Ann Agric Environ Med 2007, 14, 159-167

LEVELS OF FUNGI AND MYCOTOXINS IN THE SAMPLES OF GRAIN AND GRAIN DUST COLLECTED FROM FIVE VARIOUS CEREAL CROPS IN EASTERN POLAND

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Krysińska-Traczyk E, Perkowski J, Dutkiewicz J: Levels of fungi and mycotoxins in the samples of grain and grain dust collected from five various cereal crops in eastern Poland. *Ann Agric Environ Med* 2007, **14**, 159-167.

Abstract: During combine harvesting of 5 various cereal crops (rye, barley, oats, buckwheat, corn) 24 samples of grain and 24 samples of settled grain dust were collected on farms located in the Lublin province of eastern Poland. The samples were examined for the concentration of total microfungi, *Fusarium* species, deoxynivalenol (DON), nivalenol (NIV), and ochratoxin A (OTA). Microfungi able to grow on malt agar were present in 79.2% of grain samples and in 91.7% of grain dust samples in the concentrations of 1.0-801.3 × 10^3 cfu/g and 1.5-12440.0 × 10^3 cfu/g, respectively. The concentration of microfungi in grain dust samples was significantly greater than in grain samples ($p<0.01$). *Fusarium* strains were isolated from 54.2% of grain samples and from 58.3% of grain dust samples in the concentrations of 0.1-375.0 × 10^3 cfu/g and 4.0-7,700.0 × 10^3 cfu/g, respectively. They were found in all samples of grain and grain dust from rye, barley and corn, but only in 0-16.7% of samples of grain and grain dust from oats and buckwheat. DON was found in 79.2% of grain samples and in 100% of grain dust samples in the concentrations of 0.001-0.18 μg/g and 0.006-0.283 μg/g, respectively. NIV was detected in 62.5% of grain samples and in 94.4% of grain dust samples in the concentrations of 0.004-0.502 μg/g and 0.005-0.339 μg/g, respectively. OTA was detected in 58.3% of grain samples and in 91.7% of grain dust samples in the concentrations of 0.00039-0.00195 μg/g and 0.00036-0.00285 μg/g, respectively. The concentrations of DON, total fusariotoxins (DON + NIV) and OTA were significantly greater in grain dust samples than in grain samples ($p<0.05$, $p<0.05$, and $p<0.001$, respectively). The concentration of *Fusarium poae* in the samples of rye grain and dust was significantly correlated with the concentrations of DON ($p<0.05$), NIV ($p<0.01$), and total fusariotoxins ($p<0.05$). Similarly, the concentration of *Fusarium culmorum* in the samples of barley grain and dust was significantly correlated with the concentration of total fusariotoxins ($p<0.05$). A significant correlation was also found between the concentration of total fungi grown on malt agar and the concentration of OTA ($p<0.05$). In conclusion, although the concentration of DON, NIV and OTA in the samples of grain dust collected from 5 various cereals on farms in eastern Poland was not large, the persistent presence of these mycotoxins in over 90% of examined samples poses a potential health risk of chronic respiratory intoxication for exposed grain farmers.

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Key words: grain, grain dust, rye, barley, oats, buckwheat, corn, farmers, occupational exposure, fungi, *Fusarium*, mycotoxins, deoxynivalenol, nivalenol, ochratoxin A.

INTRODUCTION

The occupational exposure to grain dust and other organic dusts could be a cause of allergic and immunotoxic diseases such as: bronchial astma, allergic alveolitis, allergic rhinitis, atopic conjunctivitis, organic dust toxic syndrome, chronic fatigue-like syndrome [10, 11, 16, 25, 29]. Among etiological factors of these diseases an important

Received: 27 February 2007
Accepted: 8 May 2007

role is played by fungi and their products contaminating the dusts, such as protein and glycoprotein allergens, (1→3)-β-D-glucan, volatile organic compounds (VOCs), and mycotoxins [16, 17, 29, 54]. Mycotoxins are low-molecular, secondary metabolites of mould fungi which exert cytotoxic, neurotoxic, immunosuppressive, teratogenic, mutagenic and carcinogenic effects following ingestion of contaminated food [5, 28, 54, 62]. Much less is known about respiratory, work-related effects of these compounds [20, 56]. Some authors use the terms "mycotoxicoses" or "toxomycoses" for diseases resembling organic dust toxic syndrome caused by the inhalation of large quantities of mould particles [13, 24, 48]; however, the role of mycotoxins themselves in etiopathogenesis of these diseases has not always been documented.

A particular potential risk for agricultural workers is posed by mycotoxins occurring in grain: aflatoxins, ochratoxin A (OTA), as well as moniliformin (MON) and trichothecenes produced by fungi belonging to the genus *Fusarium* (fusariotoxins): deoxynivalenol (DON, vomitoxin), nivalenol (NIV), zearalenone (ZEA), T-2 toxin, HT-2 toxin and others [18, 19, 20, 21, 43, 56]. The teratogenic and cancerogenic effects of inhaled aflatoxins and trichothecenes on exposed agricultural workers [19, 20, 21, 43] and the nephrotoxic effects of ochratoxin A (OTA) [18, 20] are suggested.

In our earlier studies [26, 27] we found the presence of *Fusarium* spp., fusariotoxins (MON, DON, NIV) and OTA in samples of wheat (*Triticum aestivum* L.) and wheat dust collected during machine threshing of stored grain and combine harvesting on farms in eastern Poland. The aim of the present study was to determine the concentration of fungi, with special attention to *Fusarium* and selected mycotoxins (trichothecenes and OTA), in 5 different kinds of grain and grain dust sampled during combine harvesting in order to assess a potential risk for grain handling farmers.

MATERIALS AND METHODS

Samples of grain and grain dust. Studies were conducted during the years 2002-2006 on 24 private farms in the Lublin region of eastern Poland growing 5 various cereals: rye (*Secale cereale* L.) (5 farms), barley (*Hordeum vulgare* L.) (5 farms), oats (*Avena sativa* L.) (6 farms), buckwheat (*Fagopyrum esculentum* Moench) (6 farms), and corn (Zea mays L.) (2 farms). On each farm, 1 sample of grain and 1 sample of settled grain dust were collected during combine harvesting of a cereal crop. Thus, a total number of 48 samples were taken – 24 grain samples and 24 grain dust samples – in which the level of mould fungi was determined, as well as their species composition and level of mycotoxins.

Determination of the concentration and species composition of fungi. The concentration and species composition of microfungi in the samples of grain and grain dust were determined by the dilution plating method [10, 26]. One gram of each sample was suspended in 100 ml of sterile saline (0.85% NaCl) containing 0.1% (v/v) of Tween 80, and after vigorous shaking, serial 10-fold dilutions in saline were made up to 10^{-10}. The 0.1 ml aliquots of each dilution were spread on duplicate sets of plates. Malt agar (Difco, Detroit, MI, USA) was used for the determination of the concentration and species composition of microfungi in grain and dust, and a potato dextrose agar medium (PDA, Merck, Darmstadt, Germany) was used as a selective medium for *Fusarium* spp. The plates with malt agar were incubated for 4 days at 30ºC and 4 days at 22ºC, whereas plates with PDA – for 8 days at 22ºC. After incubation, the number of microfungi in 1 g of the sample was calculated and expressed in cfu/g. Macro- and microscopic studies of fungi were then carried out in order to determine their species composition. Fungi were determined according to the manuals by Samson and Hoekstra [54], Nelson *et al.* [42], Raper and Fennel [52], Pidopliczko and Milko [51], and Litvinov [34].

Determination of the concentration of mycotoxins. The thin-layer chromatography method (TLC) and high-performance thin-layer chromatography method (HPTLC) was used [26, 27, 46, 47]. Determination of the concentration of ochratoxin A (OTA), deoxynivalenol (DON) and nivalenol (NIV) was carried out in 24 samples of grain and 24 samples of grain dust released during combine harvesting. Each sample of 10 grams was extracted by the mixture of acetonitryle and water (75:25). The filtrate was divided into 2 parts: the first part was cleaned in Ochratest Vicam columns, then the level of ochratoxin A (OTA) was determined by the HPLC method; the second part of the extract was cleaned in columns containing activated carbon, Celite and Al_2O_3 neutral (1:1:1), and the levels of fusariotoxins of B group: deoxynivalenol (DON) and nivalenol (NIV) were determined with the use of gas chromatograph attached to a mass spectrometer (GC/MS). Chromatographic analysis of mycotoxins was performed in the Department of Chemistry at the Agricultural University in Poznań.

Statistical analysis. The data distribution was checked for normality by the Shapiro-Wilk's test. The significance of the correlations between individual variables was tested by the non-parametric Spearman's test, and the significance of differences between variables was tested by the Mann-Whitney test. A p-value of less than 0.05 was considered significant. The analyses were performed with the use of the Statistica for Windows v. 5.0 package (Statsoft©, Inc., Tulsa, Oklahoma, USA).

RESULTS

Fungi and mycotoxins in rye grain and rye grain dust. The median concentration of the total microfungi able to grow on malt agar was insignificantly greater in

Table 1. Concentrations of total fungi, *Fusarium* spp. and selected mycotoxins determined in samples of rye grain and rye dust collected on farms in eastern Poland.

Sample	Total fungi (Malt agar) cfu/g × 10^3	*Fusarium* spp. (PDA medium) cfu/g × 10^3		Mycotoxins (µg/g)			
				Fusarium toxins			Ochratoxin A
		Fusarium poae	Total *Fusarium*	DON[a]	NIV[b]	Total[c]	(OTA)
Rye grain							
1	0.0	250.0	250.0	0.026	0.004	0.03	ND
2	5.0	17.0	17.0	0.013	ND	0.013	ND
3	5.0	3.0	3.0	0.016	ND	0.016	0.00055
4	0.0	21.0	21.0	0.02	ND	0.02	0.00039
5	5.0	2.0	2.0	0.018	ND	0.018	ND
Median	5.0	17.0	17.0	0.018	0.0	0.013	0.0
Rye dust							
1	0.0	83.4	83.4	0.015	0.0	0.015	0.00252
2	12.2	120.6	120.6	0.046	0.015	0.061	ND
3	401.1	570.8	570.8	0.034	0.005	0.039	ND
4	1,636.0	308.1	308.1	0.029	0.033	0.062	0.00036
5	401.1	147.8	147.8	0.044	0.036	0.08	0.00104
Median	401.1	147.8	147.8	0.034	0.015	0.061	0.00036
Total samples							
Median	5.0	102.0[*(D)**(N)*(DN)]	102.0[*(D)**(N)*(DN)]	0.023	0.002	0.025	0.00018

DON[a] = deoxynivalenol; NIV[b] = nivalenol; Total[c] = total *Fusarium* toxins (DON + NIV); ND = not detected (considered as zero level);
[*(D)]: significantly correlated with the concentration of DON ($p<0.05$); [**(N)]: significantly correlated with the concentration of NIV ($p<0.01$);
[*(DN)]: significantly correlated with the concentration of total *Fusarium* toxins ($p<0.05$).

the samples of rye dust compared to rye grain (401.1 vs. 5.0 × 10^3 cfu/g, $0.05<p<0.1$) (Tab. 1). Mycobiota of grain consisted of *Alternaria alternata* (66.7% of total isolates) and *Monilia sitophila* (33.3%), while that of grain dust consisted of *Monosporium silvaticum* (65.9%), *A. alternata* (21.0%) and *Oidiodendron flavum* (13.1%). The genus Fusarium was represented by only one species *Fusarium poae*, which was present in all the samples of grain and grain dust. Its median concentration in the samples of grain dust was significantly greater compared to those of grain (147.8 vs. 17.0 × 10^3 cfu/g, $p<0.05$). Of the mycotoxins, DON was present in all samples examined, NIV in 20% and 80% of grain and grain dust samples respectively, and OTA in 40% and 60% of grain and grain dust samples, respectively. The concentrations of DON, NIV and OTA in the samples of grain and grain dust were within the ranges of 0.013-0.046 µg/g, 0.0-0.036 µg/g, and 0.0-0.00252 µg/g, respectively (Tab. 1). The concentration of NIV was significantly greater in the samples of grain dust compared to those of grain ($p<0.05$), whereas in the cases of DON and OTA no significant differences between both sample sets could be observed. A significant correlation was found between the concentration of *Fusarium poae* in the total samples (grain + grain dust) and the concentrations of DON ($p<0.05$), NIV ($p<0.01$), and total fusariotoxins ($p<0.05$) (Tab. 1).

Fungi and mycotoxins in barley grain and barley grain dust. The median concentration of the total microfungi able to grow on malt agar was significantly greater in the samples of barley dust compared to barley grain (632.1 vs. 5.0 × 10^3 cfu/g, $p<0.01$) (Tab. 2). Strains of *Alternaria alternata* formed a dominant part of fungal isolates from grain and grain dust (78.5% of each), followed by *Oidiodendron griseum* in grain (12.7%), and *Rhizopus artocarpi* in grain dust (17.5%). Yeast, *Cladosporium elegantulum* and *Trichoderma album* constituted the remaining 8.8% of the grain mycobiota, while yeast, *Monosporium silvaticum*, *Prophytroma tubularis* and *Cladosporium elegantulum* constituted the remaining 4.0% of the grain dust mycobiota. The genus *Fusarium* was represented by only one species, *Fusarium culmorum*, which was present in all the samples of grain and grain dust. Its median concentration in the samples of grain dust was significantly greater compared to those of grain (5100.0 vs. 4.0 × 10^3 cfu/g, $p<0.01$). Of the mycotoxins, DON and NIV were present in all samples examined, and OTA in 80% of grain samples and in all grain dust samples. The concentrations of DON, NIV and OTA in the samples of grain and grain dust were within the ranges of 0.01-0.11 µg/g, 0.01-0.09 µg/g, and 0.0-0.00285 µg/g, respectively (Tab. 2). The concentrations of total fusariotoxins and OTA were significantly greater in the samples of grain dust compared to those of grain ($p<0.05$, and $p<0.01$ respectively), whereas in the cases of DON and NIV no significant differences between both sample sets could be observed. A significant correlation was found between the concentration of total fungi grown on malt agar and the concentration of OTA ($p<0.05$). Similarly, a significant correlation was found between the concentration of *Fusarium culmorum* in the total samples (grain + grain dust) and the concentration of total fusariotoxins ($p<0.05$) (Tab. 2).

Table 2. Concentrations of total fungi, *Fusarium* spp. and selected mycotoxins determined in samples of barley grain and barley dust collected on farms in eastern Poland.

Sample	Total fungi (Malt agar) cfu/g × 10^3	*Fusarium* spp. (PDA medium) cfu/g × 10^3		Mycotoxins (µg/g)			
				Fusarium toxins			Ochratoxin A
		Fusarium culmorum	Total *Fusarium*	DON[a]	NIV[b]	Total[c]	(OTA)
Barley grain							
1	9.0	4.0	4.0	0.01	0.01	0.02	0.0
2	1.8	1.0	1.0	0.03	0.03	0.06	0.00042
3	5.0	4.7	4.7	0.02	0.05	0.07	0.00051
4	5.0	43.0	43.0	0.04	0.02	0.06	0.00046
5	2.0	0.1	0.1	0.01	0.04	0.05	0.00091
Median	5.0	4.0	4.0	0.02	0.03	0.06	0.00046
Barley dust							
1	12,440.0	1,256.0	1,256.0	0.08	0.09	0.17	0.00115
2	201.5	5,100.0	5,100.0	0.03	0.04	0.07	0.00155
3	632.1	6,000.0	6,000.0	0.11	0.01	0.11	0.0024
4	752.3	7,700.0	7,700.0	0.01	0.08	0.09	0.00285
5	601.3	220.0	220.0	0.02	0.09	0.11	0.00114
Median	632.1	5,100.0	5,100.0	0.03	0.08	0.11	0.00155
Total samples							
Median	105.2*(O)	131.5*(DN)	131.5*(DN)	0.025	0.04	0.07	0.001025

DON[a] = deoxynivalenol; NIV[b] = nivalenol; Total[c] = total *Fusarium* toxins (DON + NIV). *(O): significantly correlated with the concentration of ochratoxin ($p<0.05$). *(DN): significantly correlated with the concentration of total *Fusarium* toxins ($p<0.05$).

Table 3. Concentrations of total fungi, *Fusarium* spp. and selected mycotoxins determined in samples of oats grain and oats dust collected on farms in eastern Poland.

Sample	Total fungi (Malt agar) cfu/g × 10^3	*Fusarium* spp. (PDA medium) cfu/g × 10^3		Mycotoxins (µg/g)			
				Fusarium toxins			Ochratoxin A
		Fusarium culmorum	Total *Fusarium*	DON[a]	NIV[b]	Total[c]	(OTA)
Oats grain							
1	3.5	16.0	16.0	0.046	0.502	0.548	0.00142
2	2.0	0.0	0.0	0.026	0.031	0.057	0.00195
3	1.0	0.0	0.0	0.025	0.187	0.212	0.0
4	1.0	0.0	0.0	0.022	0.012	0.034	0.00053
5	6.0	0.0	0.0	0.001	0.016	0.017	0.0
6	0.0	0.0	0.0	0.048	0.036	0.084	0.0
Median	1.5	0.0	0.0	0.0255	0.0355	0.0705	0.000265
Oats dust							
1	1.5	0.0	0.0	0.028	0.025	0.053	0.00215
2	251.1	0.0	0.0	0.045	0.321	0.366	0.00233
3	2.0	0.0	0.0	0.086	0.022	0.108	0.00103
4	15.3	0.0	0.0	0.006	0.035	0.041	0.00142
5	4.0	4.0	4.0	0.021	0.010	0.031	0.00088
6	0.0	0.0	0.0	0.178	0.339	0.517	0.00142
Median	3.0	0.0	0.0	0.0365	0.03	0.0805	0.00142
Total samples							
Median	2.0	0.0	0.0	0.027	0.033	0.0705	0.001225

DON[a] = deoxynivalenol; NIV[b] = nivalenol; Total[c] = total *Fusarium* toxins (DON + NIV).

Fungi and mycotoxins in oats grain and oats grain dust. The median concentrations of fungi grown on malt agar were similar in the samples of oats grain and oats dust (Tab. 3). Yeast and *Geotrichum candidum* strains prevailed in grain samples, forming 51.8% and 37.1% of the total isolates respectively, followed by *Cladosporium herbarum* (7.4%) and *Alternaria alternata* (3.7%). In the samples of grain dust *Rhodotorula rubra* prevailed which constituted 92% of total isolates. *Monilia sitophila*, *Cladosporium elegantulum*, *Alternaria alternata*, and *Penicillium* spp. formed the remaining 8% of the grain dust mycobiota. The *Fusarium* strains, belonging to one species, *F. culmorum*, were recovered from only 16.7% of samples of grain and grain dust. DON and NIV were present in all samples examined, and OTA in 50% of grain samples and in all grain dust samples. The concentrations of DON, NIV and OTA

Table 4. Concentrations of total fungi, *Fusarium* spp. and selected mycotoxins determined in samples of buckwheat grain and buckwheat grain dust collected on farms in eastern Poland.

Sample	Total fungi (Malt agar) cfu/g × 10^3	*Fusarium* spp. (PDA medium) cfu/g × 10^3		Mycotoxins (µg/g)		
		Fusarium culmorum	Total *Fusarium*	*Fusarium* toxins DON[a]	*Fusarium* toxins Total[b]	Ochratoxin A (OTA)
Buckwheat grain						
1	0.0	0.0	0.0	0.074	0.074	0.0
2	113.4	0.0	0.0	0.0	0.0	0.00075
3	801.3	0.0	0.0	0.0	0.0	0.00114
4	0.0	0.0	0.0	0.087	0.087	0.00099
5	31.2	0.0	0.0	0.0	0.0	0.0
6	701.1	0.0	0.0	0.0	0.0	0.0
Median	72.3	0.0	0.0	0.0	0.0	0.00038
Buckwheat dust						
1	15.1	0.0	0.0	0.283	0.283	0.00131
2	7,511.0	4,995.0	4,995.0	0.01	0.01	0.00236
3	15.2	0.0	0.0	0.01	0.01	0.00215
4	301.1	0.0	0.0	0.114	0.114	0.00242
5	15.2	0.0	0.0	0.01	0.01	0.00103
6	101.3	0.0	0.0	0.011	0.011	0.00110
Median	58.3	0.0	0.0	0.0105	0.0105	0.00173
Total samples						
Median	66.3	0.0	0.0	0.01	0.01	0.00107

DON[a] = deoxynivalenol; Total[b] = total *Fusarium* toxins.

in the samples of grain and grain dust were within the ranges of 0.001-0.178 µg/g, 0.01-0.502 µg/g, and 0.0-0.00233 µg/g, respectively (Tab. 3). No significant differences were noted between concentrations of fungi and mycotoxins in grain and grain dust samples; likewise, no significant correlations were found between the concentrations of fungi and mycotoxins.

Fungi and mycotoxins in buckwheat grain and buckwheat grain dust. The median concentrations of fungi grown on malt agar were similar in the samples of buckwheat grain and buckwheat dust (Tab. 4). *Penicillium* spp. prevailed in the samples of grain, forming 91.1% of total isolates. *Mucor mucedo*, *Alternaria alternata*, and *Cladosporium lignicola* constituted the remaining 8.9% of the grain mycobiota. *Rhodotorula rubra* was the dominant species in the samples of grain dust (62.8% of total isolates), followed by *Mucor mucedo* (34.4%). *Alternaria alternata* and *Penicillium* spp. constituted the remaining 2.8% of the grain dust mycobiota. The *Fusarium* strains, belonging to one species *F. culmorum*, were recovered from only 16.7% of samples of grain dust and from no samples of grain. DON and OTA were present in 33.3% and 50% of grain respectively, and in all samples of grain dust. The concentrations of DON and OTA in the samples of grain and grain dust were within the ranges of 0.0-0.283 µg/g, and 0.0-0.00242 µg/g, respectively (Tab. 4). The concentration of OTA in grain dust was significantly greater than in grain ($p<0.01$). No significant correlations were found between the concentrations of fungi and mycotoxins.

Fungi and mycotoxins in corn grain and corn grain dust. The median concentration of fungi grown on malt agar was nearly 10 times greater in the samples of grain dust than in grain (Tab. 5), but the difference was not significant because of the small number of samples examined. Both in the samples of grain and grain dust the dominant fungi were *Penicillium* spp. which formed respectively 39.9% and 59% of total isolates. The other species common in grain samples were *Alternaria alternata* (17.7%), *Geotrichum candidum* (13.3%), *Mucor mucedo* (11.4%), *Rhizopus nigricans* (9.2%), and *Aspergillus ochraceus* (8.5%). In the samples of grain dust the common species was *Alternaria alternata* (25.6%), while *Aspergillus ochraceus*, *Geotrichum candidum*, *Rhizopus nigricans*, and *Aspergillus niger* constituted the remaining 15.4% of the grain dust mycobiota. The median concentration of *Fusarium* was over 10 times greater in the samples of grain dust than in grain (Tab. 5). The *Fusarium* isolates comprised the species *F. culmorum*, *F. poae*, and *F. graminearum*, which formed respectively 36.5%, 34.3%, and 29.2% of the total strains isolated from grain and grain dust. DON and NIV were found in half of grain samples and in all grain dust samples, while OTA was found in all the examined corn samples. The concentrations of DON, NIV and OTA in the samples of grain and grain dust were within the ranges of 0.0-0.18 µg/g, 0.0-0.17 µg/g, and of 0.00051-0.00226 µg/g, respectively (Tab. 5).

Summarized results. Microfungi able to grow on malt agar were present in 79.2% of grain samples and in 91.7%

Table 5. Concentrations of total fungi, *Fusarium* spp. and selected mycotoxins determined in samples of corn grain and corn grain dust collected on farms in eastern Poland.

Sample	Total fungi (Malt agar)	*Fusarium* spp. (PDA medium) cfu/g × 10^3				Mycotoxins (µg/g)			
	cfu/g × 10^3	*Fusarium culmorum*	*Fusarium graminearum*	*Fusarium poae*	Total *Fusarium*	*Fusarium* toxins DON[a]	NIV[b]	Total[c]	Ochratoxin (OTA)
Corn grain									
1 (moist grain)	101.2	53.7	43.3	34.6	131.6	0.18	0.17	0.35	0.00166
2 (dry grain)	302.3	130.9	99.3	144.8	375.0	ND	ND	ND	0.00193
Median	201.8	92.3	71.3	89.7	253.3	0.09	0.085	0.175	0.001795
Corn dust									
1 (dust from combine)	1,412.0	769.0	621.0	700.0	2,090.0	0.05	0.17	0.22	0.00226
2 (dust from dryer)	2,021.0	1,483.0	1,620.0	1,112.0	4,215.0	0.08	0.09	0.17	0.00051
Median	1,716.5	1,126.0	1,120.5	906.0	3,152.5	0.065	0.13	0.195	0.001385
Total samples									
Median	857.2	449.9	360.2	422.4	1,232.5	0.065	0.13	0.195	0.001795

DON[a] = deoxynivalenol; NIV[b] = nivalenol; Total[c] = total *Fusarium* toxins (DON + NIV). ND = not detected (considered as zero level).

of grain dust samples in the concentrations of 1.0-801.3 × 10^3 cfu/g and 1.5-12440.0 × 10^3 cfu/g, respectively. The concentration of microfungi in grain dust samples was significantly greater than in grain samples ($p<0.01$). Altogether, the following 19 species and/or genera of fungi able to grow on malt agar were isolated from samples of grain and grain dust: *Alternaria alternata*, *Aspergillus ochraceus*, *Aspergillus niger*, *Cladosporium elegantulum*, *Cladosporium herbarum*, *Cladosporium lignicola*, *Geotrichum candidum*, *Monilia sitophila*, *Monosporium silvaticum*, *Mucor mucedo*, *Oidiodendron griseum*, *Oidiodendron flavum*, *Penicillium* spp., *Prophytroma tubularis*, *Rhizopus artocarpi*, *Rhizopus nigricans*, *Rhodotorula rubra*, *Trichoderma album*, as well as unidentified yeasts. Most frequently, *Alternaria alternata* strains were isolated (from 47.9% samples), less often *Penicillium* spp. (from 25% samples) and *Mucor mucedo* (from 18.8% samples).

Fusarium strains were isolated from 54.2% of grain samples and from 58.3% of grain dust samples in the concentrations of 0.1-375.0 × 10^3 cfu/g and 4.0-7700 × 10^3 cfu/g, respectively. They were found in all samples of grain and grain dust from rye, barley and corn, but only in 0-16.7% samples of grain and grain dust from oats and buckwheat. Altogether, 3 species of *Fusarium* were isolated from examined samples: *Fusarium culmorum*, *Fusarium graminearum*, and *Fusarium poae*. Most frequently, *F. culmorum* strains were isolated (from 35.4% samples), less often *F. poae* (from 29.2% samples) and *F. graminearum* (from 8.3% samples).

DON was found in 79.2% of grain samples and in 100% of grain dust samples in the concentrations of 0.001-0.18 µg/g and 0.006-0.283 µg/g, respectively. NIV was detected in 62.5% of grain samples and in 94.4% of grain dust samples in the concentrations of 0.004-0.502 µg/g and 0.005-0.339 µg/g, respectively. OTA was detected in 58.3% of grain samples and in 91.7% of grain dust samples in the concentrations of 0.00039-0.00195 µg/g and 0.00036-0.00285 µg/g, respectively. The concentrations of DON, total fusariotoxins (DON + NIV) and OTA were significantly greater in grain dust samples than in grain samples ($p<0.05$, $p<0.05$, and $p<0.001$, respectively). For the total samples from all the examined cereals, a significant correlation was found between the concentration of total fungi grown on malt agar and the concentration of OTA ($p<0.05$), but not between the concentration of *Fusarium* and the concentration of total fusariotoxins (as noted previously for samples from rye and barley).

DISCUSSION

Mycotoxins detected in the present study are known factors of health risk for humans [18, 20, 33, 43, 49, 60]. Deoxynivalenol (DON) and nivalenol (NIV) produced by *Fusarium* spp. are trichothecene mycotoxins causing vomiting, diarrhoea and dermal reaction. An epidemic of alimentary mycotoxicosis in the Kashmir Valley caused by the consumption of mouldy wheat and rice contaminated with DON and other fusariotoxins was described by Smith *et al.* [55]. At the molecular level, DON disrupts normal cell function by inhibiting protein synthesis [49]. In animal experiments, DON adversely affects growth, immune function and reproduction [49], while NIV shows an organ specific genotoxicity [60]. Nordby *et al.* [43] have hypothesised that hormonal and immunological effects of work-related exposure to trichothecene mycotoxins may cause adverse reproductive outcomes and cancer in farmers' families.

Ochratoxin A (OTA), a nephrotoxin produced by filamentous fungi of *Aspergillus ochraceus* and *Penicillium* spp., was described as a cause of Balkan endemic nephropathy (BEN) [55]. The consumption of food contaminated with high levels of OTA results in pathologic symptoms such as: great reduction in kidney size, tubular degeneration, interstitial fibrosis and hyalinization of the glomeruli.

Chronic exposure may induce kidney cancers. In animal models, OTA is cancerogenic, teratogenic, embryotoxic

and immunosuppressive [18, 33]. Besides, OTA inhibits protein synthesis [20]. It has been shown that OTA inhalation may lead to renal failure in both humans and animals, which poses a potential risk to occupationally exposed individuals, including grain farmers [18, 20].

Measurements of mycotoxin concentration in plant materials, dust and air are important for the proper assessment of their role in the etiopathogenesis of diseases induced by exposure to organic dusts. To date, no internationally accepted threshold limit values (TLV) for the concentration of mycotoxins in grain and other food products have been established. The proposals of such values for DON range between 0.5-2.0 μg/g [46], and for OTA between 3.0-5.0 μg/g [1, 4]. In this study, the proposed threshold values were not exceeded, neither in grain nor in grain dust samples.

The concentrations of DON, NIV and OTA found in the present work in grain and grain dust are similar to those recorded by us in an earlier study concerning combine harvested wheat grain and dust [27], but smaller compared to another study in which stored, machine threshed wheat grain and dust were examined [26]. This seems to indicate that modern methods of combine grain harvesting reduce exposure to mycotoxins and/or that mycotoxin content in freshly harvested grain is smaller compared to stored grain, as pointed out by various authors [18]. Nevertheless, the frequency of mycotoxin detection in the present study was greater than in previous studies, except for the frequency of OTA detection in grain, which was similar [26, 27].

Compared to studies on the concentration of mycotoxins in grain carried out in various countries, the average concentration of DON determined in the present work for positive grain samples was low (0.0375 μg/g). It was similar to values reported for grain samples from Finland [14] and UK [57], but smaller compared to those reported for samples of grain or grain products from Germany [40, 4l], the Netherlands [59], Hungary [15], Portugal [37], Denmark [53], Russia [61], Turkey [44], Korea [22, 31], China [32], Ethiopia [2], Venezuela [39], Argentina [8], and Canada [58]. In contrast, the frequency of DON detection in grain samples found in the present work was high (79.2%). It was similar to data reported from Finland [14], Germany [40, 41], Portugal [37], Russia [61], and Argentina [8], smaller compared to those reported from the Netherlands [59], Hungary [15], China [32], and Canada [58], and greater compared to those reported from UK [57], Turkey [44], and Ethiopia [2].

The average concentration of NIV determined in the present work for positive grain samples (0.0846 μg/g) was in the mid-range of hitherto reported data. It was similar to data reported from Germany [40], the Netherlands [59], UK [57], and Ethiopia [2], smaller compared to those from Korea [22, 31], China [32], and Sweden [50], and greater compared to those reported from Finland [14], and Canada [58]. The frequency of NIV detection in grain samples found in the present work was high (62.5%) and greater compared to the data reported by most authors [2, 14, 32, 50, 57, 58].

The average concentration of OTA determined in the present work for positive grain samples (0.000933 μg/g) was low. It was similar compared to the values reported for barley grain samples from Lithuania [3], but smaller compared to those reported for grain samples from Croatia [9], Russia [1, 23], Turkey [4], Ethiopia [2], and another locality in Poland [7]. In contrast, the frequency of OTA detection in grain samples found in the present work was high (58.3%) and greater compared to hitherto published data [1, 2, 3, 6, 9, 23], except for those from Turkey [4].

The concentrations of total fungi in positive samples of grain from rye, barley and oats were of the order 10^3 cfu/g and approximated those reported by other authors [36], while in positive samples of grain from buckwheat and corn were greater, of the order 10^4-10^5 cfu/g. The concentrations of fungi in grain dust were steadily and significantly greater than in grain, being within the range of 10^3-10^7 cfu/g. Fungal species isolated in this study were commonly reported from grain, grain dust and from air polluted with grain dust [3, 25, 26, 27, 35, 36]. *Aspergillus fumigatus*, which was found by us to be a prevalent species in stored wheat grain [26], was never isolated in the course of the present study.

Fusarium strains were isolated from all samples of grain and grain dust from rye, barley and corn in the concentrations of 10^3-10^5 cfu/g and 10^4-10^6 cfu/g, respectively. In contrast, they were found only in single samples of grain and grain dust from oats and buckwheat. In our earlier studies of wheat grain, *Fusarium* strains were also very rare, but they were very common in wheat grain dust, occurring in the concentrations of 10^3-10^7 cfu/g [26, 27]. The spectrum of *Fusarium* species in wheat grain dust was more diverse than in the present study of 5 kinds of grain, comprising 5 species (*F. avenaceum*, *F. culmorum*, *F. graminearum*, *F. poae*, *F. sporotrichioides*) [26, 27], versus 3 species recovered in the present work (*F. culmorum*, *F. graminearum*, *F. poae*). *F. culmorum* was the only *Fusarium* species isolated from grain and grain dust from barley, oats, and buckwheat, whereas *F. poae* was the only *Fusarium* species isolated from grain and grain dust from rye.

A significant correlation was found in this study between the concentration of *Fusarium poae* in the samples of rye grain and rye grain dust and the concentrations of DON and NIV in these samples. In an earlier study [26], we found that the concentrations of *F. poae* were significantly correlated with the DON concentration (in grain samples) and with the concentration of moniliformin and total fusariotoxins (in total samples of grain and grain dust). Summarized results suggest that *Fusarium poae*, the species common in grain and grain dust in eastern Poland, could probably be a source of adverse mycotoxins in this area.

In the study of different cereals, we did not confirm a significant correlation between the concentrations of *F. culmorum* and *F. graminearum* and the concentration of DON that we found for wheat grain dust in an earlier study [26]. A moderately significant correlation between the concentrations of *F. culmorum* and *F. graminearum* in

grain dust samples and the concentration of DON has been reported from Norway by Halstensen *et al.* [19]. Similarly, a significant correlation between the concentration of *F. graminearum* and DON has been found by Dalcero *et al.* [8] who examined wheat samples in Argentina. In contrast, Moreno Contreras *et al.* [39] failed to find any significant correlation between the concentration of *Fusarium* spp. and DON in grain samples collected in Venezuela.

Halstensen *et al.* [18] found a significant correlation between the concentration of *Penicillium* spp. and the concentration of OTA in grain dust samples in Norway. In our study, we noted a significant correlation between the concentration of total fungi grown on malt agar and the concentration of OTA in total samples of grain and grain dust from barley and all examined cereals.

So far, very little is known about the concentrations of fusariotoxins and other mycotoxins in grain dust with respect to a potential health hazard. Low DON concentrations (0.003-0.020 μg/m^3) were found in the air during milling of grain in Finland [30]. May *et al.* [38] detected low concentration of DON (0.1-0.2 μg/g) in a sample of silage associated with a febrile illness in farmers. Palmgren *et al.* [45] examined for mycotoxins 15 samples of settled grain dust collected in elevators in the New Orleans area and found in 10 samples zearalenon at levels from 0.025 to 0.1 μg/g, but no OTA and aflatoxin. Ehrlich and Lee [12] found in 80% of examined samples of grain dust the presence of OTA, DON, secalonic acid, zearalenone, and aflatoxin in the concentrations of 0.0005-0.02 μg/g, 0.0005-0.02 μg/g, 0.0005-0.02 μg/g, 0.02 μg/g, and 0.0005 μg/g, respectively. Nordby *et al.* [43] determined the mean concentrations of DON, T-2, and HT-2 trichothecenes in settled grain dust (from barley, oats, and spring wheat) finding the values of 0.031 μg/g, 0.062 μg/g, and 0.13 μg/g, respectively. Halstensen *et al.* [18] found the median concentration of OTA in samples of settled grain dust in Norway equal to 0.004 μg/g, and estimated inhalable OTA exposure during grain handling of 4-40 pg/m^3.

The concentrations of mycotoxins in grain dust samples found in the present work were significantly greater compared to those recorded in grain samples. The mean concentration of DON in positive samples of grain dust (0.05625 μg/g) was greater compared to data reported by Ehrlich and Lee [12], and similar to that reported by Nordby *et al.* [43], while the mean concentration of OTA in positive samples of grain dust (0.001608 μg/g) was within the range reported by Ehrlich and Lee [12], and similar to the data reported by Halstensen *et al.* [18].

In conclusion, even though the concentrations of examined trichothecenes and ochratoxin A in the examined grain dust samples were not large, their persistent presence (in over 90% of samples) creates a potential hazard for farmers who could be exposed to large concentrations of grain dust, exceeding 50 mg/m^3 [25]. Considering the higher bioavailability of mycotoxins through inhalation [18] and possibility of chronic effects by steady inhaling of low mycotoxin doses [20], the present study supports the hypothesis of Norwegian authors [18, 19, 43] concerning possible adverse effects of trichothecenes and ochratoxin A on grain farmers.

Acknowledgements

The skillful assistance of Engin. Grażyna Cholewa, Ms. Wiesława Lisowska and Ms. Halina Wójtowicz in conducting the study is gratefully acknowledged.

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