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The role of wastewater treatment in reducing pollution of surface waters with zearalenone

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Zearalenone (ZEA) is a mycotoxin produced by some *Fusarium* species in food and feed. The toxicity of ZEA and its metabolites is related to the chemical structure of the mycotoxin, which is similar to naturally occurring oestrogens. Currently, there is increasing awareness of the presence of fungi and their toxic metabolites in the aquatic environment. One of the sources of these compounds are the effluents from wastewater treatment plants. The average annual efficiency of zearalenone reduction in the Łęczyca plant in our three-year study was in the range from 51.35 to 69.70 %. The three-way analysis of variance (year, month, and kind of wastewater) shows that the main effects of all factors and all interactions between them were significant for zearalenone and dissolved organic carbon content. Our findings suggest that wastewater is not the main source of surface water pollution with zearalenone. Future research should investigate the means to reduce ZEA and its migration from the fields through prevention strategies such as breeding for crops, plant debris management (crop rotation, tillage), and/or chemical and biological control.

KEY WORDS: *aquatic environment; dissolved organic carbon; HPLC; mycotoxins; water quality*

Since recently, large-scale environmental monitoring of natural toxic compounds has included the products of fungal biosynthesis - the so called mycotoxins. Mycotoxins are produced by three genera: *Aspergillus*, *Penicillium,* and *Fusarium*. The first two contaminate food during drying and storage, whereas certain *Fusarium* species produce mycotoxins before or immediately after the harvest. The most important sources of mycotoxins for humans are food of plant origin naturally contaminated with these toxins and food of animal origin contaminated with mycotoxin metabolites (1, 2).

Unfortunately, little is known about the distribution of the *Fusarium* genus and their mycotoxins in the environment. Recent efforts in the European Union to address this issue have resulted in the inclusion of surface waters in mycotoxin monitoring (3). So far, reports have only included data on the prevalence of zearalenone (ZEA) and deoxynivalenol (DON) in the aquatic environment.

According to Hartmann et al. (3), the presence of mycotoxins in the aquatic environment is the result of runoff from agricultural fields. However, Criado et al. (4) have shown that the fungi *Alternaria, Penicillium,* and *Cladosporium* can grow and synthesise mycotoxins in bottled mineral water, which is a serious threat to consumer health. Russell and Paterson (5), in turn, confirmed the ability of *Fusarium graminearum* to produce ZEA in drinking water.

There are a number of controversies about the entry routes of mycotoxins into the aquatic environment, and therefore it is necessary to conduct additional studies, which will clearly establish how mycotoxins migrate in the environment.

Studies on the prevalence of mycotoxins in the aquatic environment are mainly focused on ZEA due to its strong oestrogenic activity. Several recent publications reported the occurrence of ZEA in surface waters. Its concentrations ranged from below the detection limit to 65.2 ng L^{-1} (6-8).

Information about mycotoxins in wastewater and their removal efficiency is still scarce. Lagana et al. (9, 10) reported that ZEA concentrations in untreated and treated wastewater reached 18.0 and 10.0 ng L^{-1}, respectively, while Spengler et al. (11) reported as high 36.0 ng L^{-1} of ZEA in a wastewater treatment plant.

Even though the reported ZEA concentrations in water are not high, their accumulation in water used for food production may present a health risk for humans and animals (5, 7, 8). This risk increases with the presence of other endocrine disruptors such as natural oestrogenic steroids in water (6).

The aim of our study was to investigate the efficiency of ZEA removal during regular wastewater treatment and

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the correlation between ZEA levels in treated wastewater and the nearby river into which the treated wastewater is released.

MATERIALS AND METHODS

Reagents

Zearalenone (ZEA), acetonitrile, and methanol (HPLC grade) were purchased from Sigma-Aldrich (Steinheim, Germany). ZearalaTest WB immunoaffinity columns were purchased from Vicam (Milford, MA, USA). Water (HPLC grade) was obtained using the Millipore water purification system (Millipore, Bedford, MA, USA).

Sample collection

River water and wastewater samples were collected once a month between March 2010 and December 2012. Wastewater samples were taken from the wastewater treatment plant Łęczyca primary sedimentation basin, receiving 500,000 m^3 of wastewater per year from the Komorniki community, and from the treated effluents, which are directly released into the Warta tributary Wirynka. River water samples were collected from the Warta downstream of the treatment plant. All samples were taken in triplicate. The Łęczyca wastewater treatment plant uses standard Polish treatment infrastructure with mechanical-biological treatment, precipitation, nitrification, and denitrification.

Zearalenone analysis

All water samples were stored in the dark at 4 °C until analysis. ZEA was extracted within 24-36 h in order to keep microbiological degradation to a minimum and to avoid the addition of chemical preservatives. Water samples were filtered and analysed according to the method described by Gromadzka et al. (7, 8). In short, 1000 mL of water was filtered through four filters in a sequence and then purified using ZearalaTest affinity columns. The obtained ZEA was eluted with 3 mL of methanol, the mixture collected in a vial, and the content evaporated to dryness. Followed high-performance liquid chromatography (HPLC), in which the vial residue was dissolved in 250 µL of a water:methanol:acetonitrile mixture in the ratio of 70:20:10, respectively. Then, 20 µL of the solution was injected in a Waters (Waters Corporation, Milford, MA, USA) reversed-phase C18 column (150x3.9 mm, 4 mm particle size).

For analysis we used a Waters 2695 HPLC with a Waters 2475 fluorescence detector and a Waters 2996 photodiode detector. Millennium software was used for data processing and calculation (Edison, NJ, USA). The wavelengths of excitation and emission were 274 and 440 nm, respectively. The limit of detection (LOD) for ZEA was 0.3 ng L^{-1}, which corresponded to the concentration that gave a signal-to-noise ratio of 3:1. Method linearity, recovery, and precision have been described earlier (7).

Analysis of dissolved organic carbon

Dissolved organic carbon (DOC) was analysed with a TOC 1030 total organic carbon analyser (IO Analytical, College Station, TX, USA) using the persulphate wet oxidation method at 100 °C. The amount of carbon dioxide was measured with an IR detector and relayed to a computer. The method's detection limit was 0.1 mg L^{-1}.

Statistics

We used three-way analysis of variance (ANOVA) to determine the effects of years, months, and kind of wastewater (treated or untreated) as well as of the interactions between years×months, years×kind of wastewater, months×kind of wastewater, and years×months ×kind of wastewater on the concentrations of ZEA and DOC. Variability of ZEA and DOC concentrations was determined with coefficients of variation (CV) (12). The relationships between the concentrations of ZEA and DOC in untreated wastewater, treated wastewater, and river water were estimated using Pearson correlation.

RESULTS AND DISCUSSION

The main objective of this study was to determine ZEA levels in the untreated and treated wastewater and to evaluate ZEA removal efficiency.

The results of the three-way ANOVA show significant ($P<0.001$) effect of all three parameters and their interactions on ZEA and DOC levels (Table 1).

Table 2 shows ZEA and DOC levels in untreated and treated wastewater from 2010 to 2012. ZEA levels in

Table 1 *Mean squares from three-way analysis of variance (ANOVA) for the observed factors*

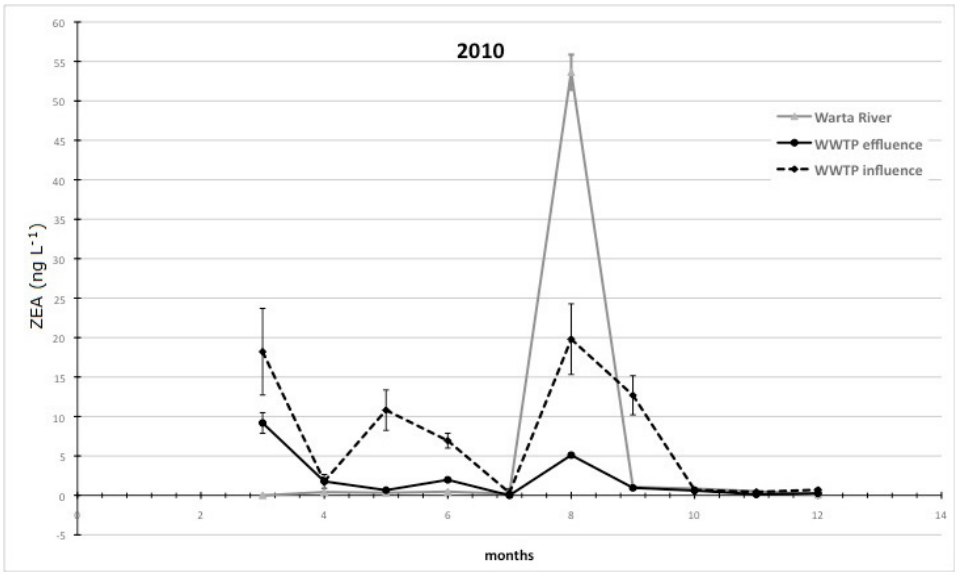
Source of variation	DF	ZEA content	DOC content
Year	2	419.8753*	1372.47*
Month	9	188.9871*	1540.01*
Kind of wastewater	2	172.1069*	31594.72*
Year×Month	18	196.1838*	1786.82*
Year×Kind of wastewater	4	58.1694*	2844.39*
Month×Kind of wastewater	18	87.643*	1133.71*
Year×Month×Kind of wastewater	36	79.4798*	1324.09*
Residual	180	0.8156	23.11

DF-degrees of freedom
*P<0.001
ZEA-zearalenone; DOC-dissolved organic carbon

Table 2 *Zearalenone and dissolved organic carbon content in untreated and treated wastewater*

Month (2010)	Untreated wastewater		Treated wastewater		Removal efficiency (%)	
	ZEA (ng L^{-1})	DOC (mg L^{-1})	ZEA (ng L^{-1})	DOC (mg L^{-1})	ZEA	DOC
March	18.21	29.87	9.18	14.18	49.59	52.53
April	1.77	90.51	1.77	90.51	0.00	0.00
May	10.80	26.20	0.66	10.66	93.89	59.33
June	6.93	22.09	1.97	16.00	71.59	27.57
July	0.33	62.85	nd	14.58	100.00	76.80
August	19.80	18.83	5.10	10.61	74.24	43.65
September	12.69	20.06	0.96	13.07	92.43	34.85
October	0.69	26.01	0.60	11.90	13.04	54.25
November	0.43	24.53	0.13	10.65	69.77	56.58
December	0.71	28.32	0.27	9.87	62.64	65.15
CV	100.61	63.04	136.86	116.40		
		Mean value			**62.72**	**47.07**
Month (2011)	**Untreated wastewater**		**Treated wastewater**		**Removal efficiency (%)**	
	ZEA (ng L^{-1})	**DOC (mg L^{-1})**	**ZEA (ng L^{-1})**	**DOC (mg L^{-1})**	**ZEA**	**DOC**
March	2.85	32.48	2.25	14.86	21.05	54.25
April	1.11	36.47	0.25	13.40	77.39	63.25
May	2.03	85.13	0.68	14.69	66.36	82.74
June	2.02	48.93	nd	13.76	100.00	71.88
July	1.02	27.42	0.15	10.55	85.29	61.53
August	0.43	36.20	0.32	11.93	25.44	67.03
September	6.65	58.82	1.50	11.43	77.46	80.57
October	0.51	26.72	nd	12.84	100.00	51.95
November	0.89	28.35	0.318	12.07	64.47	57.43
December	0.59	23.65	0.121	11.06	79.49	53.23
CV	98.21	44.88	128.98	11.22		
		Mean value			**69.70**	**64.39**
Month (2012)	**Untreated wastewater**		**Treated wastewater**		**Removal efficiency (%)**	
	ZEA (ng L^{-1})	**DOC (mg L^{-1})**	**ZEA (ng L^{-1})**	**DOC (mg L^{-1})**	**ZEA**	**DOC**
March	3.37	43.75	2.61	14.87	22.34	66.01
April	2.29	37.50	1.29	14.23	43.85	62.06
May	2.37	59.78	1.26	14.90	46.84	75.07
June	2.58	205.37	0.36	12.28	86.05	94.02
July	3.42	39.41	2.76	9.81	19.30	75.11
August	2.91	42.83	1.20	12.59	58.76	70.61
September	2.01	37.27	1.02	16.57	49.35	55.54
October	7.44	70.71	1.80	18.45	75.81	73.91
November	2.22	41.02	0.84	17.89	62.16	56.37
December	3.10	38.09	1.58	18.12	49.00	52.43
CV	47.20	79.68	48.39	18.08		
		Mean value			**51.35**	**68.11**

CV-coefficients of variation; ZEA-zearalenone; DOC-dissolved organic carbon



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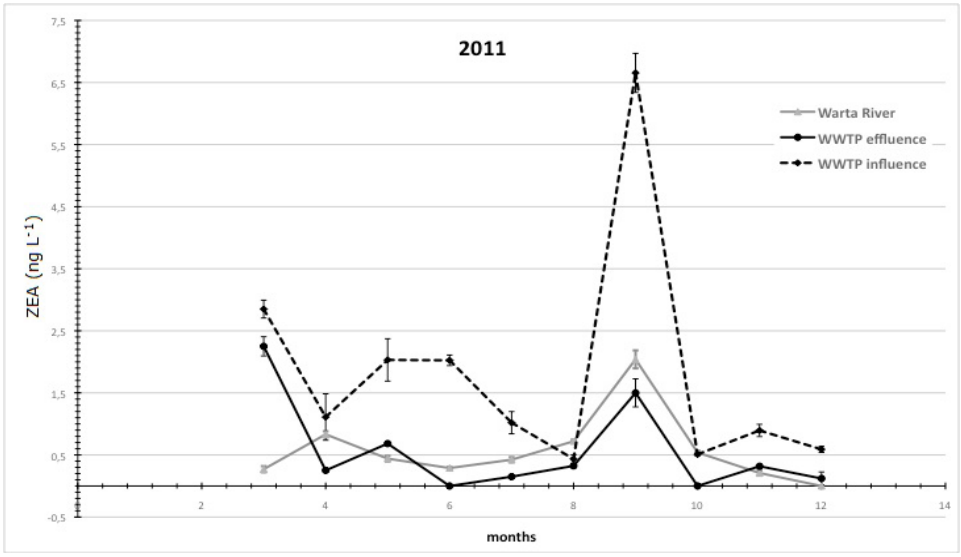

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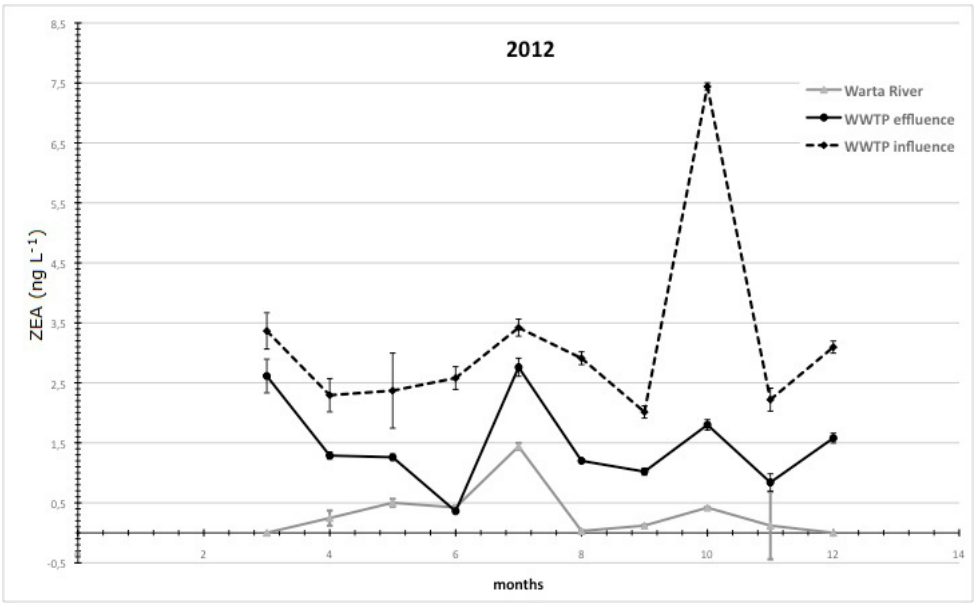

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Figure 1 *Zearalenone levels in treated and untreated wastewater and Warta River water in: a) 2010, b) 2011, c) 2012*

untreated wastewater peaked in 2010 and dropped in the subsequent years, which may reflect *Fusarium* contamination of the nearby fields. This contamination may be associated with the prevailing weather conditions of the year before. Earlier studies (7, 8, 13, 14) have shown that leaching from the fields to the aquatic ecosystem is not instantaneous. The growing season of 2009 was characterised by substantial rainfall from early June to mid-August and by high prevalence of *Fusarium* head blight (FHB). Precipitation in 2010 was lower and FHB was not observed. In 2011, the temperature was high and there was substantial rainfall in the second half of June, when spring wheat flowers, but relative humidity was lower than in 2009, and FSB was much less prevalent than in 2009 (15).

However, our findings also point to a curious behaviour of ZEA. In 2010, its levels were the highest in the Warta, while in 2011 and 2012 they were the highest in the untreated wastewater (Figure 1). This suggests that the circulation of mycotoxins in the environment is a complex process, and that it is very important to determine the contribution of individual components if we want to reduce contamination of the aquatic ecosystem. What Figure 1 also shows is a strong correlation between ZEA levels in untreated wastewater, treated wastewater, and river water ($r=0.7526$ for untreated *vs.* treated wastewater; $r=0.5729$ for untreated *vs.* river water; and $r=0.3768$ for treated *vs.* river water; $P<0.001$ for all correlations).

Wastewater treatment in our study proved relatively efficient (Table 2) compared to reports from Italy (9, 10) and Germany (11), although not as efficient as in Switzerland (13). These studies have shown that the efficiency of the toxin's removal does not depend on the initial ZEA concentration in the wastewater. An interesting alternative would be a combination of traditional wastewater treatment and an integrated photocatalysis-microfiltration-nanofiltration process. Dudziak (16) reported an over 90 % removal efficiency with this process.

Although ZEA was present in the treated water discharged into the river, its levels were a negligible source of surface water contamination (Figure 1). Even so, long-term exposure even to small doses of ZEA may affect animal and human health and its levels in aqueous environment should be monitored.

CONCLUSION

Our monitoring study has shown a seasonal pattern in ZEA levels in wastewater and river water, reaching its peak in the autumn. This is probably related to ZEA leaching from crops into the wastewater treatment basin as well as the river. This also explains a strong correlation between ZEA levels in all three waters.

Our findings suggest that ZEA levels in wastewater are not the best indicator of the toxin's presence in the environment, as wastewater is not the main source of surface water pollution. Future research should investigate the means to reduce ZEA levels and their migration from the fields through prevention strategies such as breeding for crops, plant debris management (crop rotation, tillage), and/or chemical and biological control.

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Uloga pročišćavanja otpadnih voda u smanjenju onečišćenja površinskih voda zearalenonom

Zearalenon (ZEA) je mikotoksin koji u hrani proizvode neke vrste gljivica roda *Fusarium*. Njegova toksičnost i toksičnost njegovih metabolita ovisi o kemijskoj strukturi mikotoksina, a djelovanje mu je slično onome prirodnoga estrogena. Sve smo svjesniji važnosti gljivica i njihovih toksičnih metabolita u vodenom okolišu. Jedan od izvora spoja u površinskim vodama jesu i otpadne vode. Naše je trogodišnje praćenje pokazalo da se uspješnost pročišćenja zearalenona iz otpadnih voda kreće u rasponu od 51,35 do 69,70 % na godišnjoj razini. Trostrana analiza varijance (godina, mjesec, vrsta otpadne vode - nepročišćena/pročišćena) upućuje na to da je djelovanje svih čimbenika i svih njihovih međusobnih interakcija značajno utjecalo na razine zearalenona i otopljenog organskog ugljika. Istraživanje je pokazalo da otpadne vode nisu glavni izvor onečišćenja površinskih voda zearalenonom. Buduća bi istraživanja trebala utvrditi preventivne strategije uzgoja, upravljanja ostacima biljke (rotacijom, obradom zemljišta), odnosno tretiranje kemijskim i biološkim sredstvima kojima bi se smanjile razine zearalenona i njegova migracija s polja u vodeni okoliš.

KLJUČNE RIJEČI: *HPLC; kakvoća vode; mikotoksini; otopljeni organski ugljik; vodeni okoliš*