

ZEARALENONE CONTAMINATION OF THE AQUATIC ENVIRONMENT AS A RESULT OF ITS PRESENCE IN CROPS*

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The aim of this study was to establish a relation between zearalenone contamination of crops in the Polish province of Wielkopolska and its occurrence in aquatic ecosystems close by the crop fields. Water samples were collected from water bodies such as drainage ditches, wells, or watercourses located in four agricultural areas. Moreover, control water samples were collected from the Bogdanka river, which was located outside the agricultural areas and near an urban area. Cereal samples were collected in the harvest season from each agricultural area close to tested water bodies. Zearalenone (ZEA) was found in all water and cereal samples. The highest concentrations were recorded in the post-harvest season (September to October) and the lowest in the winter and spring. Mean ZEA concentrations in water ranged between 1.0 ng L⁻¹ and 80.6 ng L⁻¹, and in cereals from 3.72 ng g⁻¹ to 28.97 ng g⁻¹. Our results confirm that mycotoxins are transported to aquatic systems by rain water through soil.

KEY WORDS: *cereals, HPLC, oestrogenic properties*

Monitoring studies throughout the world have been trying to establish the scale of water contamination with natural toxic compounds (1-5). Over the last 10 years, this investigation has included mycotoxins produced by fungi that can grow on a wide variety of crops, fruits, and vegetables, including wheat, corn, grape, pineapple, nuts, asparagus, onion, and garlic (6-7). As they pose a significant threat for human and animal health, these metabolites are subject to EU standards and regulations (8). Studies conducted to date have mainly focused on the occurrence of mycotoxins in cereals and their products, while little

attention was given to their spread in aquatic environments (9-12). Earlier studies have confirmed that zearalenone (ZEA), a major *Fusarium* mycotoxin, not only causes serious losses in agriculture, but may also contaminate aquatic ecosystems. This has been confirmed by recent reports (13-16).

Zearalenone [6-(10-hydroxy-6-oxo-trans-1-undecenyl)- β -resorcylic acid lactone; CAS No.:17924-92-4] is a natural contaminant of food of cereal origin (17, 18). Its biosynthesis has been recorded in cereals (kernels) such as corn, rice, and wheat infected by several *Fusarium* species, including *F. graminearum*, *F. culmorum*, *F. crookwellense*, *F. equiseti*, and *F. semitectum* (18, 19). The concentration of accumulated ZEA in cereals depends on several

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factors such as the substrate, temperature, duration of *Fusarium* growth, and the strain of a fungal species (20). The toxicity of zearalenone and its metabolites is related to the chemical structure of mycotoxins and is similar to naturally occurring oestrogens ER α and ER β found in mammalian tissues (21, 22). The most important toxic effect of ZEA is its oestrogenic effect, which impairs fertility and foetal development in farm animals (7, 17). A Polish study (23) in 49 women established a strong correlation between blood ZEA content and the presence of uterine pathologies (adenocarcinoma and hyperplasia of the endometrium). Moreover, an *in vitro* study (24) established the immunosuppressive effect in human peripheral blood mononuclear cells exposed to ZEA.

Data about the presence of other *Fusarium* toxins in aquatic ecosystems are scarce. However, judging by what we know about ZEA contamination of waters, it is likely that these compounds are also present in ground and surface waters.

Ecotoxicological effects of the occurrence of mycotoxins in waters have not been clarified yet. The occurrence of *Fusarium* mycotoxins in the aquatic environment, is of prime concern as *Fusaria* are the most common pathogens on crops. The aim of our study was to assess ZEA contamination of waters in the Wielkopolska region and elucidate how they got there.

MATERIALS AND METHODS

Chemicals and chromatograph

Zearalenone standard, methanol, and acetonitrile (HPLC-grade) were purchased from Sigma-Aldrich

(Steinheim, Germany). A ZearalaTestTM immuno-affinity column was purchased from Vicam (USA). Water for the HPLC mobile phase was purified in a Milli-Q system (Millipore, Bedford, MA, USA).

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 with a C₁₈ column (3.9 mm x 150 mm, 4 μ m particle size). Data were processed using the Empower software (Waters, Milford, USA). The excitation wavelength and emission wavelength were set at 274 nm and 440 nm, respectively.

Water and plant sampling

We took water samples from eight points in the province of Wielkopolska across four farmland areas (Figure 1), as follows: Cerekwica (two points: a ditch and a drainage well); Napachanie (one point: a drainage ditch); Brodnica (two points: a watercourse and a drainage ditch); and Leszno (two points: a drainage ditch and a lake in Wojnowice). We also collected water samples from the Bogdanka River on the outskirts of Poznań. This point was chosen to see if there were differences in ZEA levels between water samples collected from agricultural and non-agricultural areas. Samples were collected by immersing a 1 litre scoop 20 cm below water surface, then pouring the water into 2 litre canisters to be stored at 2 °C to 8 °C until analysis. Table 1 shows the sampling schedule.

Plant samples were collected during harvest from five random sites. From Cerekwica, Napachanie, and

Table 1 A scheme of water sampling terms (dates)

Term	Location	Bogdanka river	Cerekwica ditch	Cerekwica well	Napachanie ditch	Brodnica ditch	Brodnica watercourse	Leszno ditch	Wojnowice lake
III/2009		x							
IV/2009		x							
V/2009		x							
VI/2009		x	x	x	x	x	x	x	x
VII/2009 I		x	x	x	x	x	x	x	x
VII/2009 II		x	x	x	x	x	x	x	x
VIII/2009		x	x	x	x	x	x	x	x
IX/2009		x	x	x	x	x	x	x	x
X/2009		x	x	x	x	x	x	x	x
XI/2009		x	x	x	x	x	x	x	x
Winter 2009/2010		x	x	x	x	x	x	x	x
IV/2010		x	x	x	x	x	x	x	x



Figure 1 Distribution of sampling sites in the province of Wielkopolska. Area I – Cerekwica; Area II – Napachanie; Area III – Brodnica; Area IV – Leszno

Brodnica we sampled wheat kernels and from Leszno maize kernels.

Sample purification and extraction

Each water sample (500 mL) was filtered through three filters: Filtrak no. 389 (Munktell & Filtrak GMBH, Barenstein, Germany), Whatman no. 4, and glass filter Whatman GF/B (Whatman International Ltd, Maidstone, UK), whose pore size decreased successively. The aim was to thoroughly purify water samples for later extraction stages. The filtrate (500 mL) was passed through a ZearalaTest™ immuno-affinity column at a flow rate of one to two drops per second. Zearalenone was washed from the column using 1.5 mL methanol at a flow rate of one drop per second and evaporated in a stream of nitrogen.

Ground wheat or maize kernels (10 g) were homogenised (homogeniser H 500, Pol-Eco, Wodzisław Sl., Poland) in a glass container with 1 g of KCl and a 25 mL mixture of acetonitrile and water (90:10) at high rotation (20 000 rotation per minute) for two minutes and the homogenate mixture was filtered through the Whatman no. 4 filter paper. From the supernatant we took 10 mL and supplemented it with 40 mL of distilled water, and then repeated the filtering through Whatman no. 4 filter paper. The obtained

10 mL extracts were passed through a ZearalaTest™ affinity column at a flow rate of one to two drops per second. Zearalenone was washed from the column using 3 mL methanol at a similar flow rate and evaporated.

HPLC analysis

Evaporated extracts of water and plant samples were dissolved in a 200 µL mixture of acetonitrile: methanol:water (70:20:10), homogenised in an ultrasonic bath (Ultron, type U-505, Dywity, Poland) and applied onto the column. Zearalenone was assayed using a Waters 2695 HPLC with a Waters 2475 multi fluorescence detector system, a Waters 2996 photodiode array detector, and a C₁₈ Nova Pack 3.9 mm x 150 mm column according to the method described by Gromadzka et al. (14).

Statistical analysis

We used the two-way analysis of variance (ANOVA) to determine the effects of months, locations and months-location interaction on zearalenone concentrations in water. The least significant differences (LSDs) for ZEA were calculated. Homogeneous groups for the analysed trait were determined on the basis of LSDs.

RESULTS AND DISCUSSION

Zearalenone in water samples

Zearalenone was found in all analysed water samples. Its concentrations significantly depended on the location, month, and location-month interaction (Table 2). Figures 2-5 show mean ZEA concentrations measured in this study.

In all agricultural areas ZEA concentrations were the highest in the autumn (September - October) and the lowest in the winter and spring (Figure 2 and 5). This can be explained by summer and autumn rain

Table 2 Results of analysis of variance for concentrations of zearalenone

Source of variation	Number of degrees of freedom	Sum of squares	Mean squares	F statistics
Location	7	6157.39	879.63	43.51***
Term	8	31874.12	3984.27	197.08***
Location x Term	56	17690.66	315.9	15.63***
Residual	144	2911.23	20.22	

*** P < 0.001

washing away mycotoxins from plants through the soil to drainage ditches located close by the fields. The highest ZEA concentrations recorded in the autumn are probably related to the post-harvest period, as ZEA accumulates in drainage water. The maximum ZEA concentrations in the tested water samples recorded in the early autumn and not immediately after the harvest may be related to weather conditions. Low precipitation in the high summer does not favour the transfer of ZEA into the aquatic ecosystems.

Figure 4 and 5 show the mean level of ZEA calculated for all collection points in the experimental period. The greatest variation in ZEA water concentrations was recorded for the drainage ditch of Leszno, with both the highest (80.6 ng L⁻¹) and the lowest (0.4 ng L⁻¹) ZEA concentrations (Figure 4 and 5).

These findings significantly set apart this sampling site from the rest and point to a strong correlation with ZEA levels in maize, which was grown in the nearest vicinity of this collection point. As known, maize is the cereal with the highest recorded concentrations of ZEA (25, 26).

On the other hand, the lowest concentrations of ZEA were found in water samples collected from Cerekwica, where wheat was grown (Figure 4 and 5).

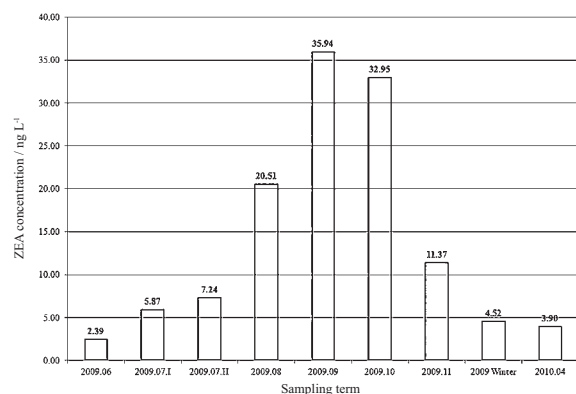


Figure 2 Mean ZEA concentrations (ng L⁻¹) in water for sampling dates

Zearalenone was also found in all Bogdanka River water samples (Figure 3). However, its levels were generally lower or similar to those measured in water samples from the agricultural areas. ZEA concentrations also varied by the season; they were low in the spring and winter and high in the summer and autumn, reaching the top in September 2009 (25.0 ng L⁻¹). Later the concentration of ZEA decreased (Figure 3).

Figure 2 and 5 show mean concentrations of ZEA calculated for individual sampling dates. These results

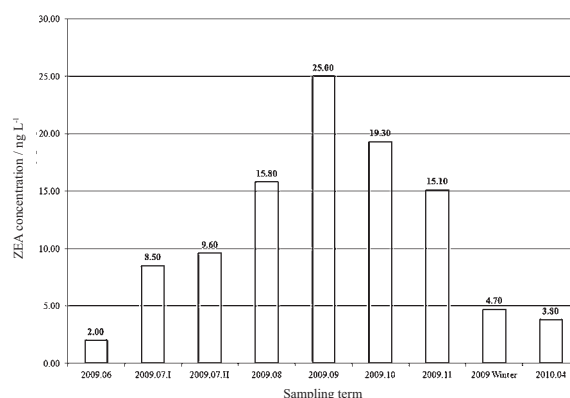


Figure 3 Mean ZEA concentrations (ng L⁻¹) in the Bogdanka River in the annual cycle

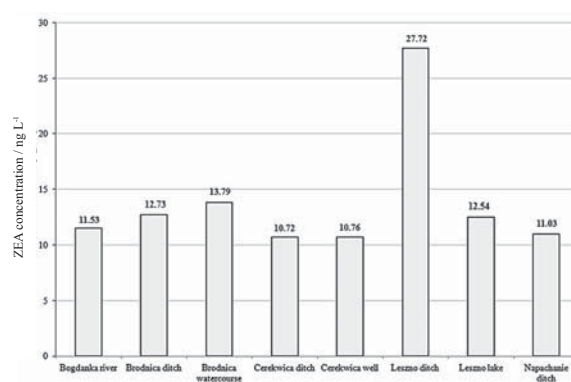


Figure 4 Mean concentrations of ZEA (ng L⁻¹) over the entire experimental period for all collection points

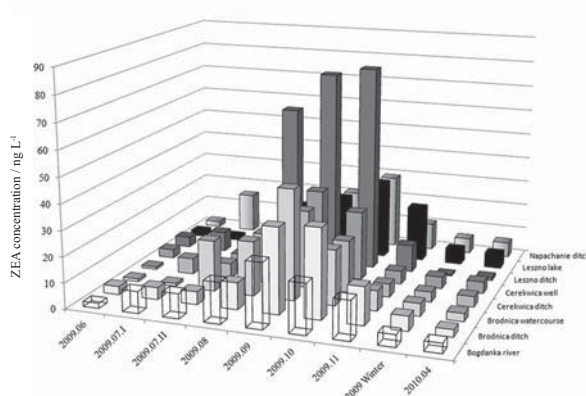


Figure 5 Mean concentrations of ZEA (ng L⁻¹) over the entire experimental period for individual sampling dates

show that ZEA levels were the highest in September (35.94 ng L⁻¹) and October 2009 (32.95 ng L⁻¹).

Our findings confirm that ZEA accumulates in the aquatic environment in the post-harvest period.

Zearalenone in cereal samples

Table 3 shows ZEA levels measured in cereal kernels. Similar to water samples, the highest mean

Table 3 Levels of zearalenone in cereal samples by location

Location	ZEA mass fraction / ng g ⁻¹		Cereal
	Mean	Range	
Cerekwica (I)	8.79	5.07 to 15.30	wheat
Napachanie (II)	3.72	2.15 to 5.55	wheat
Brodnica (III)	7.88	6.09 to 10.70	wheat
Leszno (IV)	28.97	18.90 to 39.76	maize

ZEA level was determined in maize (28.97 ng g⁻¹) from Leszno, which confirms our hypothesis that mycotoxins were washed from cropped fields through soil and transported to the nearby aquatic ecosystems by rain. The lowest mean ZEA level was found in Napachanie, while the other two sites showed similar ZEA contamination.

ZEA pollution of the aquatic ecosystems has repeatedly been repeatedly confirmed during the past decade (9, 14, 16, 27-31). However, no information is available on the pathways of toxin transport to surface waters. According to Hartmann et al. (27), the occurrence of mycotoxins in the aquatic environment is a consequence of surface run-off from cropped fields. Furthermore, mycotoxin producers may also develop in water (32-34). Criado et al. (32) reported that fungi from the genera *Alternaria*, *Penicillium* and *Cladosporium* may grow in bottled mineral water, which constitutes a serious health hazard for consumers. In another study Russell and Paterson (33) confirmed the potential of *Fusarium graminearum* for synthesising ZEA (15 ng L⁻¹) in drinking water.

Ternes et al. (35) investigated the content of ZEA in surface waters and sewage effluents, where its concentrations were up to 60 ng L⁻¹. Similar study was conducted by Hartmann et al. (27), who reported the level of ZEA in drainage ditches amounting up to 30 ng L⁻¹. Additionally, Bucheli et al. (10) monitored the content of ZEA in drainage ditches after field inoculation of winter wheat with *Fusarium graminearum*. Concentrations of ZEA in water bodies increased in the pre-harvest season. At the same time, ZEA levels in the nearby rivers were below the detection limit.

Although the results of this preliminary study show that the level of ZEA in water was not high, such an exposure might be hazardous due to potential accumulation of mycotoxins, especially if the contaminated water is used in food production for humans and animals (14, 33).

Some of the newer studies also point to the presence of other *Fusarium* toxins, for example

deoxynivalenol (36), beauvericin (BEA), enniatins (ENN), nivalenol (NIV), fusaroproliferin (FUS), and 3-acetyl-deoxynivalenol (3-AcDON) in environmental samples (37, 38).

Identification of different *Fusarium* mycotoxins in aquatic ecosystems is a rationale for further studies aiming at a precise assessment of their seasonal variability and migration routes to the aquatic environment.

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Sažetak

POJAVA MIKOTOKSINA U VODENOM OKOLIŠU ZBOG NJIHOVE PRISUTNOSTI U USJEVIMA

Cilj ovog istraživanja bio je pojasniti učestalost pojave mikotoksina u vodenim ekosustavima i njihove korelacije sa stupnjem zaraze žitarica (uzgajanih u blizini vodospremnika), čija su zrna onečišćena (kontaminirana) mikotoksinima te problem prolaska mikotoksina kroz tlo u vodeni okoliš (onečišćenje podzemnih voda mikotoksinima). Uzorci vode prikupljeni su u regiji Wielkopolska iz vodenih tijela poput odvodnih jaraka i zdenaca, odnosno vodotoka smještenih u područjima koja se rabe za poljoprivredu. Dio uzoraka vode prikupljen je iz rijeke Bogdanka, u rubnom području grada Poznania. U sezoni žetve sa svake poljoprivredne površine smještene u neposrednoj blizini testiranih vodenih tijela prikupljeni su uzorci žitarica. U svim analiziranim uzorcima vode i žitarica potvrđena je prisutnost zearalenona (ZEA). Najviše koncentracije mikotoksina u uzorcima sa svih poljoprivrednih površina zabilježene su u jesen nakon sezone žetve (rujan-listopad), dok su najniže vrijednosti izmjerene zimi i u proljeće. Srednje koncentracije zearalenona u vodi bile su u rasponu od 1,0 ng L⁻¹ do 80,6 ng L⁻¹. U žitarica je prosječna razina zearalenona iznosila 3,72 ng g⁻¹ do 28,97 ng g⁻¹, što govori u prilog vjerodostojnosti naše polazišne hipoteze o prijenosu mikotoksina kroz tlo nakon njihova ispiranja s površine u jarke za odvodnju.

KLJUČNE RIJEČI: *estrogena svojstva, HPLC, zearalenon, žitarice*

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