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DETECTION OF BETA-LACTAM RESIDUES IN ENVIRONMENTAL AND DRINKING WATER BY IMMUNOENZYMATIC ASSAY

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Abstract

In recent decades, concern over emerging organic contaminants in the environment has grown considerably because of their potentially harmful effects on organisms and ecosystems. These synthetic compounds are widely used in modern life-style and due to improvements in analytical technologies, we are now able to identify and quantify them even in small concentrations. One of the most important pharmaceutical contaminants is antibiotics, of which more than half belong to the class of beta-lactams. This research aimed to determine the beta-lactam antibiotics residues in surface water (rivers) and groundwater, which serve as public or private sources of drinking water, as well as in urban wastewater. The samples were collected from different places throughout the Western part of Romania and analyzed using commercially available ELISA kits for the detection of beta-lactams in liquid samples. The results show that beta-lactam antibiotics are ubiquitous in all categories of water and establish the ELISA method as an acceptable screening tool for antibiotic residues.

Key words: antibiotic residues, drinking water, ELISA

For a long time, the impact of chemical pollution on the environment, human and animal health, has been based on monitoring priority pollutants and in particular those classified as persistent, bioaccumulative and toxic, known as "persistent organic pollutants" (POPs). The inclusion of these compounds in the list of priority hazardous substances was made not only as a result of risk identification and analysis but mainly because they could be identified in the environment at very low concentrations, by the analytical techniques available at the time (Daughton C.G. *et al*, 2001).

The improvement of analytical techniques in recent years has made it possible to detect even small quantities of natural or synthetic substances in the environment, which are usually not monitored and pose unknown risks to human health and ecosystems. These substances are termed "emerging organic contaminants" (EOCs) (Richardson S.D. *et al*, 2011; Stuart M. *et al*, 2012). EOCs are not necessarily newly discovered substances, but rather compounds that have long been reaching the environment (water, air, soil) via various pathways, but whose consequences are only now becoming known. The class of emerging pollutants includes compounds from industry, disinfectants, detergents, pharmaceuticals, and personal care products (PPCPs), as well as lifestyle substances such as caffeine and nicotine (life-style compounds). The EOCs list remains open and as analytical detection methods are refined, new substances will be included (Meffe R. *et al*, 2014).

The PPCPs category includes also antibiotics, pharmacological agents widely used in both human and veterinary medicine. After administration, they are excreted 5% to 90% unmetabolized or as active or inactive metabolites, but may subsequently be converted back to their original compounds (Dinh Q.T. *et al*, 2011; Mirzaei R. *et al*, 2018; Sarmah A.K. *et al*, 2006; Yan Q. *et al*, 2014). Thus, a significant amount of antibiotic residues ends up in urban wastewater, through sewage treatment plant effluents, in biofertilizers used in agriculture and from here, in surface and groundwater (Lapworth D.J. *et al*, 2012).

Wastewater treatment removes biodegradable organic compounds, nitrogen, phosphorus and in certain amounts pathogens, but the technology is not designed to retain the micro-organic pollutants. Thus, antibiotic residues cannot be removed efficiently and in some cases, they have been identified in higher concentration in the

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effluent than in the influent (Yan Q. *et al*, 2014; Gao L. *et al*, 2012). The increased interest of researchers in the problem of antibiotic residues in the environment has led to the identification of these compounds in all water categories: surface water (Dinh Q.T. *et al*, 2011; Feitosa-Felizzola J. *et al*, 2009), groundwater (Barnes K.K. *et al*, 2008; Wolf L. *et al*, 2012) and drinking water (Charuaud L. *et al*, 2019; Focazio M.J. *et al*, 2008; Padhye L.P. *et al*, 2014). Antibiotics are considered "pseudo-persistent" pollutants, as their persistence in the environment is due to their continuous intrusion (Huang C.H. *et al*, 2001).

The beta-lactam class includes penicillins and their derivatives, cephalosporins, carbapenems, monobactams and beta-lactamase inhibitors (Ungureanu V.A., 2018). Although in recent years, there have been attempts to identify residues of beta-lactam antibiotics in aquatic environments: wastewater, surface waters (Bruno F. *et al*, 2001; Cha J.M. *et al*, 2006; Christian T. *et al*, 2003) and groundwater (Sacher F. *et al*, 2001), many of the targeted compounds could not be detected or were found in much lower concentrations than antibiotics belonging to other antimicrobial classes. This is explained by the instability of the beta-lactam ring, which can be easily disintegrated by bacterial beta-lactamases and by chemical reactions occurring in aquatic environments (hydrolysis, photolysis) (Christian T. *et al*, 2003). Some of the degradation compounds resulting from such reactions are more stable and thus easier to detect, which is why monitoring programs may consider determining them instead of identifying the parent structures (Sy N.V. *et al*, 2017). On the other hand, as in the case of cephalosporins, the products resulting from their photodegradation in aquatic environments are more stable but also more toxic compared to the parent compounds (Wang X-H *et al*, 2012).

The danger of antibiotic residues in drinking water is primarily due to the extent of antibiotic resistance that has developed in recent years. According to the World Health Organisation, this phenomenon is one of the greatest threats to public health (WHO, 2018). In the environment (soil, water), antibiotic residues are detected in concentrations of nanograms or micrograms per volume (liter) or mass (kilogram) (Hon N.T.N. *et al*, 2016). Although such concentrations do not inhibit bacterial multiplication, some studies claim that they can cause the selection of resistant microorganisms, in particular through mechanisms involving mutations in bacterial DNA, thus contributing to the transformation of aquatic environments into reservoirs of resistant germs (Kim S. *et al*, 2012).

On the other hand, in the case of beta-lactam antibiotics, there is a risk of allergic phenomena in sensitive individuals (Cha J.M. *et al*, 2006). No less important is the impact that these substances have on the diversity and functions of microorganisms in aquatic ecosystems, most frequently leading to the selection of resistant populations and the extinction of less tolerant ones (Rosi E.J. *et al*, 2018).

For the quantification of antibiotic traces in the environment (water, soil), animal waste, or animal products, the most commonly used analytical method is the high-performance liquid chromatography (HPLC) coupled with tandem mass spectrometry (MS/MS) (LC/MS/MS). Although the advantages of this technique in detecting extremely low concentrations of residues have consecrated it as the gold-standard method, its high costs and the impossibility of processing a large number of samples simultaneously make it less useful in the screening process (Aga D.S. *et al*, 2016; Zhang Z. *et al*, 2013).

The immunoenzymatic technique (ELISA), on the other hand, has demonstrated its usefulness both as a screening tool and in the quantitative assessment of residues. Even though assay kits are generally designed for the determination of a single substance, the antibodies used show specificity against the whole class of antibiotics, cross-reacting with all structurally similar compounds. While even small changes in the structure of the analyte make it impossible to identify it by LC/MS/MS, the cross-reactivity of ELISA is an advantage in screening processes, as it also allows the detection of metabolites of the parent substance. Thus, cross-reactivity also becomes an advantage when measuring the bioavailability of the degradation products of a particular antibiotic, especially if they also exhibit biological activity (Aga D.S. *et al*, 2016).

A number of studies have relied on the enzyme-linked immunosorbent assay method in the detection of antibiotic traces in soil and animal excreta (Aga D.S. *et al*, 2003), groundwater (Barber L.B. *et al*, 2009; Bradley P.M. *et al*, 2014), surface water (Kumar K. *et al*, 2004) and wastewater (Černoch I. *et al*, 2012). Others have aimed to identify products resulting from the degradation of antibiotics in aquatic environments and concluded that ELISA is superior to HPLC for such determinations (Hu D. *et al*, 2008). Even if ELISA cannot fully replace the gold-standard quantitative analytical methods, there are strong arguments for its use as a screening tool due to: low cost, short working time, the possibility to test a large number of samples simultaneously, and above all, due to cross-reactivity, which also

allows the detection of metabolites that have entered the environment (Aga D.S. *et al*, 2016).

The aim of the study was to determine the residues of beta-lactam antibiotics in surface water (rivers) and groundwater, the last serving as public or private drinking water sources, or being used for watering animals or irrigating vegetable crops, as well as in urban wastewater. The samples were collected from different places throughout the Western part of Romania and analyzed using commercially available ELISA kits for the detection of beta-lactams in liquid samples.

MATERIAL AND METHOD

A total of 42 water samples from various sources were collected, processed, and analyzed in two stages. The first stage of sampling (S I) took place during a period of dry weather conditions. The second stage (S II) took place during a period in which surface and groundwater flow was increased due to abundant rainfall.

Surface water samples were collected from a drainage channel that flows into the Bega Veche River in Timiș county and from the Bistra River in Caraș-Severin county, Timiș River in Caraș-Severin and Timiș counties, Bega river in Timiș county and Mureș river in Arad county.

Wastewater samples were taken from the influents and effluents of four water treatment plants located in Timiș county, more precisely Timișoara, Jimbolia, Dudeștii Noi, and Lugoj (effluent only). The effluents of all four plants are discharged into natural water bodies.

Drinking water intended for human consumption was sampled from groundwater sources. The samples were taken from artesian wells in Chizătău, Dudeștii Noi, Ohaba-Forgaci, and Sinersig, from citizens' household wells in Ciuta and Țela, from the public water distribution network (groundwater) in Dudeștii Noi, Jimbolia, Lugoj, and Ohaba-Forgaci, as well as from public wells and the public distribution network (surface water) in Lugoj and Timișoara.

Further samples were taken from two shallow drilled wells (maximum 10 meters) for the watering animals and gardens, and from a source of natural mineral water intended for human consumption at the Buziaș spa resort in Timiș county. The waters of Buziaș are characterized by a high content of minerals, especially iron, as well as a high concentration of carbon dioxide.

The water samples were collected in clean glass containers with a capacity of 750 ml. River water was collected from bridges with the help of a bathometer. The samples were kept overnight at 4°C and analyzed in the following morning.

Max-Signal® Beta-Lactam ELISA kits (PerkinElmer Inc.), which are based on a competitive enzyme immunoassay for the quantitative analysis of beta-lactams were used for the analysis of the water samples. They allow the detection of beta-lactams such as ampicillin, penicillin G, amoxicillin, cloxacillin, and ceftiofur in milk, meat, egg, urine, serum, plasma, and a variety of other samples.

Each well on the plate contained in the ELISA kits is lined with the pharmaceutical substance of interest, in this case, beta-lactam antibiotics (reference antigens). The test samples were distributed into the wells and a protein with a high affinity for beta-lactams (primary antibodies, not enzyme-conjugated) was added. If there are beta-lactam residues in the sample, they will bind to the protein, not allowing it to bind to the reference antigens in the wells. Once all of the residues have been attached to the proteins, the excess protein binds to the reference antigens. A solution with enzyme-conjugated detection antibodies (secondary antibodies, peroxidase) is then added. These antibodies attach to the protein that is bound by the control antigen in the wells. 3,3',5,5'-tetramethylbenzidine (TMB) is added in the last step to obtain the color reaction that can be read in a spectrophotometer as absorbance values. The intensity of the color reaction is inversely proportional to the concentration of beta-lactam residue in the sample.

The limit of detection for milk is 0.4 ng/ml and the limit of quantification is 0.8 ng/ml. They were obtained by multiplying the limits of detection and quantification of the test, 0.04 ng/ml and 0.08 ng/ml, respectively, with the dilution factor for milk, which is at least 10.

The water samples were processed according to the test kit manufacturer's instructions, but they were not diluted. Without a dilution factor, we defined the limit of quantification for water as 0.08 ng/ml. The device's software also provided results that were lower than 0.08 ng/ml, as the limit of detection is 0.04 ng/ml, however, these findings lie outside of the measurement range and were therefore considered insignificant.

The optical density was analyzed spectrophotometrically at 450 nm. The concentration of beta-lactams in the sample was determined by comparing the color intensity with the standard curve (log-logit), which was established using the concentration (x-axis)

relative to the absorbance (y-axis) of each standard provided by the ELISA kit: negative control standard 0 ng/ml, standard 0.08 ng/ml, standard 0.2 ng/ml, standard 0.4 ng/ml, standard 0.8 ng/ml, and standard 1.2 ng/ml. Since the determinations were made in two stages, a standard curve was established for each stage (figure 1).

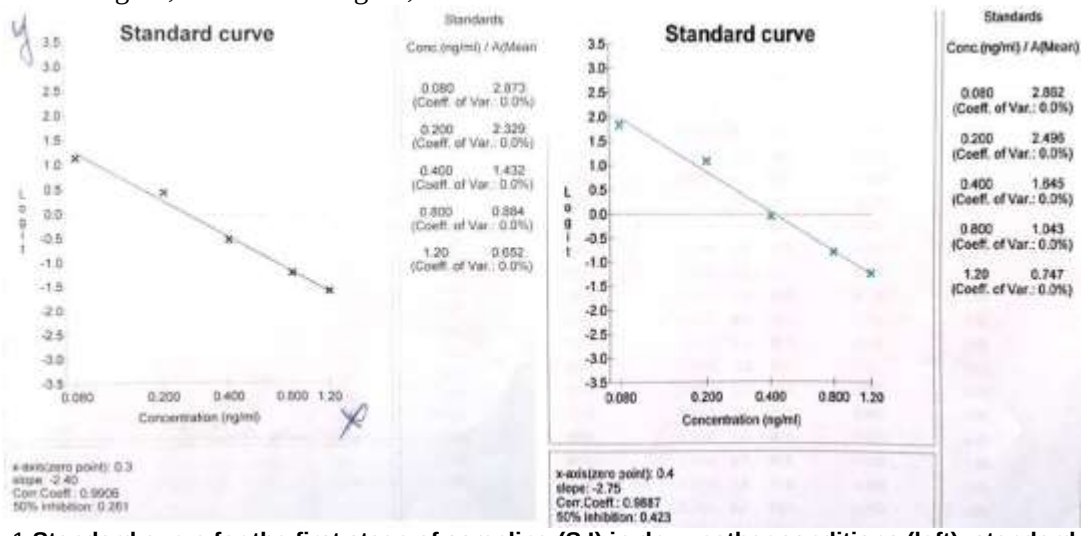

Figure 1 **Standard curve for the first stage of sampling (S I) in dry weather conditions (left); standard curve for the second stage of sampling (S II) in rainy weather conditions (right).**

The absorbance was expressed in percentage (B/B0) and calculated according to the formula:

Relative absorbance % = (standard absorbance of the sample / standard absorbance of the negative control)×100

Characteristics of the ELISA test kit that were taken into consideration were the high recovery rate of over 80% and the intra-assay and inter-assay coefficient of variation (normally below 8%). According to the data provided by the manufacturer, the test specificity is 100% for penicillin G, >100% for ampicillin, cefoperazone, ceftiofur, cloxacillin, cloxacillin, nafcillin, and oxacillin, and 44% for amoxicillin.

The removal efficiency of wastewater treatment for beta-lactam antibiotic residues was calculated according to the formula:

Removal efficiency % = [(influent value - effluent value) / influent value]×100 (Bradley P.M. et al, 2014).

RESULTS AND DISCUSSIONS

Out of the total samples that were analyzed (42), 59.52% (25) had values above the minimum limit of quantification (0.08 ng/ml). None of the samples were below the limit of detection (0.04 ng/ml). The remaining samples (17) had values between 0.055 and 0.079 ng/ml. The highest number of quantifiable samples was obtained for wastewater, followed by surface water. The lowest number was found for groundwater (*table 1*), as expected. Vulliet *et al.* (2011) analyzed surface and groundwater intended for human consumption for residues of pharmaceutical and hormonal substances and found that the detection frequency and concentrations of the target compounds are higher in surface waters. The only substance that was more frequently and in higher concentrations detected in groundwater was sulfamethoxazole (Vulliet E. *et al*, 2011).

Studies pertaining to the detection of beta-lactam antibiotic residues in the environment using enzyme-linked immunosorbent assays are few. Most of the research utilizing the ELISA method for surface water (Kumar K. *et al*, 2004), wastewater (Černoch I. *et al*, 2012), and groundwater (Barber L.B. *et al*, 2009; Bradley P.M. *et al*, 2014) analysis was done to identify substances from the class of sulfonamides (Barber L.B. *et al*, 2009; Bradley P.M. *et al*, 2014; Černoch I. *et al*, 2012). Sulfonamides are the most commonly found compounds in aquatic environments. They are found in concentrations ranging from 10 μg/L to 1 mg/L and unlike beta-lactams, which are unstable and rapidly degraded by hydrolysis or photolysis, sulfonamides are much more stable or even non-degradable (Cha J. *et al*, 2018). Recently, Ecke and Schneider (2021) presented the first example of utilizing hydrolysis of beta-lactam antibiotics for the improved immunochemical determination of these substances and their hydrolysis products and provided a method for the quick evaluation of the contamination of drinking water with pharmaceutical compounds and therefore the risk

of potential microbiological resistance development (Ecke A. *et al*, 2021).

The percentage of quantifiable samples of surface water was 69.23% (9). The values were between 0.087 ng/ml for water collected from the Bega River in the center of Timișoara in the first stage of sampling (S I) and 0.115 ng/ml for the Bistra River in the second stage of sampling (S II), which was characterized by abundant rainfall and increased river flow. Four of the 13 surface water samples had values close to the limit of quantification, between 0.072 and 0.079 ng/ml (*table 1*). Using the HPLC technique, Christian et al. (2003) found traces of amoxicillin, ampicillin, flucloxacillin, mezlocillin, and piperacillin in surface waters in concentrations of no more than 0.048 ng/ml. On average, beta-lactams were found in concentrations of less than 0.01 ng/ml (Christian T. *et al*, 2003). Using the same analytical method, Cha et al. (2006) found values of beta-lactam residues in surface water between 0.009 and 0.011 ng/ml (Cha J.M. *et al*, 2006).

Table 1

Levels of β-lactams in aqueous environmental samples

Water category and location				Concentration ng/ml		Samples with quantifiable values
				S I	S II	
Surface water (13)	Rivers	Bistra	Ciuta		0.115	**9 (69.23%)**
		Timiș	Downstream Caransebeș		0.105	
			Upstream Lugoj		0.102	
			Upstrea Lugoj drinking water treatment plant II	0.079*		
			Influent of the Lugoj drinking water treatment plant II		0.089	
		Mureș	Săvârșin		0.075*	
		Bega	Lipova		0.098	
			Făget		0.099	
			Bucovăț		0.097	
			Influent of the Timisoara drinking water treatment plant	0.093		
			Center of Timișoara	0.087		
	Drainage channel	Upstream Dudeștii Noi wastewater treatment plant		0.076*		
		Downstream Dudeștii Noi wastewater treatment plant		0.072*		
Wastewater (7)	Timișoara	Influent			0.528	**5 (71.42%)**
		Effluent			0.131	
	Jimbolia	Influent			0.471	
		Effluent			0.148	
	Dudeștii Noi	Influent		0.072*		
		Effluent		0.055*		
	Lugoj	Effluent		0.094		
Drinking water (22)	Public distribution network (surface water)	Lugoj (Timiș)		0.078*		**11 (50%)**
		Timișoara (Bega)			0.276	
	Public distribution network (groundwater)	Ohaba-Forgaci		0.082		
		Dudeștii Noi		0.075*		
		Lugoj water treatment plant I		0.069*		
		Lugoj water treatment plant III		0.074*		
		Jimbolia			0.153	
	Artesian wells/Springs	Ohaba-Forgaci		0.079*		
		Dudeștii Noi 1		0.067*		
		Dudeștii Noi 2		0.070*		
		Chizătău			0.079*	
		Sinersig			0.112	
		Buziaș			0.196	
	Public wells (groundwater)	Lugoj 1		0.078*		
		Lugoj 2		0.064*		
		Timișoara			0.085	
	Private wells (groundwater)	Ciuta 1			0.138	
		Ciuta 2			0.132	
		Țela 1			0.090	
		Țela 2			0.087	

Shallow drilled wells	Ohaba-Forgaci	0.082		
	Dudeștii Noi	0.075*		
Total no of samples		**20**	**22**	**42**
Unquantifiable values *		**15**	**2**	**17 (40.47%)**
Quantifiable values		**5**	**20**	**25 (59.52%)**

In a recent study that examined the presence of pesticides and veterinary medicines in 29 small waterways across 10 countries in the European Union, sulfonamide antimicrobials were identified in 48% of the samples, while the beta-lactams dicloxacillin and cloxacillin were found in 66% and 41% of the samples (Casado J. *et al*, 2019). These values reported by Casado et al. (2019) were detected by using the HPLC method and are similar to the percentage for beta-lactams in surface water identified by us, which validates the enzyme-linked immunosorbent assay as a much cheaper screening tool alternative compared to high-performance liquid chromatography (Aga D.S. *et al*, 2016; Zhang Z. *et al*, 2013).

Of the seven wastewater samples, 71.42% (5) were quantifiable. The samples that had values below the limit of quantification were those collected from the wastewater treatment plant serving the locality of Dudeștii Noi: 0.072 ng/ml in the influent and 0.055 ng/ml in the effluent. The data from *Table 1* suggests that the residue of beta-lactam antibiotics is reduced after the treatment process since the values of the effluent are lower than the values of the influent for all wastewater treatment plants (WWTPs) that were studied. The highest value in the influent was found for the wastewater treatment plant in Timișoara (0.528 ng/ml) and the highest value in the effluent was found for the wastewater treatment plant in Jimbolia (0.148 ng/ml). In the effluent of the wastewater treatment plant in Lugoj, 0.094 ng/ml of beta-lactam residues were found. For the wastewater treatment plant in Dudeștii Noi, the values for both influent and effluent were above the limit of detection, but below the limit of quantification. The removal efficiency of the WWTPs in this study was 75.18% for Timișoara, 68.57% for Jimbolia, and 23.61% for Dudeștii Noi. Using the HPLC method, Cha et al. (2006) found beta-lactam residues in the WWTP influents at values between 0.015 and 0.017 ng/ml. In the effluent samples, they were not detectable (Cha J.M. *et al*, 2006).

The two drinking water samples from public distribution networks (surface water) were collected in the cities of Lugoj and Timișoara. While the value for the sample from Lugoj was below the limit of quantification, the value for the sample from Timișoara was 0.276 ng/ml, which is the highest value we recorded excluding those of the WWTP influent samples (*table 1*).

Of the 20 drinking water samples from groundwater sources, 50% (10) contained quantifiable values of beta-lactam residue. The highest of these samples was found in the natural mineral water source at the Buziaș spa resort with a concentration of 0.196 ng/ml, followed by 0.153 ng/ml in water from the public distribution network in Jimbolia, which is treated with ozone. The rest of the quantifiable values were between 0.082 ng/ml from the shallow drilled well in Ohaba-Forgaci (Timiș county) and 0.138 ng/ml from one of the private wells in Ciuta (Caraș Severin county) (*table 1*). In one of the very few studies conducted in Romania on this topic, Szekeres et al. (2018) analyzed six groundwater sources with the HPLC technique and identified residues of cefepime and piperacillin with concentrations of 0.917 and 0.571 ng/ml, respectively, in two of the sources. Both of these sources were associated with animal farms in their vicinity. However, except for these two values, the overall distribution of beta-lactam antibiotic residues was low (Szekeres E. *et al*, 2018).

In Italy, Perret et al. (2006) identified traces of sulfonamides in sources of natural mineral water, from which it is collected, bottled, and marketed without any treatment (Perret D. *et al*, 2006). This is also the case with the natural mineral water at the Buziaș spa resort. The values obtained for the sample from this locality can be attributed to the presence of a recovery center, where significant quantities of pharmaceuticals are used.

For a more comprehensible presentation, the results from the shallow drilled wells in Ohaba-Forgaci (0.082 ng/ml) and Dudeștii Noi (0.075 ng/ml) were included in the category of drinking water, although these sources serve to water animals and for the irrigation of garden crops. Out of these two, only the water sample from Ohaba-Forgaci was quantifiable. Studies concluded that antibiotic residues from soil or water can be taken up by vegetables through a passive absorption mechanism and water transport. The highest concentrations of antibiotics in vegetable tissues were found in the leaves, then in the stems, and the lowest was found in the roots (Hu X. *et al*, 2010).

Balzer et al. (2016) recommended a threshold value of 0.1 ng/ml for antibiotic residues in groundwater. Exceeding this value would mean that the substance is in high concentrations and presents a risk factor due to persistence, bioaccumulation, or toxicity (Balzer F. *et al*, 2016).

In the case of Ohaba-Forgaci and Dudeștii Noi, it can be observed that the values found in water from the shallow drilled wells (maximum 10 meters) are identical to those obtained from the public distribution network of the same localities, which, according to the local authorities, were drilled deeper (minimum 100 meters). As there were no differences between the water from shallow-drilled wells (10 m) and deep-drilled wells (100 m), we can assume that this is due to human activity, more specifically the quality of the drilling.

The amount of residue decreases in the Timiș River from upstream to downstream during the period with increased water flow (S II). The values go from 0.105 ng/ml downstream Caransebeș to 0.089 ng/ml at the entrance of the Lugoj drinking water treatment plant. The same trend is observed in the first period of sampling (S I). Even when the residue values were below the limit of quantification, they decreased from 0.079 ng/ml upstream Lugoj water treatment plant to 0.078 ng/ml in tap water from this plant. In the Bistra River, higher residue concentrations (0.115 ng/ml) were detected than in the Timiș River. Water sampled from private wells in Ciuta, located to the right of the Bistra River, had higher concentrations than the river water, namely 0.132 and 0.138 ng/ml.

In the Bega River, the residue concentration decreased during the period with heavy rainfall (S II) from 0.099 ng/ml in the sampling point from Făget to 0.097 ng/ml in Bucovăț. Downstream, in the samples collected from the public drinking water network of Timișoara (potable water resulting after treatment of surface water from Bega River), we found very high concentrations (0.276 ng/ml), compared to all other drinking water sources. The beta-lactam residue concentrations from the water of the Bega River at the entrance to the water treatment plant (0.093 ng/ml) as well as at the collection point in the city center of Timișoara (0.087 ng/ml) were quantifiable even in the first stage of sampling, during normal weather (S I).

Although there are studies that claim that certain drinking water treatment technologies, such as ozonation, the use of chlorine dioxide, and UV irradiation, can reduce the load of pharmaceutical residues, very few of them are considered relevant, hence the results have to be interpreted with caution. Improvements in the experimental conditions are needed for the accuracy of the recreation of the phenomena that take place at each stage of treatment. Furthermore, the concentrations at which certain substances specific to the treatment method are used, such as oxidizing agents, must be taken into account for the recreation of different technologies. What is known with certainty is that the oxidation of some pharmaceutical substances can result in compounds that are more toxic than the original structures (Charuaud L. *et al*, 2019). The high concentration of beta-lactam residues found in the public distribution network in Timișoara can be ascribed to the lack of surveillance in the area upstream of the city's water treatment plant.

The river Mureș, in contrast, showed an increase in the residue concentration from upstream to downstream. The values change from 0.075 ng/ml (below the limit of quantification) in Săvârșin to 0.098 ng/ml in Lipova. Intermediate values of 0.09 and 0.087 ng/ml were detected in samples from private drinking water wells in Țela, located on the left side of the Mureș River, between Săvârșin and Lipova. At the time of sampling (S II), the water level in these wells was increased as well.

During the second sampling period, characterized by heavy rainfall and flooding, higher values for beta-lactam residues were recorded, regardless of the water category. The increased values can be attributed to floods that have collected residues of any kind from the respective areas, counteracting the dilution effect that the increased amount of water would have normally demonstrated. The presence of residues in floods can be attributed to the education, or lack thereof, of the inhabitants in regard to the environment or their understanding of their responsibility towards it.

Although a considerable number of valuable studies on residues of pharmaceuticals in aquatic environments, among which antibiotics hold an important position (Lamastra L. *et al*, 2016; Meffe, R. *et al*, 2014; Szekeres E. *et al*, 2018), have emerged in Europe over the past 10 years, the watch list developed by the European Commission for the Union-wide monitoring of hazardous substances currently includes only five antimicrobials, namely erythromycin, clarithromycin, and azithromycin of the macrolide class, amoxicillin from the beta-lactam class and ciprofloxacin from the fluoroquinolone class (EU Commission Implementing Decision, 2018).

CONCLUSIONS

Residues of beta-lactam antibiotics were identified in all categories of water (surface water, wastewater, and groundwater) in the territory of Timiș County.

The largest amount of residues, excluding wastewater, was detected in the tap water sample from the public distribution network in the city of Timișoara. This can be attributed to the lack of surveillance for emerging organic contaminants upstream of the city's drinking water treatment plant.

During the period of heavy rainfall, due to the formation of floods, the amount of residues increased in all categories of water.

The antibiotic residue removal efficiency of the wastewater treatment plants in this study was 75.18% for Timișoara, 68.57% for Jimbolia, and 23.61% for Dudeștii Noi.

Enzyme-linked immunosorbent assay can be successfully used as a screening tool for the presence of antibiotic residues in aquatic environments.

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