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DETERMINATION OF AZOLE COMPOUNDS IN ENVIRONMENTAL SAMPLES: THE INFLUENT AND EFFLUENT OF WASTEWATER TREATMENT PLANTS

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Introduction

Azoles represent one of the most widely used and effective classes of fungicides worldwide. They present multiple applications, including in human and veterinary medicine, agriculture, and in the cosmetic industry. The broad-spectrum antifungal activity and chemical stability of azole have made them indispensable in combating fungal infections in humans, animals, and crops. Considering the complex issue of the presence and potential toxicity of azole antifungals in the environment, within the project we proposed a sensitive and precise chromatographic method for the detection of azole compounds in wastewater. Up to the present, there has been a lack of research conducted in Romania investigating the presence and levels of azole compounds in wastewater treatment plants (WWTPs), including influent and effluent. Understanding the concentrations of these compounds in the Romanian environment is crucial due to their potential adverse effects on the aquatic organisms. Through this paper, we present the first multi-site screening of azoles in Romanian municipal WWTP influents and effluents. Thus, the method was used to assess the concentrations of these compounds in treatment plants.

Materials and methods

Twenty-four influent and twenty-four effluent 24-h composite samples were analyzed, corresponding to three consecutive sampling days at each of the eight WWTPs located in Bucharest (B), Iasi (IS), Galati (GL), Buzau (BZ), Ramnicu-Valcea (VL), Brasov (BV), Targoviste (TG), and Braila (BR), Romania. These samples were collected in 1 L bottles and transported to the laboratory under refrigerated conditions. The samples were sent daily and extracted within 24 h of sampling. Upon arrival, they were filtered using GF filters (0.45 µm) and extracted using solid phase extraction. The pretreatment of water samples containing analytes involved using the solid phase extraction (SPE) procedure. The recovery of target analytes from the water samples was conducted using an automatic solid phase extraction system called SPE AutoTrace 280 (Thermo-Scientific-Dionex). A Strata X cartridge with a styrene-divinylbenzene polymeric adsorbent phase (0.5 g / 6 mL, Phenomenex) was employed for the extraction. The analytical determinations were performed using an Agilent Technologies UHPLC-MS/MS system (model 1260-6410B) from the Waldbronn, Germany location. In positive ion mode, the liquid

chromatograph was connected to a triple quadrupole mass spectrometer using an electro-spray ionization (ESI) source. Separation of the pollutants was achieved using a Zorbax Eclipse XDB C18 column (100 × 2. mm, 3.4 µ m) maintained at a temperature of 30°C.

Results and conclusions

Maximum detection frequencies (Equation 1) of 100 were observed for fluconazole (FLU), climbazole (CLI), tebuconazole (TEB), prochloraz (PRO), clotrimazole (CLO).

Detection frequency (DF) (%)=$N_{detected}/N_{analysed}$*100; Equation 1, where N is the sample number

High concentration values were observed for TEB (92–424 ng/L, mean 191 ng/L), CLI (207–391 ng/L mean 289 ng/L), CLO (6.9–93-ng/ L, mean 28.2 ng /L), followed by FLU (49.3–76.8 ng/L, mean 67.3 ng/L), and PRO (7.3–72 ng/L, mean 25.5 ng/L) in the influent samples (Table 1). Three compounds were detected only in one station (Galati) as follows: epoxiconazole (EPO) in Galati influent 17.8 ng/L, tetraconazole (TET) in influent 16.5 ng/L and penconazole (PEN) 15.8 ng/L in influent and 3.2 ng/L in effluent. Imazalil (IMA) was detected in 37.5 % of samples (Bucharest, Iasi and Galati stations), with a minimum concentration of 16.5 ng/L and a maximum of 41 ng/L, average 25.8 ng/ L. The descending order of azole compounds according to the maximum concentration determined in the influent samples was: TEB >CLI >CLO>FLU (fluconazole)>PRO>IMA>EPO >TET >PEN (penconazole). The maximum levels of concentrations in the influent were observed in the case of Bucharest stations (TEB 424 ng/L), followed by Braila (CLI 391 ng/L) and Galati (TEB 382 ng/L).

Table 1 - The concentrations (ng/L) of azole compounds determined in the influent and effluent samples

Compound	Influent, ng/L				Effluent, ng/L			
	Cmin	Cmax	Caverage	Detection frequency (%)	Cmin	Cmax	Caverage	Detection frequency (%)
FLU	49.3	76.8	67.3	100	8.3	17.6	13.4	100
CLI	207	391	288.6	100	123	247	185	100
EPO	17.8	n.d.	n.d.	12.5	n.d.	n.d.	n.d.	12.5
TET	6.8	16.5	10.3	25	n.d.	n.d.	n.d.	25
TEB	92	424	191	100	39	116	76.7	100
IMA	16.5	41	25.8	37.5	2	6.5	4	37.5
PEN	15.8	n.d.	n.d.	25	3.2	n.d.	n.d.	25
PRO	7.3	72	25.5	100	4.3	31	11.8	100
CLO	6.9	93	28.2	100	1.8	22	7.9	100

CLI, TEB, CLO were the most commonly occurring compounds in both influent and effluent water samples, with FLU and PRO having intermediate concentrations in the tens of ng/L range.

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