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Possible way of zearalenone migration in the agricultural environment

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ABSTRACT

The aim of this study was to elucidate the zearalenone (ZEA) migration in the agricultural environment, from the field (wheat, corn), through soil and ending in water. All samples were collected in the agricultural area of Western Poland in period between March 2010 and December 2012 (three-year study). Determination of ZEA in environmental samples, after extraction and purification, was carried out with use of high-performance liquid chromatography. The toxin content in water samples was between 0.5 and 4.9 ng/L and increases in the late summer and autumn (after harvest). The results clearly indicate that ZEA concentration in surface waters depends on many factors, of which the most important include weather conditions, the seasons and intensity of rainfall, and it is possibly related to scab severity of cereals and the toxin biosynthesis.

Keywords: mycotoxins; water pollution; estrogenic properties; toxicity; *Fusarium*

Mycotoxins are compounds naturally occurring as secondary metabolites of *Aspergillus*, *Penicillium* and *Fusarium* species. Because of their toxicity to animals and humans, their presence in cereals, vegetables and fruits or in products of plant and animal origin is still being investigated (Shier et al. 2001, Bottalico and Perrone 2002, Binder et al. 2007).

The presence of zearalenone (ZEA) in the aquatic environment has been repeatedly confirmed (Paterson et al. 1997, Pawlowski et al. 2004, Bucheli et al. 2005, 2008, Hartmann et al. 2007, Gromadzka et al. 2009, 2012, 2008a,b, Waśkiewicz et al. 2012); however, there is a shortage of information on input pathways of toxins into surface and ground water. According to Hartmann et al. (2007), the presence of mycotoxins in the aquatic environment is a result of water runoff from agricultural fields; however, it was proven (Criado et al. 2005, Russell and Paterson 2007) that the fungus able to mycotoxin biosynthesis could also develop in water (Pereira et al. 2010). Few studies on the prevalence of mycotoxins in the aquatic environment focused mainly on zearalenone due to its strong estrogenic activity (Spengler et al. 2001, Lagana et al. 2004, Hartmann et al. 2007, Bucheli et al. 2008). As it was shown by preliminary investigations, zearalenone content in water is not high, but of prime concern is the potential to accumulate mycotoxins because of possible use of such water in foods and/or feeds technologies (Russell and Paterson in 2007, Gromadzka et al. 2009, 2012, Waśkiewicz et al. 2012).

The aim of this study was to elucidate the possible way of zearalenone migration in the environment, from the zearalenone content in field (wheat, corn), through soil and ending in water.

MATERIAL AND METHODS

Chemicals and apparatus. Standard of zearalenone was purchased from the Sigma-Aldrich

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(Steinheim, Germany) as well as acetonitrile and methanol (high-performance liquid chromatography (HPLC) grade). Water (HPLC grade) was supplied from the own Millipore water purification system. The chromatographic system consisted of a Waters 2695 high-performance liquid chromatograph (Waters, Milford, USA), a Waters 2475 multi λ fluorescence detector and a Waters 2996 photodiode array detector. Millenium software (Waters, Milford, USA) was used for data processing. The excitation and emission wavelengths were 274 and 440 nm, respectively. The reversed-phase Nova Pak C18 column was (150 mm × 3.9 mm, 4 µm particle size) (Waters, Milford, USA), working at room temperature.

Sample collection and preparation. Water samples were collected in the agricultural area of Western Poland (river Tczek and drainage ditches: Grzybno, Żabinko, Napachanie and Cerekwica, nearby Poznan, Poland) in period between March 2010 and December 2012. Sampling points were located directly at cultivated agronomic fields. Water samples were collected monthly in triplicate (from March to December), directly from the drainage ditches and river by a flow proportional sampler (n = 30/year/sampling point).

Cereal samples (wheat and corn) were collected from an area of 10 hectares located in the nearest vicinity surface waters. In three years study, grains were sampled in triplicate directly before harvest in three randomly selected locations on the field. Each replication consisted of three maize ears or ten wheat heads (n = 9/year).

Soil samples were collected directly after harvest in the upper soil layer (up to 5 cm in depth) on the fields. Samples were collected in triplicate in the three randomly selected locations on the field (n = 9/year).

The studies were conducted in three years period.

All samples were stored in the dark at 4°C until extraction. Raw water samples were filtered according to the method described previously by Gromadzka et al. (2009), transferred to 1 L glass bottles and stored in the dark at 4°C until extraction. Extraction was carried out within 24–36 h in order to keep microbiological degradation to a minimum and to avoid the addition of chemical preservatives. 500 mL of this filtrate was passed through a ZearalaTest column (Vicam, Milford, USA) at a flow rate of 1–2 drops/s. ZEA was eluted with 3 mL of methanol. After evaporation of the solvent the sample was dissolved in 200 µL of water/methanol/acetonitrile (70:20:10, v/v/v). Soil and cereal samples, after grinding, were extracted with acetonitrile/water (90:10, v/v) using 2.5 mL of solvent per 1 g of soil sample. ZEA was determined by HPLC with a fluorescence detector according to the method described by Visconti and Pascale (1998).

HPLC analysis of zearalenone. Twenty µL of extract were injected to the chromatographic system. The mobile phase consisted of a mixture of acetonitrile/water/methanol (46:46:8, v/v/v) eluted at a flow rate of 0.5 mL/min. A photodiode array detector was used to confirm the presence of ZEA. The detection limit for ZEA determination in water and soil/cereals samples was 0.3 ng/L and 0.5 ng/g, respectively. Detection parameters such as linearity, recovery, and precision of the analytical method were described earlier (Gromadzka et al. 2009).

Statistical analysis. A two-way fixed-effects model analysis of variance (ANOVA) was carried out to determine the effects of years, locations and years × locations interaction on the content of ZEA in the grain, soil and water. Mean values and standard deviations (Kozak et al. 2013) were estimated. The least significant differences (*LSD*) were calculated and – on this basis – homogeneous groups for the analyzed traits were determined. The relationships between the content of ZEA in the grain, soil and water were estimated on the basis of correlation coefficients (Kozak et al. 2013). All data analysis was performed using the statistical package GenStat v. 15 (VSN International Ltd., Hemel Hempstead, UK).

RESULTS AND DISCUSSION

Zearalenone occurrence in the surface water of the agricultural areas. Up to now studies on mycotoxins are mainly focused on their appearance in the cereals, where the concentrations can be quite high. However, exposure through less obvious routes, such as water, also needs to be explained and determined.

It was initially expected that the highest concentration of toxins would be found in waters close to cultivated fields. However, previous studies have shown that the toxin content in the water in the vicinity of agricultural areas varies greatly (Gromadzka et al. 2012). Therefore it was decided

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to elucidate the migration of mycotoxins in the agricultural environment. The investigations consisted of surface water monitoring in agricultural areas such as river (Tczek) and drainage ditches (Grzybno, Żabinko, Napachanie, Cerekwica). To determine the possible path of ZEA migration in the environment the toxin content was also monitored in the grain (wheat and corn) as well as the soil samples.

Figure 1 presents mycotoxin contents changes within the year and among years. The highest ZEA content was observed in 2010 (Figure 1a). The authors assume that the high concentration of toxins in the water in 2010 could be due to the high severity of *Fusarium* cereal diseases during vegetation season.

Climatic conditions caused that toxin residues were leached from the soil and migrated to the water during the next year. The highest ZEA concentrations were recorded in the drainage ditches Grzybno and river Tczek amounting to 45.1 and 16.7 ng/L, respectively, in the samples collected at the end of August (after harvest). In the other months the toxin concentration ranged from below the detection limit to 1.6 ng/L. During next years of the study the content of ZEA was significantly lower especially in the case of ditches Grzybno and Napachanie as well as river Tczek. In the drainage ditch in Żabinko it was rather stable during the three-year study period (Figure 1b, Table 1). The study showed that the significant increase in the ZEA concentration in surface water in agricultural areas occurs directly after harvest (cereal debris) and in a period of the intense rainfall in spring and/or autumn (leaching from the soil). The results clearly indicate that the mycotoxin content in surface waters depends on many factors, of which the most important include weather conditions, the seasons and intensity of rainfall, properties of soil, topography and is possibly related to scab severity of cereals and the toxin biosynthesis.

A number of research groups have examined ZEA levels in surface waters such as rivers, streams

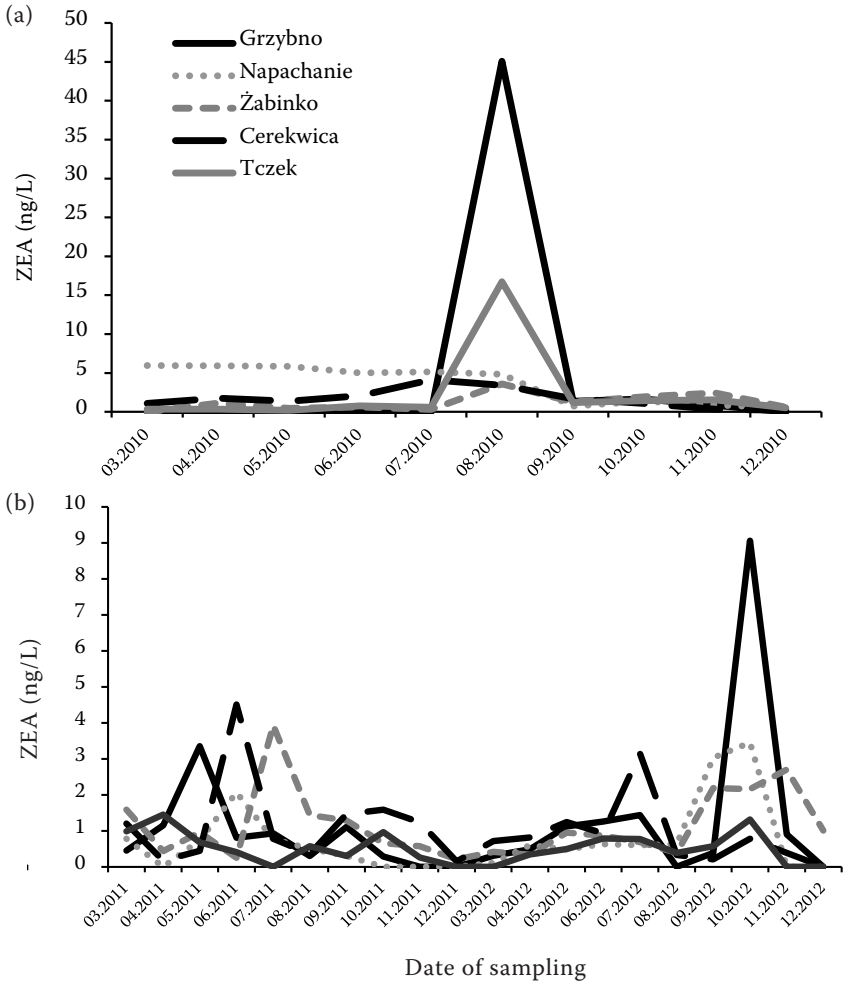

Figure 1. The changes of mycotoxin concentration during the three-years study in (a) 2010, and (b) 2011–2012. ZEA – zearalenone

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Table 1. The average zearalenone (ZEA) content ± standard deviation in grains (ng/g), soil (ng/g) and water samples (ng/L)

	Wheat		Corn		Soil		Water		
	frequency of positive samples (%)	the average ZEA content*	frequency of positive samples (%)	the average ZEA content*	frequency of positive samples (%)	the average ZEA content*	frequency of positive samples (%)	maximum ZEA content	the average ZEA content**
Grzybno									
2010	100	11.4 ± 3.1	–	–	100	6.9 ± 2.2	50	51.1	4.9 ± 14.1
2011	100	10.7 ± 2.2	–	–	100	2.0 ± 0.7	83	3.4	0.8 ± 0.9
2012	100	11.0 ± 4.7	–	–	100	4.2 ± 1.9	100	9.3	1.5 ± 2.7
Żabinko									
2010	100	17.7 ± 6.5	100	11.6 ± 2.5	100	14.4 ± 3.3	90	3.8	1.2 ± 1.1
2011	100	16.0 ± 5.9	100	12.8 ± 7.1	100	12.2 ± 4.5	100	4.2	1.1 ± 1.0
2012	100	10.1 ± 4.2	100	10.5 ± 4.2	100	2.2 ± 0.7	100	2.8	1.2 ± 0.8
Napachanie									
2010	100	45.2 ± 11.8	–	–	100	15.7 ± 6.2	100	6.7	3.6 ± 2.4
2011	100	17.9 ± 7.5	–	–	100	8.3 ± 2.7	80	2.2	0.5 ± 0.6
2012	100	15.3 ± 6.6	–	–	100	10.3 ± 4.0	97	3.5	1.0 ± 1.2
Cerekwica									
2010	100	27.2 ± 8.6	–	–	100	12.1 ± 3.7	100	4.3	1.7 ± 1.2
2011	100	20.5 ± 5.9	–	–	100	10.8 ± 4.2	100	4.8	1.2 ± 1.2
2012	100	21.2 ± 6.8	–	–	100	4.1 ± 1.8	100	3.3	0.9 ± 0.8
Tczek									
2010	–	–	100	57.1 ± 13.6	100	15.4 ± 4.9	100	17.0	2.4 ± 5.0
2011	–	–	100	35.7 ± 10.8	100	16.2 ± 3.8	100	1.5	0.6 ± 0.4
2012	–	–	100	40.9 ± 9.2	100	25.5 ± 9.7	73	1.4	0.5 ± 0.4

*The average ZEA content from three replicates in the month of sampling (once per year); **The average ZEA content from three replicates every month (March–December) in a three-year period

and lakes, but the ZEA content in drainage ditches was studied only in Switzerland (Bucheli et al. 2008, Hartmann et al. 2008a,b) and in Poland (Dudziak 2011). According to the authors, ZEA concentration in the drainage water from cereals (fields) inoculated with *Fusarium graminearum* were in the low nanogram level, with the highest being 35 ng/L (Bucheli et al. 2008, Hartmann et al. 2008). Dudziak (2011) showed ZEA concentration equal to 1.52 and 1.14 ng/L in the lake sample and in the water from melioration ditch located near allotment gardens, respectively. Monitoring studies show that the toxin levels in rivers, streams and drainage ditches are generally low. However, our results show that under favourable weather conditions the ZEA concentration could increase up to 45 ng/L in the natural environment without artificial inoculation. Similar results were described by Hartmann et al. (2008a), where ZEA concentration in puddle water from a field heavily infected with *F. graminearum* reached 250 ng/L. Therefore, despite low levels of ZEA in surface water, ZEA might contribute substantially to the total estrogenicity of water in case of *F. graminearum* occurrence (Hartmann et al. 2008a).

Possible zearalenone migration in the environment. Previous studies showed that ZEA is present both in surface and groundwater (Gromadzka et

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Table 2. Correlation coefficients between zearalenone (ZEA) concentration in different matrices (*P*-values)

	Grain	Soil	Water
All years			
Grain	1		
Soil	0.7144 (0.0028)	1	
Water	–0.1377 (0.6246)	–0.0416 (0.8830)	1
2010			
Grain	1		
Soil	0.6911 (0.1963)	1	
Water	–0.1220 (0.8451)	–0.5980 (0.2868)	1
2011			
Grain	1		
Soil	0.7449 (01486)	1	
Water	–0.4478 (0.4495)	–0.0324 (0.9587)	1
2012			
Grain	1		
Soil	0.8824 (0.0475)	1	
Water	–0.9401 (0.0174)	–0.7989 (0.1050)	1

al. 2009, 2012, Waśkiewicz et al. 2012). However, an evident toxin migration path to aquatic ecosystems has not been confirmed. Additionally our studies have eliminated the possibility of direct synthesis of toxins in the aquatic environment (data not published). Therefore, the possibility of ZEA migration was studied. Presence of ZEA was found in all cereal grain samples and in all soil samples examined in the concentration range from 2.0 ng/g up to 57.1 ng/g (Table 1).

The toxin concentration found in the soil was from 5–63% of the level in the grain. However, statistical analysis (Table 2) showed a significant correlation between the ZEA concentration in the grain and soil for all years (r = 0.7144; P = 0.0028) and in 2012 (r = 0.8824; P = 0.0475).

The toxin content in soil was also studied by Hartmann et al. (2008a). ZEA level in the topsoil varied between non-detectable and 3.8 ng/g after cereal inoculation with *F. graminearum*. ZEA concentrations in the wheat and maize samples it ranged from 0.1 up to 16.6 µg/g and 0.1 and 1.4 µg/g, respectively. According to the authors, because of the continuous ZEA elution from either *F. graminearum* infected maize or wheat plants on the soil surface, no clear concentration trend in the topsoil could be observed. A comparison of the results recorded in Poland and Switzerland shows that in spite of inoculation reported by Hartmann et al. (2008a) as well as significantly higher content of toxins in plants ZEA concentration in soil and water was present at similar levels. The results indicate that toxin adsorption occurs in trace amounts; therefore we have to consider the possibility of toxin degradation by microorganisms such as *Trichoderma* and *Clonostachys* (Popiel et al. 2008, Gromadzka et al. 2012) as well as the possibility of ZEA migration into the deeper soil layers. However, the results confirm a relationship between the severity of *Fusarium* diseases and toxin content in soil and water. Additionally, we have observed a statistically significant negative relationship between ZEA occurrence in water and in grain as well as in water and in soil (Table 2).

Negative correlation can be explained by very low possibility of ZEA synthesis in water indicating that toxin leaching from the soil and cereal debris causes the increase of its content in aquatic ecosystems. Figure 1a best illustrates the rate of mycotoxins migration to the surface water in agricultural areas. The presented chart shows that the highest concentration of toxin occurs immediately after the harvest. The next increases in the concentration of toxins in the water are due to climatic conditions because heavy rainfall during the spring and/or autumn causing toxins leaching from the soil.

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