

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

Agnieszka WAŚKIEWICZ<sup>1</sup>, Karolina GROMADZKA<sup>1</sup>, Jan BOCIANOWSKI<sup>2</sup>, Paulina PLUTA<sup>1</sup>,  
and Piotr GOLŃSKI\*

*Department of Chemistry<sup>1</sup>, Department of Mathematical and Statistical Methods<sup>2</sup>, Poznan University of Life Sciences,  
Poznań, Poland*

Received in February 2012  
CrossChecked in August 2012  
Accepted in October 2012

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<sup>-1</sup> and 80.6 ng L<sup>-1</sup>, and in cereals from 3.72 ng g<sup>-1</sup> to 28.97 ng g<sup>-1</sup>. 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)- $\beta$ -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

\* The subject of this article has partly been presented at the International Symposium "Power of Fungi and Mycotoxins in Health and Disease" held in Primošten, Croatia, from 19 to 22 October 2011.





**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, Wodzislaw 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 C<sub>18</sub> 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	

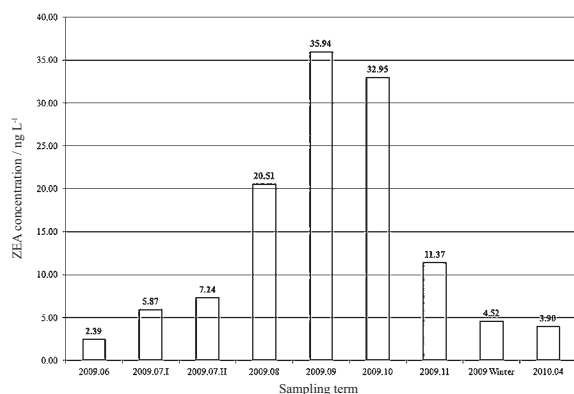
\*\*\* P < 0.001

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<sup>-1</sup>) and the lowest (0.4 ng L<sup>-1</sup>) 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).

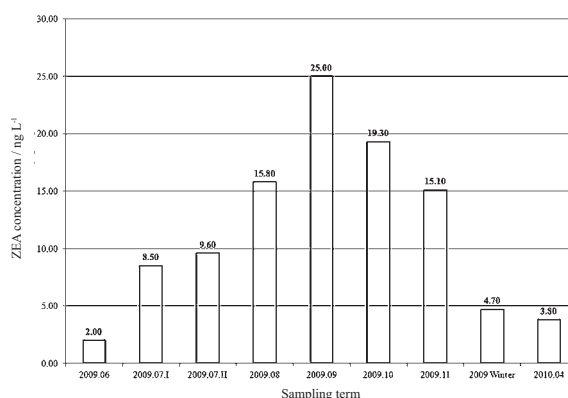
On the other hand, the lowest concentrations of ZEA were found in water samples collected from Cerekwica, where wheat was grown (Figure 4 and 5).



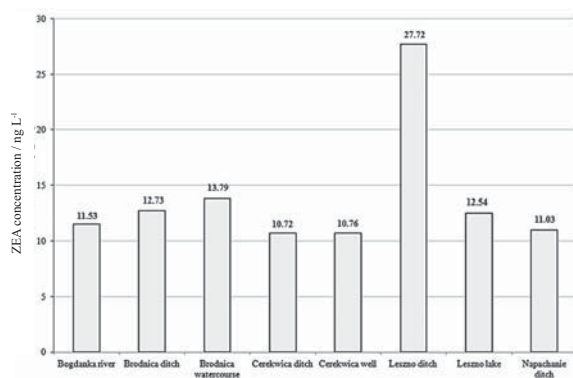
**Figure 2** Mean ZEA concentrations (ng L<sup>-1</sup>) in water for sampling dates

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<sup>-1</sup>). Later the concentration of ZEA decreased (Figure 3).

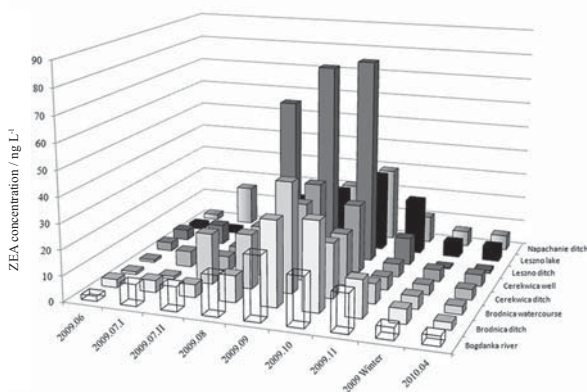
Figure 2 and 5 show mean concentrations of ZEA calculated for individual sampling dates. These results



**Figure 3** Mean ZEA concentrations (ng L<sup>-1</sup>) in the Bogdanka River in the annual cycle



**Figure 4** Mean concentrations of ZEA (ng L<sup>-1</sup>) over the entire experimental period for all collection points



**Figure 5** Mean concentrations of ZEA (ng L<sup>-1</sup>) over the entire experimental period for individual sampling dates

show that ZEA levels were the highest in September (35.94 ng L<sup>-1</sup>) and October 2009 (32.95 ng L<sup>-1</sup>).

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 <sup>-1</sup>		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<sup>-1</sup>) 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<sup>-1</sup>) 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<sup>-1</sup>. Similar study was conducted by Hartmann et al. (27), who reported the level of ZEA in drainage ditches amounting up to 30 ng L<sup>-1</sup>. 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 (ENNs), 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.

#### Acknowledgments

This study was supported by Polish Ministry of Science and Higher Education (Project no: NN 305 1655 37).

#### REFERENCES

1. Laganà A, Fago G, Marino A, Santarelli D. Development of an analytical system for the simultaneous determination of anabolic macrocyclic lactones in aquatic environmental samples. *Rapid Commun Mass Spectrom* 2001;15:304-10.
2. Laganà A, Bacaloni A, De Leva I, Faberi A, Fago G, Marino A. Analytical methodologies for determining the occurrence of endocrine disrupting chemicals in sewage treatment plants and natural waters. *Anal Chim Acta* 2004;501:79-88.
3. Pawlowski S, Ternes TA, Bonerz M, Rastall AC, Erdinger L, Braunbeck T. Estrogenicity of solid phase-extracted water samples from two municipal sewage treatment plant effluents and river Rhine water using the yeast estrogen screen. *Toxicol in Vitro* 2004;18:129-38.
4. Spengler P, Körner W, Metzger JW. Substances with estrogenic activity in effluents of sewage treatment plants in southwestern Germany. 1. Chemical analysis. *Environ Toxicol Chem* 2001;20:2133-41.
5. Hoerger CC, Wettstein FE, Hungerbuhler K, Bucheli TD. Occurrence and origin of estrogenic isoflavones in Swiss river waters. *Environ Sci Technol* 2009;43:6151-7.
6. Stępień Ł, Koczyk G, Wańkiewicz A. Genetic and phenotypic variation of *Fusarium proliferatum* isolates from different host species. *J Appl Genet* 2011;52:487-96.
7. Golinski P, Wańkiewicz A, Gromadzka K. Zearalenone and its derivatives: known toxins in new aspects. In: Rai M, Varma A, editors. *Mycotoxins in food, feed and bioweapons*. Berlin, Heidelberg: Springer-Verlag; 2011. p. 113-29.

8. Commission Regulation (EC) No 1126/2007 of 28 September 2007 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards *Fusarium* toxins in maize and maize products.
9. Bucheli TD, Erbs M, Hartmann N, Vogelgsang S, Wettstein FE, Forrer HR. Estrogenic mycotoxins in the environment. *Mitt Lebensm Hyg* 2005;96:386-403.
10. Bucheli TD, Wettstein FE, Hartmann N, Erbs M, Vogelgsang S, Forrer HR, Schwarzenbach RP. *Fusarium* mycotoxins: overlooked aquatic micropollutants? *J Agric Food Chem* 2008;56:1029-34.
11. Schwartz P, Thorpe KL, Bucheli TD, Wettstein FE, Burkhardt-Holm P. Short-term exposure to the environmentally relevant estrogenic mycotoxin zearalenone impairs reproduction in fish. *Sci Total Environ* 2010;409:326-33.
12. Hoerger CC, Schenzel J, Strobel BW, Bucheli TD. Analysis of selected phytotoxins and mycotoxins in environmental samples. *Anal Bioanal Chem* 2009;395:1261-89.
13. Maragos CM. Zearalenone occurrence in surface waters in central Illinois, USA. *Food Addit Contam Part B Surveillance* 2012;5:55-64.
14. Gromadzka K, Waškiewicz A, Goliński P, Świetlik J. Occurrence of estrogenic mycotoxin – zearalenone in aqueous environmental samples with various NOM content. *Water Res* 2009;43:1051-9.
15. Hartmann N, Erbs M, Wettstein FE, Hoerger CC, Schwarzenbach RP, Bucheli TD. Quantification of zearalenone in various solid agroenvironmental samples using D<sub>6</sub>-zearalenone as the internal standard. *J Agric Food Chem* 2008;56:2926-32.
16. Gromadzka K, Waškiewicz A, Goliński P, Świetlik J, Bocianowski J. Dissolved organic carbon as an indicator of the presence of zearalenone in the aquatic environment. *World Mycotox J* 2012;5:357-64.
17. Goliński P, Waškiewicz A, Gromadzka K. Mycotoxins and mycotoxicoses under climatic conditions of Poland. *Pol J Vet Sci* 2009;12:581-8.
18. Goliński P, Waškiewicz A, Wisniewska H, Kiecana I, Mielniczuk E, Gromadzka K, Kostecki M, Bocianowski J, Rymaniak E. Reaction of winter wheat (*Triticum aestivum* L.) cultivars to infection with *Fusarium* spp.: mycotoxin contamination in grain and chaff. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess* 2010;27:1015-24.
19. Waškiewicz A, Gromadzka K, Wiśniewska H, Goliński P. Accumulation of zearalenone in genotypes of spring wheat after inoculation with *Fusarium culmorum*. *Cereal Res Commun* 2008;36(Suppl 6):401-4.
20. Zinedine A, Soriano JM, Moltó JC, Mañes J. Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone: An oestrogenic mycotoxin. *Food Chem Toxicol* 2007;45:1-18.
21. Shier WT, Shier AC, Xie W, Mirocha CJ. Structure-activity relationships for human estrogenic activity in zearalenone mycotoxins. *Toxicol* 2001;39:1435-8.
22. Tiemann U, Tomek W, Schneide F, Vanselow J. Effects of the mycotoxins  $\alpha$ - and  $\beta$ -zearalenol on regulation of progesterone synthesis in cultured granulosa cells from porcine ovaries. *Reprod Toxicol* 2003;17:673-81.
23. Tomaszewski J, Míturski R, Semczuk A, Kotarski J, Jakowicki J. [Tissue zearalenone concentration in normal, hyperplastic and neoplastic human endometrium, in Polish]. *Ginekologia Polska* 1998;69:363-6.
24. Berek L, Petri IB, Mesterházy A, Téren J, Molnár J. Effects of mycotoxins on human immune functions *in vitro*. *Toxicol in Vitro* 2001;15:25-30.
25. Pleadin J, Sokolović M, Perši N, Zdravec M, Jaki V, Vulić A. Contamination of maize with deoxynivalenol and zearalenone in Croatia. *Food Control* 2012; 28:94-98.
26. Hadiani MR, Yazdanpanah H, Ghazi-Khansari M, Cheraghali AM, Goodarzi M. Survey of the natural occurrence of zearalenone in maize from northern Iran by thin-layer chromatography densitometry. *Food Addit Contam* 2003; 20:380-385.
27. Hartmann N, Erbs M, Wettstein FE, Schwarzenbach RP, Bucheli TD. Quantification of estrogenic mycotoxins at the ng/L level in aqueous environmental samples using deuterated internal standards. *J Chromatogr A* 2007;1138:132-40.
28. Koplín DW, Hoerger CC, Meyer MT, Wettstein FE, Hubbard LE, Bucheli TD. Phytoestrogens and mycotoxins in Iowa streams: an examination of under investigated compounds in agricultural basins. *J Environ Qual* 2010;39:2089-99.
29. Paterson RRM, Kelley J, Gallagher M. Natural occurrence of aflatoxins and *Aspergillus flavus* (LINK) in water. *Lett Appl Microbiol* 1997;25:435-6.
30. Hartmann N, Erbs M, Forrer HR, Vogelgsang S, Wettstein FE, Schwarzenbach RP, Bucheli TD. Occurrence of zearalenone on *Fusarium graminearum* infected wheat and maize fields in crop organs, soil and drainage water. *Environ Sci Technol* 2008;42:5455-60.
31. Hartmann N, Erbs M, Wettstein FE, Hörgner CC, Vogelgsang S, Forrer HR, Schwarzenbach RP, Bucheli TD. Environmental exposure to estrogenic and other myco- and phytotoxins. *Chimia* 2008;62:364-7.
32. Criado MV, Fernández PVE, Badessari A, Cabral D. Conditions that regulate the growth of moulds inoculated into bottled mineral water. *Int J Food Microbiol* 2005;99:343-9.
33. Russell R, Paterson M. Zearalenone production and growth in drinking water inoculated with *Fusarium graminearum*. *Mycol Progress* 2007;6:109-13.
34. Hageskal G, Lima N, Skaar I. The study of fungi in drinking water. *Mycol Res* 2009;113:165-72.
35. Ternes TA, Eggert T, Meisenheimer M. [Pflanzliche Hormonell wirksame Stoffe in der aquatischen Umwelt und deren Verhalten bei der Trinkwasseraufbereitung. Abschlussbericht, in German]. Wiesbaden: ESWE-Institut für Wasserforschung und Wassertechnologie GmbH; 2001.
36. Wettstein FE, Bucheli TD. Poor elimination rates in waste water treatment plants lead to continuous emission of deoxynivalenol into the aquatic environment. *Water Res* 2010;44:4137-42.
37. Monti SM, Fogliano V, Logrieco A, Ferracane R, Ritieni A. Simultaneous determination of beauvericin, enniatins, and fusaproliferin by high performance liquid chromatography. *J Agric Food Chem* 2000;48:3317-20.
38. Schenzel J, Schwarzenbach RP, Bucheli TD. Multi-residue screening method to quantify mycotoxins in aqueous environment samples. *J Agric Food Chem* 2010;58:11207-17.

### **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 Poznańa. 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<sup>-1</sup> do 80,6 ng L<sup>-1</sup>. U žitarica je prosječna razina zearalenona iznosila 3,72 ng g<sup>-1</sup> do 28,97 ng g<sup>-1</sup>, 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*

#### **CORRESPONDING AUTHOR:**

Piotr Goliński  
Department of Chemistry  
Poznan University of Life Sciences  
Wojska Polskiego 75  
60-625 Poznań, Poland  
E-mail: [piotrg@up.poznan.pl](mailto:piotrg@up.poznan.pl)