>Alternaria alternata</i> , <i>Cladosporium sp.</i>	[6]
	Poland	<i>Aspergillus aculeatus</i> , <i>Aspergillus parasiticus</i> , <i>Fusarium moniliforme</i> , <i>F.verticillioides</i>	[15]
	Argentina	<i>Fusarium verticillioides</i> , <i>F.proliferatum</i> , <i>F.subglutinans</i> , <i>F.dlamini</i> , <i>F.nygamai</i> , <i>Alternaria alternata</i> , <i>Penicillium funiculosum</i> , <i>P.citrinum</i> , <i>Aspergillus flavus</i>	[16, 17]

Commodities	Country	Fungal species	Ref
	Croatia	<i>Fusarium verticillioides</i> , <i>F.graminearum</i>	[11]
Oats	Poland	<i>Cladosporium</i> sp., <i>Aspergillus</i> sp., <i>Penicillium</i> sp.	[18]
Breakfast cereals	Poland	<i>Aspergillus versicolor</i> , <i>A.flavus</i> , <i>A.sydowi</i> , <i>A.niger</i> , <i>A.ochraceus</i> , <i>Fusarium graminearum</i> , <i>Penicillium chrysogenum</i> , <i>Eurotium repens</i>	[19]
Wheat flour	Germany	<i>Aspergillus candidus</i> , <i>A.flavus</i> , <i>A.niger</i> , <i>Eurotium</i> sp. <i>Penicillium</i> <i>aurantogriseum</i> , <i>P.brevicompectum</i> , <i>P.citrinum</i> , <i>P.griseofulvum</i> , <i>P.verrucosum</i> , <i>Cladosporium cladosporioides</i>	[14]

**Table 1.** Fungal species dominated in cereals and cereal products

*Fusarium graminearum* is associated with cereals and soybean growing in warmer areas such as South and North America or China, and *F.culmorum* in cooler areas such as Finland, France, Poland or Germany. Mechanical damage of kernels by birds or insects, e.g. European corn borer and sap beetles, predisposes corn to infections caused by *Fusarium* and other "field fungi". *Fusarium moniliforme* and *F.proliferatum* are the most common fungi associated with maize. It was found that the levels of contamination with *Fusarium* sp. were significantly greater on the conventional than the transgenic cultivars in 2000, but in 1999 the difference between the cultivars was not statistically significant. In case of *Alternaria*, a greater frequency of contamination in transgenic varieties was observed. The authors concluded that the isolation frequency can vary by years and is more dependent on the environmental and cultural practices than on varieties [9]. The isolation frequencies of fungi from seeds and pods of soybean cultivars varied annually, in part due to some differences in environmental conditions (rainfall) [8].

*Fusarium* species occur worldwide in a variety of climates and on many plant species as epiphytes, parasites, or pathogens. *Fusarium*-induced diseases of soybean have been attributed to different species: *Fusarium oxysporum* (fusarium blight, wilt and root rot), *Fusarium semitectum* (pod and collar rot), *F.solani* (sudden death syndrome) [21, 22]. *Fusarium* infections are spread by air-borne conidia on the heads or by a systemic infection. The species belonging to *Fusarium* genera are of particular interest due to the formation of a wide range of secondary metabolites, many of which are toxic to humans or animals. Infections by *Fusarium* spp. were determined by [11] in different crops. The contamination expressed as the percentage of seeds with *Fusarium* colonies ranged from 5% to 69% for wheat, from 25% to 100% for maize, from 4% to 17% for soybean. The dominant species were *F.graminearum* on wheat (27% of isolates), *F.verticillioides* on maize (83 % of isolates), and *F.sporotrichioides* on soybean (34 % of isolates) [11]. This study suggested that the risk of contamination with *Fusarium* toxins is higher for maize and wheat than for soybean.

The mycological state of grain can be considered as good when the number of CFU is within the range  $10^3$ - $10^5$  per gram [23]. In our research, the contamination of feed components such as barley, maize and wheat was in the range from  $10^2$  to  $10^4$  CFU/g, depending on the crop, region and mills [15]. It was found that wheat from organic farms was contaminated

with fungi by 70.5% more and barley by 24.8% less as compared to the crops from conventional farms [24]. Similarly, the total number of fungi in Polish ecological oat products was about a hundred times higher than in conventional ones. In samples of ecological origin, the mean value of fungi was  $1.1 \times 10^4$  CFU/g, whereas for conventional grains it was  $5.0 \times 10^2$  CFU/g [18].

The results obtained by [14] showed that the most common moulds isolated from whole wheat and wheat flour belong to the *Aspergillus* and *Penicillium* genera. From the whole wheat flour, 83.7% of *Aspergillus* followed by *Penicillium* (7.6%), *Eurotium* (2.9%) and *Alternaria* (2.5%) species were isolated. The white flour contained 77.3% of *Aspergillus*, 15% of *Penicillium* and 4.1% of *Cladosporium* genera. *Aspergillus candidus* was the dominant species. Among all the isolated fungal species, 93.2% belonged to the group of toxigenic fungi. Several toxin-producing *Aspergillus* species were reported to dominate on cereals, especially *A.flavus*, *A.candidus*, *A.niger*, *A.versicolor*, *A.penicillioides*, and *Eurotium* sp. at lower water activity [25]. Among *Aspergillus* species isolated from Ecuadorian soybean seeds, *Aspergillus flavus* and *A.ochraceus* were the most prevalent ones. The most frequent *Fusarium* species were *F.verticillioides* and *F.semitecium*. All the examined samples were contaminated with these species [6]. The presence of mycobiota in raw materials and finished fattening pig feed was determined in eastern Argentina. All samples of soybean seeds were contaminated with fungi in the range from 10 to  $9.0 \times 10^2$  CFU/g, depending on the sampling period. The most prevalent species in soybean and wheat bran were *Aspergillus flavus* and *Fusarium verticillioides* [12].

The fungal microflora changes during post-harvest drying and storage. The field fungi are adapted to growth at high water activity and they die during drying and storage, to be replaced by storage fungi that are capable of growing at lower  $a_w$ . For most grains, moisture content in the range from 10% to 14% is recommended, depending on the grain type and desired storage life [1].

A wide range of microorganisms have been isolated from storage grains, including psychrotolerant, mesophilic, thermophilic, xerophilic and hydrophilic species. The extremely xerophilic species are *Eurotium* spp. and *Aspergillus restrictus*, the moderate xerophilic ones include *A.candidus* and *A.flavus*, and the slightly xerophilic one is *A.fumigatus*. An example of psychrotolerant species belonging to *Penicillium* genera is *P.aurantiogriseum* and *P.verrucosum*, mesophilic species can be represented by *P.corylophilum*, and thermophilic species by *Talaromyces thermophilus*. Among the hydrophiles, the most common are *Fusarium* and *Acremonium* species [25]. The minimum  $a_w$  for conidial formation is influenced by temperature, for instance, *P.aurianogriseum* produces conidia to a minimum of 0.86  $a_w$  at 30°C, but to 0.83  $a_w$  at 23°C. Many species belonging to *Aspergillus* and *Penicillium* genera are highly adapted to the rapid colonisation of substrates of reduced water activity. Modifying several factors in grain storage may facilitate safe storage. Stores should be monitored for relative humidity, temperature and airflow efficiency. Moisture migration may occur during storage and create damp pockets. In addition to this, insect infestations may cause heating and the generation of moisture. Aeration with cool air may help to protect the stored commodities against fungal development.

## 2. Conditions affecting mycotoxin production

Cereals in the field are exposed to fungi from the soil, birds, animals, insects, organic fertilizers, and from other plants in the field. Mechanical damage of raw material or food due to insects and pests is a disturbing problem mainly in tropical regions, particularly as food contaminants are present in the field more abundantly than in the storage. Many different insects, e.g. European corn borer and sap beetles have the capability of promoting infections of various crops with mycotoxigenic fungi [25].

Mycotoxin production is determined by genetic capability related to strain and environmental factors including the substrate and its nutritious content. Toxin production is dependent on physical (temperature, moisture, light), chemical (pH value, nutrients, oxygen content, preservatives), and biological factors (competitive microbiota). Each fungus requires special conditions for its growth and other conditions for its toxin production.

### 2.1. Physical factors

The most important factor governing colonisation of grains and mycotoxin production is the availability of water which on the field comes mainly with rainfall. The second important factor is temperature. The moisture and temperature effects on mycotoxin production often differ from those on germination and growth. Table 2 presents the moisture and temperature requirements of most common toxigenic fungi for their growth and mycotoxin production.

It was found that optimal temperature for *F.graminearum* growth on soybean contained in the range 15-20°C (in isothermal temperature) and 15/25°C (in cycling temperature). The optimal temperature for mycotoxin production on soybean was 20°C for deoxynivalenol (DON) and 15°C for zearalenone (ZEA). After 15 days of incubation, the maximum levels 39 ppm and 1040 ppm for ZEA and DON, respectively, were detected. Fumonisin were produced by *Fusarium graminearum* only the on culture medium at 30°C; on soybean no fumonisins were detected [31].

Most fungi need at least 1-2% of O<sub>2</sub> for their growth. The influence of high carbon dioxide and low oxygen concentrations on the growth and mycotoxin production by the foodborne fungal species was investigated by [32]. Three groups of species were distinguished: first, which did not grow in 20% CO<sub>2</sub> <0.5% O<sub>2</sub> (*Penicillium commune*, *Eurotium chevalieri* and *Xeromyces bisporus*); second, which grew in 20% CO<sub>2</sub> <0.5% O<sub>2</sub>, but not 40% CO<sub>2</sub> <0.5% O<sub>2</sub> (*Penicillium roqueforti* and *Aspergillus flavus*); and third, which grew in 20%, 40% and 60% CO<sub>2</sub> <0.5% O<sub>2</sub> (*Mucor plumbeus*, *Fusarium oxysporum*, *F.moniliforme*, *Byssoschlamys fulva* and *B.nivea*). The production of aflatoxin, patulin, and roquefortine C was greatly reduced under all of the atmospheres tested. For example, aflatoxin was not produced by *A. flavus* during growth under 20% CO<sub>2</sub> for 30 days. Patulin was produced by *B.nivea* in the atmospheres of 20% and 40% CO<sub>2</sub>, but only at low levels [32].

### 2.2. Chemical factors

Nutritional factors such as carbohydrate and nitrogen sources and microelements (copper, zinc, cobalt) affect mycotoxin production, but the mechanisms of this impact are still

Species	For growth		For mycotoxin production			Ref.	
	Temperature [°C]		Minimal a <sub>w</sub>	Temperature [°C]			Minimal a <sub>w</sub>
	Range	Optimum	Range	Range	Optimum		Range
<i>Alternaria alternata</i>	0 – 35	20 – 25	0.88	5-30	20-25	0.95-1.0 AOH 0.90 TeA	[25, 26, 28]
<i>Fusarium culmorum</i>	<0 – 31	21	0.89	11-30	25-26	Nd	[25]
<i>Fusarium graminearum</i>	Nd	24 – 26	0.89	Nd	24-26	Nd	[25]
<i>Fusarium sporotrichoides</i>	-2 – 35	22 – 28	0.88	6-20	Nd	Nd	[25]
<i>Penicillium verrucosum</i>	0-31	20	0.81-0.83	4-31	20	0.86	[28, 29]
<i>Penicillium expansum</i>	-6 – 35	25 – 26	0.82 – 0.85	0-31	25	0.95	[25]
<i>Aspergillus ochraceus</i>	8-37	24-30	0.76-0.83	12-37	25-31	0.85 OTA 0.88 PA	[28, 29]
<i>Aspergillus parasiticus</i>	10-43	32-33	0.84	12-40	25-30	0.87	[28, 29]
<i>Aspergillus flavus</i>	6 – 45	35 – 37	0.78	12-40	30	0.82	[25]
<i>Aspergillus versicolor</i>	4 – 39	25 – 30	0.75	15-30	23 – 29	">0.76	[25, 30]

OTA – ochratoxin A; PA – penicillic acid AOH – alternariol, TeA – tenuazonic acid, ND – no data

**Table 2.** Environmental requirements for growth and mycotoxin production

unclear. A relationship between mycotoxin production and sporulation has been documented in several toxigenic fungi. For example, chemical substances that inhibit sporulation of *Aspergillus parasiticus* have also been shown to inhibit the production of aflatoxin [33]. Chemical preservatives such as organic acids (sorbic, propionic, acetic, benzoic) or fungicides have been used to restrict the growth of mycotoxigenic fungi. It was found that propionic acid at the concentration of up to 0.05% inhibited the growth and ochratoxin production by *Penicillium auriantogriseum*. A more effective result in higher temperature was observed [34]. Inhibiting fungal growth and toxigenic properties by organic acids is connected with lowering the pH value. It was found that ammonium and sodium bicarbonate at the concentration of 2% fully inhibited the development of the cultures of *Aspergillus ochraceus*, *Fusarium graminearum* and *Penicillium griseofulvum* inoculated into corn. The production of ochratoxin A by *Aspergillus ochraceus* was reduced from 26 ppm in untreated corn to 0.26 ppm in bicarbonate-treated corn samples [35].

### 2.3. Biological factors

The simultaneous presence of different microorganisms, such as bacteria or other fungi, could disturb fungal growth and the production of mycotoxins. For instance, *Alternaria* and *Fusarium* are antagonistic, and *Alternaria* was less abundant in grain with a high incidence rate of *F.culmorum*. *Epicoccum* is a strong antagonist too [25].



At 30°C, the ochratoxin production by *Aspergillus ochraceus* was inhibited by *A.candidus*, *A.flavus*, and *A.niger* in 0.995 a<sub>w</sub>. At 18°C and 0.995 a<sub>w</sub>, the interaction between *Aspergillus ochraceus* and *Alternaria alternata* resulted in a significant stimulation of ochratoxin A production [36]. Therefore, several microorganisms were reported as effective biocontrol agents against several fungal plant pathogens [37]. It was determined that *Trichoderma harzianum* produces a lytic enzyme, chitinase, which manifests antifungal activity against a wide range of fungal strains. It was found that non-toxicogenic *T.harzianum* isolates significantly reduce the production of six types of A trichothecenes in cereals [38].

According to [39], soybean is not a favourable medium for ZEA production since it possesses some features that limit the production of this toxin by *Fusarium* isolates. Similarly, the production of aflatoxin B<sub>1</sub> by *Aspergillus flavus* was suppressed by soybean phytoalexin – glyceollin [40].

### 3. Main mycotoxins

The worldwide contamination of foods and feeds with mycotoxins is a significant problem. It was estimated that 25% of the world's crops may be contaminated with these metabolites. Mycotoxigenic fungi involved with the human food chain belong mainly to three genera *Aspergillus*, *Penicillium* and *Fusarium*. The toxins produced by *Alternaria* have recently been of particular interest. The biochemistry, physiology and genetics of mycotoxigenic fungi have been discussed in several review articles [28, 41, 42].

Mycotoxins diffuse into grain and can be found in all grind fractions and, due to their thermo-resistant properties, also in products subjected to thermal processing [43].

The characteristics of major toxins that contaminate foods and feeds in the EU, described from the economic and toxicological point of view, are presented below.

#### 3.1. Aflatoxins (AFs)

Aflatoxins are difuranocoumarin derivatives. The main naturally produced aflatoxins based on their natural fluorescence (blue or green) are called B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub>. Aflatoxin M<sub>1</sub> is a monohydroxylated derivative of AFB<sub>1</sub> which is formed and excreted in the milk of lactating animals. AF<sub>s</sub> are very slightly soluble in water (10–30 µg/mL); insoluble in non-polar solvents; freely soluble in moderately polar organic solvents (e.g. chloroform and methanol) and extremely soluble in dimethyl sulfoxide. They are unstable under the influence of ultraviolet light in the presence of oxygen, to extremes of pH (<3, >10) and to oxidizing agents [44].

Aflatoxins are produced only by a closely related group of aspergilli: *Aspergillus flavus*, *A.parasiticus*, and *A.nomius* strains [45]. These species are very widespread in the tropical and subtropical regions of the world. Other species such as *A.bombycis*, *A.ochraceoroseus*, and *A.pseudotamari* are also aflatoxin-producing species, but they are found less frequently [46, 47]. Aflatoxins constitute a problem concerning many commodities (nuts, spices), however, in terms of grain they are primarily problematic in case of maize. This is because only maize

can be colonised by *A.flavus* and related species in the field. Out of the other grains, rice is an important dietary source of aflatoxins in tropical and subtropical areas. In regions with moderate climate, the problem is connected with imported commodities or the local crops that are wet or stored in improper conditions [45]. The carcinogenicity, mutagenicity and acute toxicology of AFB<sub>1</sub> have been well documented. The IARC determined it to be a human carcinogen (group 1A).

### 3.2. Ochratoxin A (OTA)

Ochratoxin A is a chlorinated isocoumarin derivative, which contains a chlorinated isocoumarin moiety linked through a carboxyl group to L-phenylalanine via an amide bond. It is colourless, crystalline, and soluble in polar organic solvents compounds. This toxin is more stable in the environment than AFs. The studies of [45] reported that thermal destruction of OTA occurs after exceeding 250°C. OTA is produced by *Penicillium* species such as *P.verrucosum*, *P.aurantiogriseum*, *P.nordicum*, *P.palitans*, *P. commune*, *P.variabile* and by *Aspergillus* species e.g. *A.ochraceus*, *A.melleus*, *A.ostianus*, as well as the aspergilli species of section *Nigri*. In moderate climates, the main producers of OTA are *Penicillium* species, while *Aspergillus* species dominate in tropical and subtropical climates. Ochratoxin A is often found with citrinin produced by *Penicillium aurantiogriseum*, *P.citrinum*, and *P.expansum* [48]. Significant human exposure comes from the consumption of grape juice, wine, coffee, spices, dried fruits and cereal-based products, e.g. whole-grain breads, and in addition to this from products of animal origin, e.g. pork and pig blood-based products. The Scientific Panel on Contaminants in the Food Chain of the European Food Safety Authority (EFSA) has derived an OTA tolerable weekly intake (TWI) on the level of 120ng/kg b.w. The IARC [49] determined it to be a possible human carcinogen (group 2B). Ochratoxins are the cause of urinary tract cancers and kidney damage. In ruminants, ochratoxin A is divided to non-toxic ochratoxin alfa and phenylalanine [44].

### 3.3. Citrinin

Citrinin is a polyketide nephrotoxin produced by several species of the genera *Aspergillus*, *Penicillium* and *Monascus*. Some of the citrinin-producing fungi are also able to produce ochratoxin A or patulin. Citrinin is insoluble in cold water, but soluble in aqueous sodium hydroxide, sodium carbonate, or sodium acetate; in methanol, acetonitrile, ethanol, and most other polar organic solvents. Thermal decomposition of citrinin occurs at >175 °C under dry conditions, and at > 100 °C in the presence of water. The known decomposition products include citrinin H<sub>2</sub> which did not show significant cytotoxicity, whereas the decomposition product citrinin H<sub>1</sub> showed an increase in cytotoxicity as compared to the parent compound [50]. The most commonly contaminated commodities are barley, oats, and corn, but contamination can also occur in case of other products of plant origin e.g. beans, fruits, fruit and vegetable juices, herbs and spices, and also in spoiled dairy products [50].

### 3.4. Fumonisin (F<sub>1</sub>)

Fumonisin are a group of diester compounds with different tricarboxylic acids and polyhydric alcohols and primary amine moiety. There are several fumonisins, but only fumonisins

B<sub>1</sub> (FB<sub>1</sub>) and B<sub>2</sub> (FB<sub>2</sub>) have been found in significant amounts. Some technological processes hydrolyze the tricarboxylic acid chain in fumonisin B<sub>1</sub>. The product of this reaction is more toxic than fumonisin [51].

FB<sub>1</sub> is produced by fungi from *Fusarium* genera, especially by *F.moniliforme* and *F.proliferatum*. The study of [11] suggests that the risk of contamination with *Fusarium* toxins is higher for maize and wheat than for soybean and pea. High concentrations of fumonisins are associated with hot and dry weather, followed by the periods of high humidity. Studies on fumonisin residues in milk, meat and eggs are incomplete [52, 53]. Human exposure assessments on fumonisin B<sub>1</sub> have rarely been reported. The mean daily intake in Switzerland is estimated to be 0.03 µg/kg bw/day. In the Netherlands the exposure estimates ranged from 0.006 to 7.1 µg/kg bw/day. In South Africa, the estimates ranged from 14 to 440 µg/kg bw/day, showing that the exposure to FB<sub>1</sub> is considerably higher than in the other countries in which exposure assessments were performed [54]. It was concluded that for F<sub>s</sub> there was inadequate evidence in humans for carcinogenicity. Therefore, the IARC classified *Fusarium moniliforme* toxins, including fumonisins, as potential carcinogens to humans (group 2B).

### 3.5. Zearalenone

Zearalenone is a macrocyclic lactone with high binding affinity to oestrogen receptors. ZEA is produced mainly by *Fusarium graminearum* and *F.sporotrichoides* in the field and during storage of commodities such as maize, barley, sorghum, and soybean. The IARC has evaluated the carcinogenicity of zearalenone and found it to be a possible human carcinogen (group 2B). Residues of zearalenone in meat, milk and eggs do not appear to be a practical problem [53, 54].

### 3.6. Trichothecenes

Trichothecenes constitute a group of 50 mycotoxins produced by *Fusarium*, *Cephalosporium* and *Stachybotrys* genera in different commodities. There are including T-2 toxins, deoxynivalenol, nivalenol, and diacetoxyscirpenol. Beside trichothecenes, deoxynivalenol (DON, vomitoxin) is probably the most widely distributed in cereal and soybean foods and feeds. In contaminated cereals, DON derivatives such as 3-acetyl DON and 15-acetyl DON can occur in significant amounts (10 – 20%) with DON. DON is produced by closely related *Fusarium graminearum*, *F.culmorum* and *F.crokwelense* species [55].

T-2 toxin produced mainly by *F.sporotrichoides* and *F.poaie* is primarily associated with mould millet, wheat, rye, oats, and buckwheat. This toxin can be transmitted from dairy cattle feed to milk [56].

### 3.7. Alternaria toxins

*Alternaria* species, besides *Fusarium*, is the most isolated fungi from soybean and other cereals. Several species are known producers of toxic metabolites called *Alternaria* mycotoxins. The most important *Alternaria* mycotoxins include alternariol (AOH), alternariol monomethyl ether (AME), altertoxins I, II, and III (ATX-I, -II, III), tenuazonic acid (TeA), and altenuene

(ALT). They belong to three structural classes: dibenzopyrone derivatives, perylene derivatives, and tetramic acid derivatives. Alternariol and related metabolites (AME and ALT) are produced by *Alternaria alternata*, *A.brassicae*, *A.citri*, *A.cucumerina*, *A.dauci*, *A.kikuchiana*, *A.solani*, *A.tenuissima*, and *A.tomato*. These strains are known as plant, especially fruit and vegetable pathogens. In cereals, soybean and oilseeds, AOH, AME and ALT are produced mainly by *Alternaria alternata*, *A.tenuissima*, and *A.infectoria*. AOH has been reported to possess cytotoxic, genotoxic, mutagenic, carcinogenic, and oestrogenic properties [27]. Tenuazonic acid (TeA) is a mycotoxin and phytotoxin produced primarily by *Alternaria alternata* and other phytopathogenic *Alternaria* species. The overview of the chemical characterisation, producers, toxicity, analysis and occurrence in foodstuffs was summarised by [27].

### 3.8. Sterigmatocystin

Sterigmatocystin (STC) is a precursor of the aflatoxins produced mainly by many *Aspergillus* species such as *A.versicolor*, *A.chevalieri*, *A.ruber*, *A.aureolatus*, *A.quadrilineatus*, *A.sydowi*, *Eurotium amstelodami*, and less often by *Penicillium*, *Bipolaris*, *Chaetomium*, and *Emericella* genera [30]. Sterigmatocystin was reported as a fungal metabolite in mouldy wheat, rice, barley, rapeseed, peanut, corn, and cheeses or salami. The STC producers, occurrence and toxic properties were reviewed by [30, 57].

## 4. Contamination level in cereal and soybean-based food and feed products

Food security strategy in the European Union (EU) includes the Rapid Alert System for Food and Feed. The RASFF was established by the European Parliament and Council Regulation No. 178/2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and specifying the procedures in matters concerning food safety [58].

In 2002 – 2011, the number of notifications to the RASFF system due to mycotoxin contamination of food was respectively: 302, 803, 880, 996, 878, 760, 933, 669, 688, 631 notifications identifying the presence of aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) and the amount of AFB<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>, AFM<sub>1</sub>, ochratoxin A (OTA), fumonisins B<sub>1</sub> and B<sub>2</sub> (FB<sub>1</sub>, FB<sub>2</sub>), patulin, deoxynivalenol (DON) and zearalenone (ZEA) in such groups of foods, as nuts and milk, oilseeds, cereal, dried fruit, fruit, cocoa, coffee, herbs and spices, wine, milk, products for children. Approximately 95% of the notifications concerned foodstuffs contaminated with aflatoxins. During this period, the number of notifications regarding mycotoxin contamination of grains did not exceed 15% of the total number of notifications. The data in Figure 1 show that in 2002-2011 aflatoxins, ochratoxin A and fumonisins were the main contaminants isolated from cereals [59].

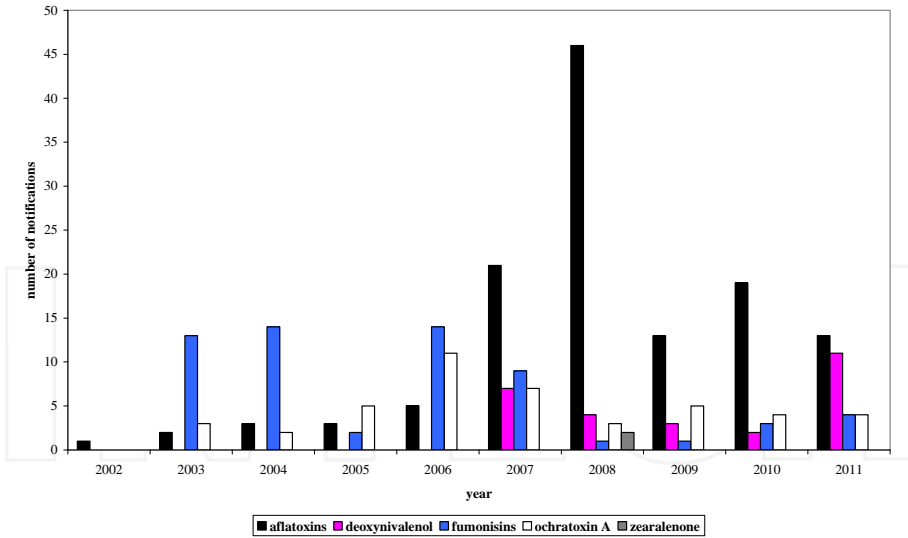
In the research of [60], ninety-five cereal samples from retail shops and local markets of different locations in Pakistan were examined in terms of the presence of aflatoxins. The results showed the percentage of aflatoxin contamination samples in the commodities such as in:

Mycotoxin	Produced species	Commodities
Aflatoxins	<i>Aspergillus flavus</i> , <i>A.parasiticus</i> , <i>A.nomius</i> , <i>A.bombycis</i> , <i>A.ochraceoroseus</i> , <i>A.pseudotamari</i>	Nuts, spices, Cereals, maize, soybean, rice
Ochratoxin A	<i>Penicillium verrucosum</i> , <i>P.aurantiogriseum</i> , <i>P.nordicum</i> , <i>P.palitanis</i> , <i>P.commune</i> , <i>P.variabile</i> , <i>Aspergillus ochraceus</i> , <i>A.melleus</i> , <i>A.niger</i> , <i>A.carbonarius</i> , <i>A.sclerotiorum</i> , <i>A.sulphureus</i>	Cereals, fruits, spices, coffee, Food of animal origin
Citrinin	<i>Penicillium citrinum</i> , <i>P.verrucosum</i> , <i>P.viridicatum</i> , <i>Monascus purpureus</i>	Oats, rice, corn, beans, fruits, fruit and vegetable juices, herbs and spices
Sterigmatocystin	<i>Aspergillus versicolor</i> , <i>A.nidulans</i> , <i>A.chevalieri</i> , <i>A.ruber</i> , <i>A.aureolatus</i> , <i>A.quadrilineatus</i> , <i>Eurotium amstelodami</i>	Cereals, cheese
Zearalenone	<i>Fusarium graminearum</i> , <i>F.sporotrichoides</i> , <i>F.culmorum</i> , <i>F.cerealis</i> , <i>F.equiseti</i> , <i>F.incarnatum</i>	Maize, soybean, cereals
Deoxynivalenol	<i>Fusarium graminearum</i> , <i>F.culmorum</i> , <i>F.crokwwellense</i>	Maize, soybean, cereals
Fumonisin	<i>Fusarium proliferatum</i> , <i>F.verticillioides</i> ,	Maize, soybean, cereals
Alternariol, alternariol monomethyl ether	<i>Alternaria alternata</i> , <i>A.brassicae</i> , <i>A.capsici-anui</i> , <i>A.citri</i> , <i>A.cucumerina</i> , <i>A.dauci</i> , <i>A.kikuchiana</i> , <i>A.solani</i> , <i>A.tenuissima</i> , <i>A.tomato</i> , <i>A.longipes</i> , <i>A.infectoria</i> , <i>A.oregonensis</i>	Vegetables, fruit, cereals, soybean
Tenuazonic acid	<i>Alternaria alternata</i> , <i>A.capsici-anui</i> , <i>A.citri</i> , <i>A.japonica</i> , <i>A.kikuchiana</i> , <i>A.mali</i> , <i>A.solani</i> , <i>A.oryzae</i> , <i>A.porri</i> , <i>A.radicina</i> , <i>A.tenuissima</i> , <i>A.tomato</i> , <i>A.longipes</i>	Vegetables, fruit, cereals, soybean

**Table 3.** Mycotoxigenic fungi and mycotoxins

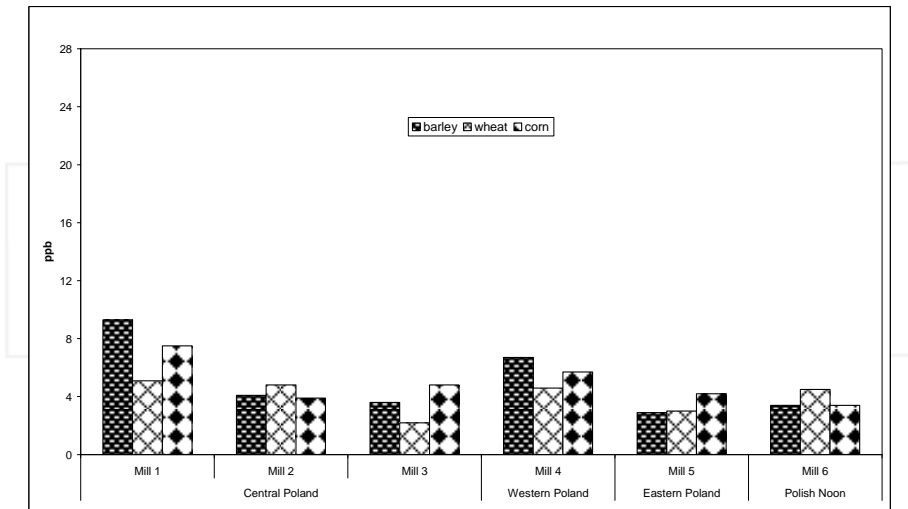
rice (25%), broken rice (15%), wheat (20%), maize (40%), barley (20%) and sorghum (30%), while in soybean (15%). The highest contamination levels of aflatoxins were found in one wheat sample (15.5 ppb), one maize sample (13.0 ppb) and one barley sample (12.6 µg/kg). In the research of [61], seventeen samples of wheat grain from Morocco were tested for OTA and DON contamination. The results show that only two samples (11.76%) out of 17 were contaminated with OTA, at the mean concentration of 29.4 ppb. However, seven samples (41.17%) were contaminated with DON at the mean concentration of 65.9 ppb.

The aim of our own research [15] was mycotoxic analysis of grains included in the standard mixtures used in feed formulations. Eighteen samples were tested containing seeds evenly divided into three types: barley, wheat and corn. The tested seeds were from randomly selected Polish mills: the central, western, eastern and south ones (Figure 2). The aflatoxins content in 51% of the screened barley samples and in 34% of the screened wheat and maize samples did not exceed the limit set in the European Union Regulation, i.e. 4 ppb [62]. In reference to the grain origin, it was established that grains from the central and western parts of Poland exhibited the highest extent of AF<sub>s</sub> contamination. To compare, the AFs level in wheat grains from various regions of Turkey was very low, ranging from 10.4 to 634.5

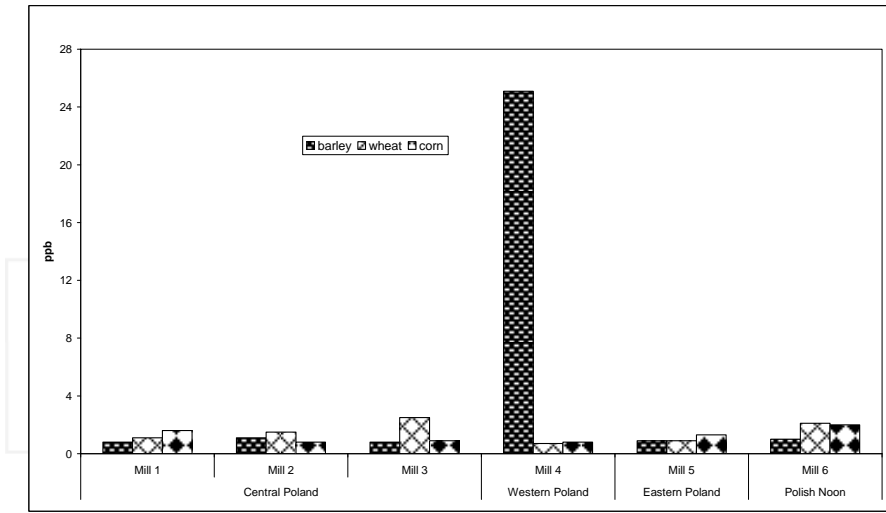


**Figure 1.** The number of notifications received by RASFF on mycotoxins in cereals in 2002-2011

ng/kg [63], whereas in the samples of barley, wheat, and oat grains from Sweden it was contained between 50 and 400 ppb [64].



**Figure 2.** Level of contamination with aflatoxins in grains coming from different regions of Poland



**Figure 3.** Level of contamination with ochratoxina A in grains coming from different regions of Poland

The OTA level in the examined grains collected from mills in central, eastern and southern Poland was low and ranged from 0.5 to 2.5 ppb (Figure 3). Therefore, it did not exceed the permissible limit set by the European Union (Commission Regulation No. 105/2010), i.e. 5 ppb [65]. Only in barley coming from a mill located in western Poland, the OTA level exceeded the limits fivefold. The extent of OTA contamination of barley, wheat, and maize grain from various regions of Mexico was also low and recorded 0.17 ppb, 0.42 ppb, and 1.08 ppb, respectively. Only 1 out of 20 examined maize grains showed the OTA level of 7.22 [66]. To compare, the OTA concentration in barley and wheat grain from the UK equalled from 1 to 33 ppb [67]. In the research of [68], among others, the levels of AFs and OTA in 532 grain and feed samples from Poland from 2002 and 2003 were determined. The average mycotoxin concentration levels were similar and quite low, i.e. AFs - 0.3 ppb and OTA - 1.1 ppb in grains and feeds from 2002, and respectively, AFs 3.1 and 1.0 ppb and OTA 0.5 and 0.7 OTA in samples from 2003. The authors of the study stressed that in 2002 and 2003 the harvesting seasons were hot and dry, which might have resulted in the low extent of fungi contamination of the examined grain. Although the extent of mycotoxin contamination of grain in the quoted studies varies, their authors concur that it is a serious issue whose scale depends on the microclimate during arable farming and the subsequent phases, i.e. grain storage. It was reported that no mycotoxins were found in barley samples stored for 20 weeks at 15% seed humidity, whereas the samples of wheat stored for the same period of time at 19% humidity recorded relatively high concentration levels: OTA - 24 ppb, citrinin - 38 ppb, and sterigmatocystin even up to 411 ppb [69].

The aim of our research was the assessment of cereal products available in trade and meant for direct consumption as for contamination with selected mycotoxins. The research included corn flakes, corn flakes with nuts and honey, various kinds of breakfast cereal products

and muesli containing dried fruit, nuts as well as cereal and coconut flakes (15 samples). None of the products was contaminated with  $AB_1$  on the level exceeding the acceptable limits (2 ppb). The presence of ochratoxin A exceeding the amount of 3 ppb was discovered in four samples (two kinds of corn flakes, exotic muesli and traditional muesli). The contamination with that toxin equalled 4.5 ppb on average. According to the current regulation, contamination of breakfast flakes with deoxynivalenol DON should not exceed 500 ppb. Four samples (containing corn) exceeded this limit by 50%. In case of one sample, DON contamination was very high, almost three times higher than the acceptable level [19].

Mycotoxin contamination of soybean is not considered a significant problem as compared to commodities such as corn, cottonseed, peanuts, barley and other grains. In the early surveys conducted by the U.S. Department of Agriculture (USDA), 1046 soybean samples collected from different regions of the United States were examined for aflatoxins contamination. Aflatoxin presence was confirmed at low levels (7-14 ppb) in only two of the tested samples [70]. In the research of [71], fifty-five samples of soybean meals were analysed for the content of aflatoxins, deoxynivalenol (DON), zearalenone (ZEA) and ochratoxin A (OTA). Regarding aflatoxins, only  $AFB_1$  was detected in 32 out of the 51 non-suspicious samples, but the maximal concentration found was only 0.41 ppb. ZEA was detected in 23 out of the 51 samples with a maximum concentration of 18 ppb. DON could be detected only in one suspicious sample in a low concentration of 104 ppb. OTA was found in 5 samples, with the greatest concentration being only 1 ppb.

The research of [72] tested 122 soybean samples that came from Asia and the Pacific region. Aflatoxin was found in only in 2% (maximum of 13 ppb, median 9 ppb), zearalenone in 17% (maximum 1078 ppb, median 57 ppb), ochratoxin in 13% (maximum 11 ppb, median 7 ppb), and DON and fumonisins each in 7% of the analyzed samples (DON: maximum 1347ppb, median 264 ppb; fumonisins: maximum 331 ppb, median 154 ppb). In maize and maize products, the levels of fumonisins varied from 0.07 to 38.5 ppm in Latin America, from 0.004 to 330 ppm in North America, from 0.02 to 8.85 ppm in Africa, and from 0.01 to 153 ppm in Asia. The data available for Europe varied from 0.007 to 250 ppm in maize, and from 0.008 to 16 ppm in maize products. [54].

## 5. Influence of mycotoxins on human and animal organisms

Effects of mycotoxins on human and animal health are now increasingly recognised. Mycotoxins enter human and animal dietary systems mainly through ingestion, but increasing evidence also points to inhalation as another entry route. Mycotoxins exhibit a wide array of biological effects and individual mycotoxins can be [73]:

- carcinogenic - aflatoxins, ochratoxins, fumonisins, and possibly patulin;
- mutagenic - aflatoxins and sterigmatocystin;
- hematopoietic - aflatoxins and trichothecenes. Hematopoiesis refers to the production of all types of blood cells from the primitive cells stem cells in the bone marrow. The dys-



function of hematopoiesis leads firstly to the decrease in the number of neutrophils, thus perturbing the animal's immune system and subsequently to the decrease in red blood cells, which leads to anemia;

- hepatotoxic - aflatoxins, ochratoxins, fumonisins. All of them induce significant liver damage when given to animals;
- nephrotoxic - ochratoxins, citrinin, trichothecenes, and fumonisins;
- teratogenic - aflatoxin B<sub>1</sub>, ochratoxin A, T-2 toxin, sterigmatocystin, and zearalenone;
- oestrogenic - zearalenone;
- neurotoxic - ergot alkaloids, fumonisins, deoxynivalenol. The effects of mycotoxins are best evidenced by vomiting and taste aversion produced by DON, seizures, focal malata and liquefaction of the brain tissue, possibly mediated by sphingolipid synthesis under the influence of fumonisins, staggering and trembling produced by many tremorgenic penitrem mycotoxins seizures and other neural effects of ergot alkaloids and parasympathomimetic activity resulting from the effects of the metabolite slaframine for selected receptors in the nervous system
- immunosuppressive - several mycotoxins. The predominant mycotoxins in this regard are aflatoxins, trichothecenes, and ochratoxin A. However, several other mycotoxins such as fumonisins, zearalenone, patulin, citrinin, and fescue and ergot alkaloids have been shown to produce some effects on the immune system.

Table 4 presents the groups of mycotoxins which are most harmful to human and animal organisms, together with the chosen disease symptoms they cause.

### 5.1. Negative effects of mycotoxins on humans

Mycotoxicoses can be divided into acute and chronic. Acute toxicity usually has a rapid onset and obvious toxic response, chronic exposure is characterized by chronic doses over a long period of time and may lead to cancer and other effects that are generally irreversible. The symptoms of mycotoxicosis depend on the type, amount and duration of exposure, age, health and sex of the exposed individual, and many poorly understood synergistic effects involving genetics, dietary status, and interaction with other toxic contaminants. Thus, the severity of mycotoxin poisoning can be compounded by factors such as vitamin deficiency, caloric deprivation, alcohol abuse, and infectious disease status. Mycotoxicosis is difficult to diagnose because doctors do not have experience with this disease and its symptoms are so wide that it mimics many other conditions [74, 75].

Aflatoxicosis is toxic hepatitis leading to jaundice and, in severe cases, death. AFB<sub>1</sub> has been extensively linked to human primary liver cancer and was classified by the International Agency for Research on Cancer (IARC) as a human carcinogen (Group 1A - carcinogens) [49]. Although acute aflatoxicosis in humans is rare, several outbreaks have been reported. In 2004, one of the largest aflatoxicosis outbreaks in Kenya, resulting in 317 cases and 125 deaths was observed. Contaminated corn was responsible for the outbreak, and officials

Mycotoxin	Toxicity class according to International Agency for Research on Cancer (IARC)	Symptoms and diseases
Aflatoxins	I *	aflatoxicosis, primary liver cancer, lung neoplasm, lung cancer, failure of the immune system, vomiting, depression, hepatitis, anorexia, jaundice, vascular coagulation
Ochratoxins	II B **	renal diseases, nephropathy, anorexia, vomiting, intestinal haemorrhage, tonsillitis, dehydration
Fumonisin	II B **	diseases of the nervous system, cerebral softening, pulmonary oedema, liver cancers, kidney diseases, oesophagus cancers, anorexia, depression, ataxia, blindness, hysteria, vomiting, hypotension
Zearalenone	-	reproduction disruptions, abortions, pathological changes in the reproductive system
Trichothecenes	-	nausea, vomiting, haemorrhages, anorexia, alimentary toxic aleukia, failure of the immune system, infants' lung bleeding, increased thirst, skin rash

\*The agent (mixture) is carcinogenic to humans. The exposure circumstance entails exposures that are carcinogenic to humans

\*\*The agent (mixture) is possibly carcinogenic to humans. The exposure circumstance entails exposures that are possibly carcinogenic to humans.

**Table 4.** The list of adverse effects of the chosen mycotoxins.

found the level of aflatoxin B<sub>1</sub> as high as 4400 ppb [76]. Research in Gambian children and adults reported a strong association between aflatoxin exposure and impaired immunocompetence suggesting that the consumption of aflatoxin reduces resistance to infections in human populations [77, 78]. In 1974, an epidemic of hepatitis in India affected 400 people resulting in 100 deaths. The death was due to consumption of corn that was contaminated with *A. flavus* containing up to 15000 ppb of aflatoxins [79].

Ochratoxin A was the cause of epithelial tumours of the upper urinary tract in the Balkans [80, 81]. The condition is known as Balkan endemic nephropathy. Despite the seriousness of the problem, the study did not explain the mechanism of action and the size of OTA carcinogenicity in humans [82]. Ochratoxin has been detected in blood in 6-18% of the human population in some areas where Balkan endemic nephropathy is prevalent. Ochratoxin A has also been found in human blood samples from outside the Balkan Peninsula. In some survey, over 50% of the tested samples were contaminated. A highly significant correlation was observed between Balkan nephropathy and urinary tract cancers, particularly tumours of the renal pelvis and ureter. However, no data have been published that establishes a direct causal role of ochratoxin A in the etiology of these tumours [81].

Fumonisin B<sub>1</sub> was classified by the IARC as a group 2B carcinogen (possibly carcinogenic for humans) [44]. Fumonisin, which inhibit the absorption of folic acid through the foliate receptor, have also been implicated in the high incidence of neural tube defects in the rural population known to consume contaminated corn, such as the former Transkei region of South Africa and some areas of Northern China [75, 83].

Trichothecenes have been proposed as potential biological warfare agents. In the years 1975-1981, T-2 toxin was implicated as a chemical agent "yellow rain" used against the Lao Peoples Democratic Republic. A study conducted from 1978 to 1981 in Cambodia revealed the presence of T-2 toxin, DON, ZEA, and nivalenol in water and leaf samples taken from the affected areas [75, 84]. Clinical symptoms proceeding to death included vomiting, diarrhoea, bleeding, and difficulty with breathing, pain, blisters, headache, fatigue and dizziness. There also occurred necrosis of the mucosa of the stomach as well as the small intestine, lungs and liver [85]. One disease outbreak was recorded in China and was associated with the consumption of scabby wheat containing 1000-40000 ppb of DON. The disease is characterized by gastrointestinal symptoms. Also, in India there took place a reported infection associated with the consumption of bread made from contaminated wheat (DON 350-8300 ppb, acetyldeoxynivalenol 640-2490 ppb, NIV 30-100 ppb and T-2 toxin 500-800 ppb). The disease is characterized by gastrointestinal symptoms and throat irritation, which developed within 15 minutes to one hour after ingestion of the contaminated bread [81].

## 5.2. Negative effects of mycotoxins on animal

Animals may show varied symptoms upon contact with mycotoxins, depending on the genetic factors (species, breed, and strain), physiological factors (age, nutrition) and environmental factors (climatic conditions, rearing and management). The natural contamination with mycotoxins in animal feed usually does not occur at the levels that may cause acute or overt mycotoxicosis, such as hepatitis, bleeding, nephritis and necrosis of the oral and enteric epithelium, and even death. It is often difficult to observe and diagnose the symptoms of the disease, but it certainly is the most common form of mycotoxicosis in farm animals, affecting such parameters as productivity, growth and reproductive performance, feed efficiency, milk and egg production.

The negative effects of mycotoxins on the performance of poultry have been shown in numerous studies. For example, feeding the broilers with feed containing an AF<sub>s</sub> mixture (79% AFB<sub>1</sub>, 16% AFG<sub>1</sub>, AFB<sub>2</sub> 4% and 1% AFG<sub>2</sub>) in the concentration of 3.5 ppm decreased their body weight and increased their liver and kidney weight [75, 86]. Feeding OTA (0.3-1 ppm) to broilers reduced glycogenolysis and dose-dependent accumulation of glycogen in the liver. These negative metabolic reactions were attributed to inhibition of cyclic adenosine 3',5'-monophosphate-dependent protein kinase, and were reflected in reduced efficiency of feed utilization and teratogenic malformations [75].

*Fusarium* mycotoxins proved to be harmful to poultry. In addition to reduced feed intake and weight gain, sore mouth, cheeks and plaque formation was observed after 7-day-old chicks were exposed to T-2 toxin (4 or 16 ppm) [75, 87]. Pigs are among the most sensitive species to mycotoxins. In the study by [88], pigs in response to AF<sub>s</sub> (2 ppm), OTA (2 ppm),

or both were evaluated. Compared to the control group, the body weight gains were reduced by 26, 24 and 52% for animals consuming diets containing AF<sub>1</sub>, OTA, or both, respectively. Additional symptoms in pig ochratoxicosis were anorexia, fainting, uncoordinated movements, and increased water consumption and urination. Pigs also are susceptible to other mycotoxins, such as fumonisins and ergot alkaloids. Fumonisin B<sub>1</sub>, for example, has been shown to cause pulmonary oedema and heart and respiratory dysfunction. The symptoms of swine pulmonary oedema included dyspnoea, cyanosis, and death [89, 90]. Mycotoxic porcine nephropathy is a serious disease, often associated with pigs consuming feed contaminated with OTA, especially in Scandinavian region. In addition to the enlarged and pale kidneys (with vascular lesions and white spots), morphological changes include a proximal tubular injury, epithelial atrophy, fibrosis and hyalinization of renal glomerular [80, 81]. Negative effects of ZEA on pigs' reproductive function have also been demonstrated [91]. Oestrogenic effects of ZEA on gilts and sows include oedematous uterus and ovarian cysts, increased maturation of follicles, more numerous litters or decreased fertility [92].

Aflatoxins affect the quality of the milk produced by dairy cows and result in a carry-over of AFM<sub>1</sub> with AFB<sub>1</sub>-contaminated feed. Ten ruminally-cannulated Holstein cows received AFB<sub>1</sub> (13 mg per cow daily) through a hole in the rumen for 7 days. The AFM<sub>1</sub> levels in the milk of the treated cows ranged from 1.05 to 10.58 ng/L. The carry-over rate was higher in early lactation (2-4 weeks) compared to late lactation (34 -36 weeks) [75, 93]. The T-2 toxin causes necrosis of the lymphoid tissues. Bovine infertility and natural abortion in the last trimester of pregnancy also result from consumption of feed contaminated with T-2 toxin. Calves consuming T-2 toxin in the amount of 10-50 mg/kg of feed showed abomasal ulcers and sloughing of papillae in the rumen [75, 94, 95].

## 6. Current EU regulations concerning mycotoxins

Since the discovery of aflatoxins in the 1960s, regulations have been established in many countries to protect consumers from harmful mycotoxins that can contaminate foods. Maximum levels of mycotoxins have been established by the European Commission after consultations with the Scientific Committee for Food, based on the analysis of scientific data collected by EFSA and the Codex Alimentarius.

These data include [73, 96]:

- toxicological properties of mycotoxins,
- mycotoxin dietary exposure,
- distribution of concentrations of mycotoxins in raw materials or a product batch
- availability of analytical methods,
- regulations in other countries with which trade contacts exist.

The first two factors provide the information necessary for risk assessment and exposure assessment, respectively. Risk assessment is the scientific evaluation of the likelihood of

known or potential adverse health effects resulting from human exposure to food-borne hazards. It is a fundamental scientific basis for the notification of regulations. The third and fourth factors are important factors in enabling the practical enforcement of mycotoxins, through appropriate procedures as regards sampling and analysis. The last factor is the only one economic in nature, but it is equally important in decision-making to establish reasonable rules and restrictions for mycotoxins in foods and feeds [96].

According to the Commission Regulations, the maximum levels should be set at a strict level, which is reasonably achievable by following good agricultural and manufacturing practices and taking into account the risk related to the consumption of food. Health protection of infants and young children requires establishing the lowest maximum levels, which is achievable through the selection of raw materials used for the manufacturing of foods for this vulnerable group of consumers. Development of international trade, progress in research focused on mycotoxin food contamination and their toxicological properties cause changes in the mycotoxin-related legislation across the European Union. The Commission Regulation 466/2001 [97] setting the maximum levels for certain contaminants in foodstuffs has been substantially amended many times. The current maximum levels for mycotoxins in food are specified by the Commission Regulation EU 1881/2006 and the Commission Regulation EU 105/2010 as regards OTA, the Commission Regulation EU 165/2010 as regards aflatoxins, and the Commission Regulation EU 1126/2007 as regards *Fusarium* toxins [62, 65, 98, 99]. There have also been established maximum levels for aflatoxins, ochratoxin A, patulin, and *Fusarium* toxin (fumonisin, deoxynivalenol, zearalenone) in different products: nuts, cereals, dried fruit, unprocessed cereals, processed cereal-based food, coffee, wine, spices, and liquors [62, 65, 97-99].

The number of countries that have regulations concerning mycotoxins is continuously increasing, and at least 100 countries are known to have founded specific limits for different combinations of mycotoxins and commodities, often accompanied by the prescribed or recommended procedures for sampling and analysis [100]. Specific regulations for food in different world regions were summarized by [101].

As for feeds, the legal situation is somewhat different and only aflatoxin B<sub>1</sub> is regulated by the Directive 2002/32/EC on undesirable substances in animal food amended by the Commission Directive (EC) 100/2003 [102, 103]. For other mycotoxins, such as deoxynivalenol, zearalenone, ochratoxin A and fumonisin B<sub>1</sub> and B<sub>2</sub> - only non-binding recommendation values in the Commission Recommendation 2006/57/EC [104] are determined for feeds (Table 6). This results from the fact that with the exception of aflatoxin-contaminated feed which either directly or indirectly affects human health, there is only a slight transfer to animal products [104, 105].

Table 5 presents the current maximum levels of mycotoxin content as regards cereals and cereal-based foods and feeds.

Mycotoxins in agricultural commodities are distributed heterogeneously. Therefore, sampling plays a crucial role in making the estimation of the levels of mycotoxin presence more precise. In order to obtain representative samples, sampling procedures, and particularly

homogenisation, for different matrix types have been regulated. The EU Commission Regulation (EC) 401/2006 established the methods of sampling and analysis for the official control of mycotoxins in foodstuffs [106]. Official sampling plans for aflatoxins in dry figs, groundnuts, peanuts, oilseeds, apricot kernels and tree nuts and for ochratoxins in coffee and liquorice root are provided in the Commission Regulation (EU) No 178/2010 [107]. The sampling frequency and the method of sampling for cereals and cereal products for lots >50 tonnes and <50 tonnes, as well as for retail packed products were presented. Moreover, the procedures of subdivision of lots into sublots depending on the product and lot weight were also summarised [106, 107].

According to the current regulations where no specific methods for the determination of mycotoxin levels in food are required by the EU regulations, laboratories may select any method provided that they meet the relevant criteria presented in [106, 107]. These criteria are different in relation to individual mycotoxins, and the limit of detection, precision, and recovery depends on the concentration range. The analytical results must be submitted corrected or uncorrected for recovery and the level of recovery expressed in % must be reported too.

The main analytical procedures for the determination of the major mycotoxins from complex biological matrices consist of the following steps: sampling, extraction, purification, detection, quantification, and finally confirmation. The current development in mycotoxin estimation was reviewed by [108-110].

Regulation	Matrix	Maximum levels [ppb]				
		AFB <sub>1</sub>	OTA	DON	ZEA	F
<b>FOOD</b>						
Commission Regulation (EU) 165/2010	All cereals and all products derived from cereals	2.0	-	-	-	-
	Maize and rice	5.0	-	-	-	-
	Processed cereal-based foods for infants and young children	0.10	-	-	-	-
Commission Regulation (EC) 1126/2007	Unprocessed cereals	-	-	1250	100	-
	Unprocessed durum wheat and oats	-	-	1750	-	-
	Pasta (dry)	-	-	750	-	-
	Bread (including small bakery wares), pastries, biscuits, cereal snacks and breakfast cereals	-	-	500	50	-
	Maize-based breakfast cereals and maize-based snacks	-	-	-	-	800
	Unprocessed maize with the exception of unprocessed maize intended to be processed by wet milling	-	-	1750	350	4000
	Cereals intended for direct human consumption, cereal flour, bran and germ as an end product marketed for direct human consumption	-	-	750	75	-

Regulation	Matrix	Maximum levels [ppb]				
		AFB <sub>1</sub>	OTA	DON	ZEA	F
	Milling fractions of maize and milling products with particle size $\geq$ 500 micron not used for direct human consumption	-	-	750	200	1400
	Milling fractions of maize and maize milling products with particle size $\leq$ 500 micron not used for direct human consumption	-	-	1250	300	2000
	Processed cereal-based foods for infants and young children	-	-	200	20	200
	Processed maize-based foods for infants and young children	-	-	-	20	-
Commission Regulation (EC) 1881/2006	Unprocessed cereals	-	5.0	-	-	-
	All products derived from unprocessed cereals, including processed cereal products and cereals intended for direct human consumption	-	3.0	-	-	-
	Processed cereal-based foods for infants and young children	-	0.50	-	-	-
<b>FEED</b>						
Commission Recommendation (EC) 576/2006	Cereals and cereal products with the exception of maize by-products	-	250	8000	2000	-
	Maize by-products	-	-	12000	3000	-
	Complementary and complete feedingstuffs for pigs	-	50	900	250	-
	Complementary and complete feedingstuffs for calves, lambs and kids	-	-	2000	500	-
	Complementary and complete feedingstuffs for poultry	-	100	-	-	-
Commission Directive (EC) 100/2003	All feed materials	20	-	-	-	-
	Complete feedingstuffs for dairy animals	5	-	-	-	-
	Complete feedingstuffs for calves and lambs	10	-	-	-	-
	Complete feedingstuffs for pigs, poultry, cattle, sheep and goats	20	-	-	-	-

(-) limit not established; AFB<sub>1</sub> – aflatoxin B<sub>1</sub>; OTA – ochratoxin A; ZEA – zearalenone; DON – deoxynivalenol; F – fumonisins

**Table 5.** Legislation on mycotoxins as regards cereals and cereal-based foods and feeds

## 7. Prevention strategies of exposure to mycotoxins

Several codes of practice have been developed by Codex Alimentarius for the prevention and reduction of mycotoxins in cereals, peanuts, apple products, and other raw materials. In order for this practice to be effective, it will be necessary for the producers in each country to consider the general principles given in the Code, taking into account their local crops, cli-

mate, and agronomic practices, before attempting to implement the provisions specified in the Code. The recommendations for the reduction of various mycotoxins in cereals are divided into two parts: recommended practices based on Good Agricultural Practice (GAP) and Good Manufacturing Practice (GMP); a complementary management system to consider in the future is the use of Hazard Analysis Critical Control Point (HACCP) [111].

Recommendations to be taken into account before the harvest in order to reduce the risk of mould contamination and mycotoxin production include [112]:

- use certified seed or ensure it is free from fungal infections;
- avoid drought stress – irrigate if possible;
- sow the seed as early as possible, so that crop matures early;
- when practising minimum or zero tillage, remove crop residues;
- weed regularly;
- control insect and bird pests;
- rotate crops;
- avoid nutrient stress – apply the appropriate amount of organic or inorganic fertiliser;
- plant resistant varieties where these are available

The main mycotoxin hazards associated with pre-harvest in Europe are the toxins that are produced by fungi belonging to the genus *Fusarium* in the growing crops. It is important to note that although *Fusarium* infection is generally considered to be a pre-harvest problem, it is certainly possible for poor drying practices to lead to crops' susceptibility in storage and mycotoxin contamination [113]. This part of the book will discuss some pre-harvest strategies appropriate to reduce the prevalence of fungi belonging to the genus *Fusarium* and their mycotoxins.

### 7.1. Resistance

There are inherent differences in the susceptibility of cereal species to *Fusarium* infections. The differences between crop species appear to vary between countries. This is probably due to the differences in the genetic pool within each country's breeding program and the diverse environmental and agronomic conditions in which crops are cultivated [114, 115]. It was observed that oats had higher levels of DON than barley and wheat in Norway from 1996 to 1999, whereas the DON levels in wheat, barley and oats were similar when grown under the same field conditions in Western Canada in 2001 [116].

### 7.2. Field management

#### Crop rotation

Numerous studies have shown that fumonisins or DON contamination in wheat is affected by the previous crop. It was shown that a higher incidence of  $F_s$  occurred in wheat after



maize and, in particular, in wheat after a succession of two maize crops and in wheat following grain maize compared to silage maize. In Ontario, Canada, in 1983, the fields where maize was the previous crop had a significantly higher incidence of fumonisins than the fields where the previous crop was a small grain cereal or soybean [117]. In a repeated study, the following year, the fields where maize was the previous crop had a 10-fold DON content than the fields following a crop other than maize [118]. The research of [119] found higher levels of fumonisins in wheat following wheat rather than wheat following fallow.

An observational study performed using commercial fields in Canada [120] identified significantly lower DON content in wheat following soybean or wheat, compared to wheat following maize. In New Zealand, an observational study determined that higher levels of DON occurred in wheat grown after maize (mean = 600 ppb) and after grass (mean = 250 ppb), compared to small grain cereals (mean = 90 ppb) and other crops (mean = 70 ppb). The highest levels were recorded in wheat-maize rotations [121].

Codex recommends that crops such as potatoes, other vegetables, clover and alfalfa that are not hosts to *Fusarium* species should be used in rotation to reduce the inoculum in the field [122].

### 7.3. Soil cultivation

Soil cultivation can be divided into ploughing, where the top 10-30 cm of soil are inverted; minimum tillage, where the crop debris is mixed with the top 10-20 cm of soil; and no till, where seed is directly drilled into the previous crop stubble with minimum disturbance to the soil structure [111]. In the 1990s, a large observational study of  $F_s$  and DON was conducted in Germany (n=1600). The DON concentration of wheat crops after maize was ten-times higher in the field that was min-tilled compared to the ploughed one [123]. In wheat the DON concentration after min-till was 1300 ppb, after no-till it was 700 ppb and after ploughing it was 500 ppb [120]. Studies in France have determined that crop debris management can have a large impact on the DON concentration at harvest, particularly after maize. The highest DON concentration was found after no-till, followed by min-till, whereas the lowest DON levels were recorded after ploughing. The reduction in DON has been linked to the reduction in crop residue on the soil surface [124]. Large replicated field trials in Germany identified that there was a significant interaction between the previous crop and the cultivation technique [125]. Following sugar beet, there was no significant difference in the DON concentration between wheat plots receiving different methods of cultivation; however, following a wheat crop without straw removal, direct drilled wheat had a significantly higher DON level compared to wheat from plots which were either ploughed or min-tilled [125].

In accordance with the guidelines contained in the Codex Alimentarius, soil should be tested to determine if there is need to apply a fertilizer and/or soil conditioners to assure adequate soil pH and plant nutrition to avoid plant stress, especially during seed development [122].

Research of [126] showed that supplementary nitrogen and a plant growth regulator increased, by up to 125%, the incidence of infection by *Fusarium* species in the seed of wheat,

barley and triticale. Similarly, in the studies of [127], a significant increase in fumonisins and deoxynivalenol contamination in the grain of wheat and kernels was observed with increasing N fertilizer from 0 to 80 kg/ha. That research concluded that in practical crop husbandry,  $F_s$  cannot be sufficiently controlled by only manipulating the N input [111]. The study of [128] showed that the use of six different combinations of agricultural practices (sowing time, plant density, N fertilization and European corn borer (ECB) control with insecticide) can effectively lead to good control of fumonisins and deoxynivalenol in maize kernels.

#### 7.4. Use of chemical and biological agents

In accordance with the guidelines contained in the Codex Alimentarius [122], farmers should minimize insect damage and fungal infections of the crop by proper use of registered insecticides, fungicides and other appropriate practices within an integrated pest management program.

Some studies have been conducted to examine the effectiveness of the fungicides which are applied during flowering can reduce *Fusarium* infections and subsequent DON in the harvested grains. The results of [129] provided that azoles, tebuconazole, metconazole and prothioconazole significantly reduced the *Fusarium* disease symptoms and *Fusarium* mycotoxin concentrations. The greatest reduction in the DON concentration occurred with prothioconazole (10-fold). Azoxystrobin had little impact on the mycotoxin concentration in the harvested grain infected by *Fusarium* species, but could increase the mycotoxin concentration in grains when *F. nivale* was the predominant species present [130, 131]. Fungicide mixtures of azoxystrobin and azole resulted in a lower reduction of DON, compared to azole alone [120, 132]. A number of trials in Germany have indicated that some strobilurin fungicides applied before anthesis can also result in increased DON compared to unsprayed plots [133]. Reductions in DON observed in field experiments using fungicides against natural infections of *Fusarium* are lower and inconsistent [134]. This is probably due to the fact that during a natural infection, the infection occurs over a longer period of time.

Alternatively, a limited number of biocompetitive microorganisms have been shown useful for the management of *Fusarium* infections [111]. Research has demonstrated the successful use of bacteria in biocontrol of mycotoxigenic fungi. One bacterium, *Enterobacter cloacae* was discovered as an endophytic symbiont of corn [135]. Corn plants with roots endophytically colonized by these bacteria were observed to be fungus-free and *in vitro* control of *F. verticillioides* and other fungi with this bacterium was demonstrated. An endophytic bacterium, *Bacillus subtilis* showed promising for reducing the mycotoxin contamination with *F. verticillioides* during the endophytic growth phase [136]. Yeast antagonists such as *Cryptococcus nodaensis* were isolated from wheat anthers. The antagonists reduced *Fusarium* head blight severity by up to 93% in greenhouse and by 56% in field trials when sprayed onto flowering wheat heads [137]. The most successful antagonists reduced the DON content of grain more than 10-fold in greenhouse studies [138].

Actions to be taken during harvest in order to reduce the risk of mould contamination and mycotoxin production include [112]:

- harvest as quickly as possible
- avoid field drying
- transport the crop to the homestead as soon as possible
- if lack of labour force or time prevents removal from the field, then dry the crops on platforms raised above ground (if climate is hot and the drying crop can be left to stay on the field on a platform or cut and tied into stooks) to dry
- bundles of stover should also be placed on platforms to dry and not left lying on the soil

The post-harvest strategies include improving the drying and storage conditions together with the use of chemical, physical or biological methods.

## 8. Methods of removing mycotoxins from cereals

When mycotoxin prevention is not satisfactory, some decontamination methods are needed. The use of detoxification methods is allowed only in the case of feed and feed components. Foodstuffs containing contaminants exceeding the maximum levels should not be placed on the market either as such, in the form of a mixture with other foodstuffs or used as an ingredient in other foods. Food contaminated with mycotoxins is not safe for consumers and no decontamination methods can be used.

According to FAO [111, 139, 140] the feed decontamination process must:

- destroy, inactivate or remove mycotoxins
- not produce toxic, carcinogenic or mutagenic residues in decontaminated final products
- not decrease the nutritive value and organoleptic properties
- destroy all fungal morphological forms
- not significantly increase the cost of production

There are some physical methods of decontamination of feed components such as sorting grains, washing procedures, gamma radiation and UV treatment and also extraction with organic solvents. These methods are summarized by [140]. Physical removal of damaged, mouldy or discoloured kernels significantly decreased the concentration of AF in peanuts. Sorting is not effective for maize and cottonseed. Washing with water or sodium carbonate solutions could decrease the concentration of DON, ZEA and fumonisins in wheat and maize.

High temperature is not used for decontamination of agricultural products, due to thermostability of mycotoxins. Different types of radiation were tested for mycotoxin detoxification, but the results were not effective enough.

Chemical compounds such as organic acids, ammonium, sodium hydroxide, hydrogen peroxide, ozone, chloride and bisulphite were tested for their efficacy in mycotoxin decontami-

nation [141, 142]. Chemical decontamination is very effective, but these methods are expensive and affect the feedstuff quality. Among the chemical methods, only peroxide and ammonia are mostly used for aflatoxin removal from feed. Ammoniation works by irreversibly converting AFB<sub>1</sub> to less toxic products such as AFD<sub>1</sub> [143]. Data show that treatment of maize contaminated with 1000 or 2000 ppb aflatoxins with 1% of aqueous ammonia for 48 h removed 98% of the aflatoxins. There was no significant change in the dietary intake, body weight gain, and feed conversion ratio in chickens fed with ammonia-treated aflatoxin-contaminated maize, whereas these parameters were suppressed in birds fed with aflatoxin-containing diet [142]. Atmospheric ammoniation of corn does not appear to be an effective method for the detoxification of *F.moniliforme*-contaminated material. In the research of [144], the levels of fumonisin B<sub>1</sub> in naturally contaminated corn were reduced by about 45% due to the ammonia treatment. Despite this, the toxicity of the culture material in rats was not altered by ammoniation.

A recent and promising approach to protect animals against the harmful effects of mycotoxin-contaminated feed is the use of mycotoxin binders (MB). They are added to the diet in order to reduce the absorption of mycotoxins from the gastrointestinal tract and their distribution to blood and target organs. These feed additives may act either by binding mycotoxins to their surface (adsorption), or by degrading or transforming them into less toxic metabolites (biotransformation). Various inorganic adsorbents, such as hydrated sodium calcium aluminosilicate, zeolites, bentonites, clays, and activated carbons, have been used as mycotoxin binders. The use of mycotoxin binders is discussed in some review articles [145-147]. The best aflatoxin adsorbent seems to be HSCAS (hydrated sodium calcium aluminosilicate), which rapidly and preferentially binds aflatoxins in the gastrointestinal tract [148-150]. The prevention of aflatoxicosis in broiler flocks was examined by [150]. HSCAS and activated charcoal were incorporated into the diets for broilers containing purified aflatoxin B<sub>1</sub> (7.5 ppm), or natural aflatoxin produced by *Aspergillus parasiticus* on rice (5 ppm). The authors showed that HSCAS significantly decreased the growth-inhibitory effects of AFB<sub>1</sub> or AF<sub>s</sub> on the growing chicks, namely by 50 to 67%. The authors suggest that HSCAS can modulate the toxicity of aflatoxins in chickens; however, adding activated charcoal to the diet did not appear to have protective properties against mycotoxicosis [150].

Physical and chemical methods have a lot of disadvantages; in many cases they do not meet the FAO requirements. Therefore, the use of other methods is considered. Biological methods, involving decontamination with microorganisms or enzymes, give promising results. Recently, an increase in the research connected with mycotoxin detoxification by microorganisms has been observed. Several studies have shown that some bacteria, moulds and yeasts such as *Flavobacterium aurantiacum*, *Corynebacterium rubrum*, lactic acid bacteria (*Lactobacillus acidophilus*, *L.rhamnosus*, *L.bulgaricus*), *Aspergillus niger*, *Rhizopus nigricans*, *Candida sp.*, *Kluyveromyces sp.*, etc. are able to conduct detoxification of mycotoxins (Tab. 6). Unfortunately, few of these findings have practical application.

Already in 1966, a review of microorganisms was conducted by [151] as for their capability of degrading aflatoxins. It was found that yeasts, actinomycetes and algae did not show this trait, but some moulds, such as *Aspergillus niger*, *A. parasiticus*, *A. terreus*, *A. luchuensis*, and

*Penicillium reistrickii*, partially transformed aflatoxin B<sub>1</sub> to a new product. Among them, only the bacteria *Flavobacterium aurantiacum* (now *Nocardia corynebacterioides*) is able to remove aflatoxin, both from the media and from the natural environments such as milk, oil, cocoa butter and grain. It was shown that to obtain the apparent loss of the toxin, it was necessary to use the bacterial population with the density of more than 10<sup>10</sup> CFU/ml [154, 188].

Mycotoxin	Microorganism	References
Aflatoxin B <sub>1</sub>	<i>Flavobacterium aurantiacum</i> ( <i>Nocardia corynebacterioides</i> ), <i>Lactobacillus acidophilus</i> , <i>L.johnsonii</i> , <i>L.salivarius</i> , <i>L.crispatus</i> , <i>L.gasserii</i> , <i>L.rhamnosus</i> , <i>Lactococcus lactis</i> , <i>Bifidobacterium longum</i> , <i>B.lactis</i> , <i>Mycobacterium luoranthenvivorans</i> , <i>Rhodococcus erythropolis</i> , <i>Bacillus megaterium</i> , <i>Corynebacterium rubrum</i> , <i>Kluyveromyces marxianus</i> , <i>Saccharomyces cerevisiae</i> , <i>Aspergillus niger</i> , <i>A. terreus</i> , <i>A.luchuensis</i> , <i>Penicillium reistrickii</i> , <i>Trichoderma viride</i>	[151-165]
Ochratoxin A	<i>Lactococcus salivarius</i> subsp. <i>thermophilus</i> , <i>Lactobacillus delbrueckii</i> subsp. <i>Bulgarius</i> , <i>L. acidophilus</i> , <i>Bifidobacterium animalis</i> , <i>B. bifidum</i> , <i>Lactobacillus plantarum</i> , <i>L. brevis</i> , <i>L. sanfranciscensis</i> , <i>L.acidophilus</i> , <i>Acinetobacter calcoaceticus</i> , <i>Rhodococcus erythropolis</i> , <i>Oenococcus oeni</i> , <i>Saccharomyces cerevisiae</i> , <i>Kluyveromyces marxianus</i> , <i>Rhodotorula rubra</i> , <i>Phaffia rhodozyna</i> , <i>Xanthophyllomyces dendrorhous</i> , <i>Metschnikowia pulcherrima</i> , <i>Pichia guilliermondii</i> , <i>Trichosporon mycotoxinivorans</i> , <i>Rhizopus sp.</i> , <i>Aureobasidium pullulans</i> , <i>Aspergillus niger</i> , <i>A.carbonarius</i> , <i>A. fumigatus</i> , <i>A. versicolor</i>	[166-183]
Fumonisin B <sub>1</sub>	<i>Lactobacillus rhamnosus</i> , <i>Lactococcus lactis</i> , <i>Leuconostoc mesenteroides</i> , <i>Saccharomyces cerevisiae</i> , <i>Kluyveromyces marxianus</i> , <i>Rhodotorula rubra</i>	[176, 184]
Trichothecenes	Ruminant bacteria, chicken intestinal microflora, <i>Saccharomyces cerevisiae</i> , <i>Kluyveromyces marxianus</i> , <i>Rhodotorula rubra</i>	[176, 185, 186]
Zearalenone	Soil bacteria, <i>Propionibacterium fraudenreichii</i> , <i>Rhizopus sp.</i> , <i>Trichosporon mycotoxinivorans</i>	[179, 183, 187]

**Table 6.** Decontamination abilities of microorganisms

It was observed that cultures of toxinogenic *Aspergillus flavus* and *Aspergillus parasiticus* were able to reduce aflatoxin contamination. Aflatoxins were degraded by the strains that produce them, but only after the fragmentation of the mycelium. The cause of this phenomenon was absorption into the cell wall of mycelium [165]. In the research of [176], 10 yeast strains of the *Saccharomyces*, *Kluyveromyces* and *Rhodotorula* genera were studied for their ability to perform biodegradation of fumonisin B<sub>1</sub>, ochratoxin A and trichothecenes. Significant differences were demonstrated between the strains, but there were no preferences as to the types of mycotoxins. Fumonisin B<sub>1</sub> was removed by the majority of the strains in 100%, the removal rate for deoxynivalenol ranged from 63 to 100%, and for ochratoxin A from 69 to 100%. The possibility of using moulds to remove ochratoxin A was studied by [179, 182]. The au-

thors selected two out of 70 isolates of the *Aspergillus* species - *Aspergillus fumigatus* and *Aspergillus niger*, which transformed ochratoxin A to ochratoxin  $\alpha$  and phenylalanine within 7 days of incubation on both liquid and solid media.

In vitro studies conducted by [186] demonstrated the degradation of 12 trichothecene mycotoxins conducted by bacteria isolated from the digestive tract of chickens. The transformation of the toxin led to their partial or total deacylation and de-epoxidation. Similarly, it was shown, that the strains of anaerobic bacteria - isolated from the rumen, Gram positive, pre-classified to the genus *Eubacterium* - are able to perform the transformation of type A trichothecenes to non-toxic forms [185].

The above-presented examples of microbial activity aimed at removal of mycotoxins are mainly of scientific nature, allowing for a better understanding of the strains, their properties and the mechanisms of the processes. Their limited practical application made that research turned in the direction of such organisms, which can be used in biotechnological processes during production, such as fermented food production, where the raw material may be contaminated with mycotoxins. The most important among them are lactic acid bacteria and yeasts *Saccharomyces cerevisiae* [163].

Literature data indicate the existence of strains of lactic acid bacteria with different abilities to remove mycotoxins, as demonstrated both in *in vitro* and *in vivo* studies conducted by various authors with the use of some strains of probiotic *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, *B. longum*, and *Streptococcus* spp., *Lactococcus salivarius*, *Lactobacillus delbrueckii* subsp. *bulgaricus* [155, 156, 158, 160, 169, 189, 190]. According to [191], the decontamination process is very fast; after 4h the toxin concentration was reduced from 50 to 77%. It was observed that heat-inactivated cells were more effective than living cells, which results from the changes in the surface properties of cells, which occur under high temperature [191]. The capacity to reduce the content of ochratoxin A in milk by lactic acid bacteria belonging to the species *Lactococcus salivarius*, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Bifidobacterium bifidum* was confirmed in [167]. The content of patulin in the medium decreased in the level from 10 to 82% under the influence of bacteria belonging to the genus *Lactobacillus* and *Bifidobacterium*. The decontamination process depends on the inoculum density, pH and the concentration of toxins. Among the studied strains, *L.acidophilus*, removes up to 96% of the toxin added to the medium in an amount of 1ppm [166].

Our *in vivo* experiments indicate that the use of probiotics as feed additives limited the effects of mycotoxins in animals, as well as reduced the accumulation of toxins in the tissues, thus reducing the contamination of food of animal origin with the toxins [192]. It was shown that *Lactobacillus rhamnosus* bacteria limited by 75% the adsorption of aflatoxin B<sub>1</sub> in the digestive tract of chickens [189].

The second group of organisms with a potential application in detoxification is constituted by *Saccharomyces cerevisiae* yeasts. Our own research demonstrated that these organisms are capable of eliminating ochratoxin A from the plant raw material during fermentation and chromatographic analysis did not show any products of OTA metabolism, which proves that it was not the case of biodegradation. The amount of ochratoxin A removed by bakery yeasts after 24-

hour contact equalled from 29% to 75% for 5 mg d.m./ml and 50 mg d.m./ml, respectively. The process of adsorption proved to be very fast; immediately after mixing the cells with the toxin its amount significantly decreased, and lengthening the contact up to 24 hours did not bring further notable changes. The presence of physiologically active cells is not necessary in order to remove the toxin; the dead biomass also removed OTA from the buffer and the amount of the toxin removed was much bigger than in the case of the active biomass. In the case of the 5 mg/ml density, 54% of the toxin was adsorbed, i.e. twice more than in the case of the active biomass [171]. The reason for OTA removal was adsorption of the toxin to the yeast cell wall. This mechanism was independent of the type of toxin, as demonstrated in relation to aflatoxin B<sub>1</sub>, zearalenone and T-2 toxin and patulin. The compounds of the cell wall that are involved in the binding process are probably  $\beta$ -D-glucan and its esterified form [193, 194]. Yeasts and their cell wall components are also used as feed additives for animals, and as adsorbents, which effectively limits mycotoxicosis in farm animals [195, 196].

The potential application of yeasts as adsorbents for foods and feeds depends on the stability of the toxin binding to the cells in the conditions of the gastrointestinal tract. According to [194], zearalenone adsorption is most effective at a pH close to neutral and acidic, and therefore those which prevail in some regions of the gastrointestinal tract. The result of the use of yeasts to remove ochratoxin A is detoxification of the environment, as demonstrated in the cytotoxicity and genotoxicity tests using pig kidney cell lines [197]. Some yeasts also exhibit features of probiotic activity, which is an additional argument for the use of these organisms

The use of microorganisms or their cell components for decontamination of foods and feeds has raised high hopes, but also the controversy from the perspective of the consumer. There are no legal regulations devoted to this issue, and the data referring to the stability of the microorganism-toxin connection in the gastrointestinal tract, as well as toxicological data are still incomplete. The only group of microorganisms, which in addition to other advantageous features of health promotion has the ability to remove toxins, is probably that of probiotic lactic acid bacteria. Also, *Saccharomyces cerevisiae* yeast and its cell wall component - glucan can be used for this purpose. These factors can be applied both as human dietary supplements and ingredients in animal nutrition, as well as during biotechnological processes.

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