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O 52. SOME PHARMACEUTICAL ACTIVE COMPOUNDS IN SURFACE WATERS OF TIRANA CITY

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ABSTRACT: Personal care products and Pharmaceutical Active Compounds (PhACs) used for health treatments are commonly reported as pollutants in urban waters and especially in hospital waste waters. PhACs are present not only primary substances used as drugs for the treatment of various diseases but also by the secondary substances which are the products of the metabolism of these drugs. These substances are found in surface waters, urban waters and hospital waters due to their excretion by patients who use them both in home use and in hospital treatments. A large number of these substances have harmful effects on the environment and on living organisms. The determination of antibiotics (amoxicillin, ciproflax and tetracycline) and anti-inflammatory drugs (diclofenac, ibuprofen and nimesulide) in water samples of Lana and Tirana rivers was performed by HPLC/DAD technique. After the validations of analytical method, were taken water samples on 7 stations of Lana River and 5 stations of Tirana River. Samples were taken in February 2021. Drugs isolation is achieved by using the SPE extraction technique using C18 cartriges. Qualitative and quantitative determination is performed using Agilent model 1260 HPLC equipped with quaternary pump and DAD detector. S8 Zorbax-C18 column (15 m x 4 mm x 0.5 um) was used for their separation. Pharmaceutical compounds (antibiotics and anti-inflammatory drugs) taken in the study were detected in almost in all water samples of Lana and Tirana rivers. Their presence was result of direct discharges into this river from both urban and hospital wastewaters. The highest level was found for amoxicillin (antibiotic) and ibuprofen (anti-inflammatory). This may be related to their various uses especially during this Covid-19 pandemic period.

Keywords*: Pharmaceutical active compounds, antibiotics, anti-inflammatories, HPLC/DAD.*

INTRODUCTION

In 2005, EPA began studying environmental contamination by pharmaceuticals, detergents, natural and synthetic hormones, and other chemicals. These contaminants are commonly referred to collectively as contaminants of emerging concern. Hospital wastewater represents a particular type of effluent; this assumption has been often objected and rejected since 1980. Scientists have been demonstrated and confirmed that the hospital effluents present really different qualitative and quantitative characteristics (Liu et al., 2010; Verlicchi et al., 2010a) in compared with the urban wastewater. Hospitals generate 2-5 times higher than urban flow rates, which refer to one inhabitant equivalent. As a result, the collection of hospital wastewater together with domestic wastewater has been criticised and a dedicated pre-treatment of hospital wastewater has been recommended (Verlicchi et al., 2010; Pauwels and Verstraete, 2006).

Referring to pharmaceuticals, large amounts of different compounds are used worldwide and, in the last years because of Covid-19 Pandemic, their sales have been continuously increasing. In particular, the annual consumption of most used drugs such as ibuprofen (an analgesic), amoxicillin (antibiotic), etc was grow up in very higher quantities in almost all countries. Thousands of pharmaceutical chemicals are in use today, particularly in developed countries (Rounds *et al.,* 2009); approximately 3000 to 4000 different pharmaceuticals ingredients are used in the EU today, including painkillers, antibiotics, blockers, contraceptives, lipid regulators, antidepressants, antineoplastic, tranquilizers, impotence drugs and cytostatic agents. Pharmaceuticals are used extensively in human and veterinary medicine. More than 3000 different chemical substances are used as human medicines and in farming and aquaculture applications, in which antibiotic is one of the most important groups of common pharmaceuticals in our daily lives. The use of pharmaceuticals it is a necessity but we must consider also their environmental and adverse health effects. Pharmaceutical compounds when arrived to the environment are one of the

main emerging contaminant groups, as long as those can be biologically active at very low concentrations, which imply the use of advanced analytical techniques to study their occurrence. Furthermore, beside the parent molecule, they are often excreted as metabolites that can be even more toxic than the native molecule.

Pharmaceutical active compounds (PhACs) are molecules that enter to the environment as active remain, either as unmetabolized parent compounds or as pharmaceutical active metabolites. Drugs are administrated to humans and reach the environment via excretory system in unmodified, partially metabolized or completely metabolized state (Ebele et al, 2017). The main source of their presence in environment are hospital wastewaters. These molecules can promote drug tolerance or resistance to the target organisms (e.g. antibiotic resistance in bacteria, or analgesic tolerance in humans) and unwanted effects in non-target organisms (alteration of sex ratio and decreased fertility) even in low concentration. Their levels in hospital wastewaters and environment are reported from ppb to ppm levels. Study of Pharmaceutical Active Compound and also Personal Care Products (PPCPs) have obtained increasing attention over the past decade. These compounds are a set of chemicals pollutants resulting from pharmaceutical and products for personal hygiene. They include a wide and diverse range of chemicals, including prescription drugs and medicines, perfumes, cosmetics, sunscreens, cleansers, shower gel, shampoo, deodorant and other. When these substances are freely discharged into the environment, they could cause some impact on aquatic and terrestrial organisms (Fent *et al.,* 2006; Jjemba, 2006), since they have been specifically designed to produce biological effects even at very low concentrations. This broad collection of substances includes any products consumed by individuals or domestic animals for any number of countless reasons pertinent to health, performance, cognitive and physical function, or appearance (Petrovic and Barcelo, 2007). The intended biological active allowed scientist to categorize several compounds into families: analgesics and anti-inflammatory, antibiotics, psychiatric drugs, beta-blockers or lipid regulators among many others.

Hospital waste waters are of major concern as they contain pharmaceuticals residues, pathogens, chemical reagents, radionuclide, and other harmful matter. Even though, these specific effluents are usually discharged in the municipal sewer asystem and co-treated with domestic wastewaters. Recently, with the development of sensitive analytical techniques, which make possible the detection of more and more active pharmaceutical compounds, it is well established that pharmaceuticals and their metabolites are present in the environment (Kümmerer, 2004b) with wastewater being the primary entry route. Sources that include HWW are often pointed out as a hot spot to pharmaceutical residues in influents of municipal wastewater treatment plant. Hospital wastewaters mainly comprise products used in everyday life in large quantities, such as endocrine disrupting compounds (EDCs), pharmaceutical and personal care products (PPCPs), surfactants and surfactants residues, and various industrial additives.

MATERIAL AND METHODS

Reagents and standards

For this study were used analytical standards of 1 mg/ml dissolved in methanol for amoxicillin, ciprofloxacin, tetracycline, diclofenac, ibuprofen, and nimesulide. Methanol, trifluoracetic acid and acetic acid suitable for chromatography were purchased by Sigma Aldrich. Bi-distillated water was used to prepare all standard working solutions.

Standard solutions

For preparing calibration solutions of each antibiotic (amoxicillin, ciprofloxacin, tetracycline) and anti-inflammatory (diclofenac, ibuprofen, nimesulide), the standard solution of 1 mg/ml were dissolved in 100 ml of methanol. This stock solution (10 ug/ml or ppm) was used to prepare 5 ppm, 2.5 ppm, 1 ppm 0.5 ppm, 0.25 ppm and 0.125 ppm working solutions. These solutions were used to prepare calibration curves for each of the analytes taken in the study. Stock solution and standard solutions were stored in the refrigerator at + 4ºC.

Water sampling in Lana and Tirana rivers

Water samples from Lana and Tirana rivers were taken according to the protocol established by Water Resources Company Management which takes into account the sampling time, sampling stations, sampling vessel, sample pre-treatment, notes and label accompanying the sample, mode of transport of samples and storage of the sample before their analysis. Water samples from 7 stations of the Lana River and 5 stations of Tirana River (two rivers of Tirana city) were taken in this study. Samples were taken in February 2021. Water samples of Lana River were taken starting from New Maternity - LN1 to Astiri - LN7 while stations of Tirana River were taken from Paskuqani – TR1 to Instituti – TR5. Sampling stations of both rivers are shown in the map below (Figure 1).

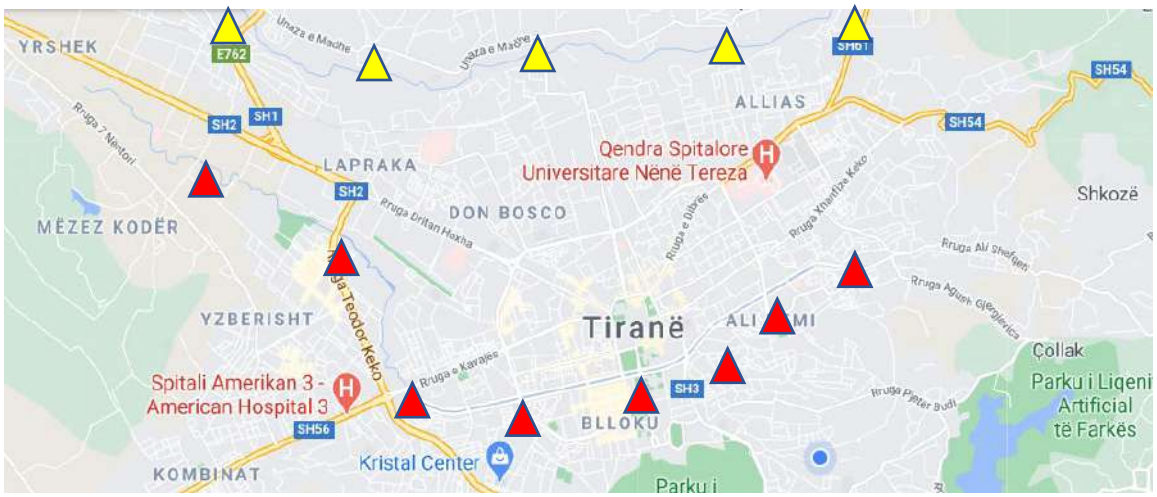

Figure 1. Sampling stations for water samples in Lana and Tirana rivers, February 2021

Pretreatment of water samples for HPLC-DAAD analyze

1 liter of water was taken at each station. Water samples were filtered to remove suspension particles and then 5 ml of glacial acetic acid was added to each sample. Water samples were stored at + 4ºC until their analysis. Water samples were degassed for 30 minutes using ultrasonic bath. Then the water samples passed to a C18, SPE column. The column was conditioned with 10 ml methanol / water (50/50) and eluted with 15 ml methanol. The samples were evaporated to a volume of 3 ml. 20 ul of each sample was injected at 30ºC in HPLC/DAAD equipment.
Each sample was analyzed in three parallel injections. Recovery of antibiotics and anti-inflammatory drugs for C18 column was as follows: for amoxicillin 83.2 ± 11.5%, for ciprofloxacin 75.3 ± 14.2%, for tetracycline 82.9 ± 9.7%, for diclofenac 77.5 ± 17.1%, for ibuprofen 79 6 76.6 n. ± 16.6%. A mixture of antibiotics and anti-inflammatory drugs with a concentration of 1 ppm in 1 liter of distilled water (9; 10) was used to calculate the recovery of analytes.

Liquid chromatography analyzes of PhACs in water samples

The analysis of antibiotics and anti-inflammatory medicaments in water samples of Lana and Tirana rivers was performed using the HP 1260 Series Chromatograph (Agilent Technologies, USA) equipped with internal degasser, Radeon manual injector (20ul loop), quaternary pump, thermostat for the column and diode detector (DAD). PhACs separation was performed on the S8 Zorbax C18 column (150 mm × 4.6 mm x 0.5 um). The mobile phases used for the separation and isolation of antibiotics were: Phase A - 0.02M aqueous solution of trifluoroacetic acid (TFA) and Phase B - methanol.
For simultaneous determination of antibiotics and anti-inflammatory in water sample, the mobile phase was initially with 30% (Phase A) and 70% (Phase B) at a flow of 1 ml/min. After 1 minute, the 0.02M TFA solution changed from 30% to 60% for 15 minutes with the same flow of 1ml/min. In this percentage of phases, A and B (60/40) the column was rinsed for 10 minutes. Qualitative and quantitative analyze of amoxicillin, tetracycline, diclofenac, ibuprofen and nimesulide were performed

at wavelength λ = 220 nm. Determination of ciprofloxacin was performed at wavelength λ = 280 nm (11; 12).

Method validation

Method validation was done according to Commission Decision 2002/657/EC. Parameters such as linearity, specificity, precision, accuracy, Limit Detection (LOD) and Quantification Limit (LOQ) (5; 6) were evaluated for each analyte (analyzed antibiotics and anti-inflammatory medicaments). Linearity was tested using test solutions from 0.125 - 5 ppm. Blank solution and "home standards" (pure bi-distilled water and fortified in several concentrations) were done frequently. Some validation data of the method are given in the following table 1:

Table 1. Validation parameters of the HPLC/DAD method used for analyzes of pharmaceutical compounds in Lana and Tirana rivers, 2021

Medicaments	Correlation coefficient (R)	LOD (ppm)	LOQ (ppm)
Amoxicillin	0.9945	0.037	0.114
Ciprofloxacin	0.9522	0.095	0.298
Tetracycline HCl	0.9847	0.038	0.102
Diclofenac	0.9044	0.074	0.223
Ibuprofen	0.9207	0.042	0.133
Nimesulide	0.9819	0.085	0.247

RESULTS AND DISCUSIONS

Some of the most widely used antibiotics and anti-inflammatory drugs were taken in the analysis, especially during the Covid-19 Pandemic period, the period in which the work coincides. These pharmaceutical compounds have been analyzed in water samples of Lana and Tirana rivers. These aquatic ecosystems are important for Tirana city but unfortunately in waters of these rivers discharged directly in many points' hospital and urban wastewaters. Hospital and urban wastewaters are the main sources of pharmaceutical compounds in these surface waters. These drugs are used for the treatment of various diseases (in hospital and home treatments). The main parts of these excreted unchanged by patients and a part of these drugs are metabolized by the body. Both unchanged drugs or primary products and metabolized products (by-products) have been observed to cause various environmental problems (2; 5; 12). Antibiotics that were analyzed in surface waters of Tirana city were: amoxicillin, ciprofloxacin and tetracycline as well as anti-inflammatory drugs: diclofenac, ibuprofen and nimesulide. Their analysis was performed simultaneously combined SPE (C18 cartridge) followed by HPLC/DAD technique. Separation of these pharmaceutical compounds was performed in column S8 Zorbax C18 (150 mm × 4.6 mm x 0.5um). The mobile phases used for the separation and isolation of PhACs was 0.02M solution of trifluoroacetic acid and methanol.
Tables 2 and 3 provide the average data obtained for antibiotics and anti-inflammatory drugs found, respectively in water samples of Lana and Tirana rivers. It was noted the presence of analyzed pharmaceutical compounds in all analyzed samples. Figure 2 shows the total antibiotics in water samples of Lana and Tirana rivers. The highest level in Lana River was found for the LN5 station (near the "Palace of the Arrows" station) with a total of 0.221 ppm while for Tirana River the higher level was found for TR5 station (Institute station) with 0.158 ppm. The lowest level for Lana River was found for sample LN1 (on New Maternity station) while for Tirana River the low level was found for TR1 station (Paskuqani station) with 0.025 ppm. The distribution of Antibiotics in water samples of Lana and Tirana rivers was shown in Figure 3. It was observed an individual distribution for each of the antibiotics taken in the analysis. For amoxicillin and tetracycline were found high levels of them starting from LN2 station (below New Maternity station) to LN7 station (Astiri station) in Lana River and for Tirana River was observed almost the same starting from RT3 to TR5 stations. The increase of concentrations for these antibiotics can be related to the inflows of these compounds from both hospital spills and urban spills

near these stations. The profile of antibiotics in the water samples of both, Lana and Tirana rivers (Figure 4) was: amoxicillin > tetracycline > ciprofloxacin. This profile should be related to the relative amounts of each individual for medical treatments. It should be noted that the Covid-19 Pandemic has significantly increased the use of antibiotics which are used in different stages of treatment of patients with this virus. Note that, their use in many cases in Albania is done without a doctor's recommendation in home treatments.

Figure 5 shows the total of the anti-inflammatory drugs (diclofenac, ibuprofen and nimesulide) analyzed in the water samples of Lana and Tirana rivers. Also, their lowest level was found in the LN1 station with 0.047 ppm for Lana River and in TR3 station with 0.023 ppm of Tirana River. The maximum level of anti-inflammatory drugs was found in the LN7 station (0.354 ppm) for Lana River and in TR5 station (0.116 ppm) for Tirana River. As for the antibiotics, this should be linked to hospital and urban spills into the Lana and Tirana rivers, leading to an increase in their concentrations from stations in the first part to the stations in the second part of both rivers. Figure 6 shows the distribution of anti-inflammatory drugs in water samples of Lana and Tirana rivers. Again, it was observed an individual profile of them in both rivers which have the same upward trend of their concentrations from starting stations to the lowest parts of the rivers. Even for this group of drugs can be said that there is an increase in their use, especially for Covid-19 treatments in both hospital and home treatments. Figure 7 shows the profile of anti-inflammatory drugs in water samples of Lana and Tirana rivers. It was notable the highest level of ibuprofen followed by diclofenac in both rivers. Nimesulide is found in smaller amounts. This is related to the degree of their use but also to the stability and other physical-chemical properties of these molecules.

Table 2. Average data of antibiotics (ppm) in water samples of Lana and Tirana rivers

Antibiotic's	**Lana River**							**Tirana River**				
	LN1	**LN2**	**LN3**	**LN4**	**LN5**	**LN6**	**LN7**	**TR1**	**TR2**	**TR3**	**TR4**	**TR5**
Amoxicillin	0.011	0.052	0.073	0.009	0.115	0.082	0.164	0.009	0.014	0.032	0.026	0.065
Ciproflaxin	N.D.	N.D.	0.014	N.D.	0.025	0.006	0.007	0.001	N.D.	0.007	N.D.	0.012
Tetracycline HCl	0.005	N.D.	0.066	0.087	0.081	0.113	0.043	0.015	0.054	0.062	0.059	0.081
Total	**0.016**	**0.052**	**0.153**	**0.096**	**0.221**	**0.201**	**0.214**	**0.025**	**0.068**	**0.101**	**0.085**	**0.158**

Table 3. Average data of anti-inflammatories (ppm) in water samples of Lana and Tirana rivers

Anti-inflammatory	**Lana River**							**Tirana River**				
	LN1	**LN2**	**LN3**	**LN4**	**LN5**	**LN6**	**LN7**	**TR1**	**TR2**	**TR3**	**TR4**	**TR5**
Diclofenac	N.D.	N.D.	0.017	0.009	0.013	0.056	0.223	0.052	0.066	N.D.	N.D.	0.089
Ibuprofen	0.044	0.050	0.041	0.063	0.027	0.142	0.093	0.011	0.051	0.023	0.047	0.073
Nimesulide	0.003	N.D.	0.004	0.027	0.058	0.008	0.038	N.D.	N.D.	0.004	0.002	0.005
Total	**0.047**	**0.05**	**0.062**	**0.099**	**0.098**	**0.206**	**0.354**	**0.063**	**0.111**	**0.027**	**0.049**	**0.166**

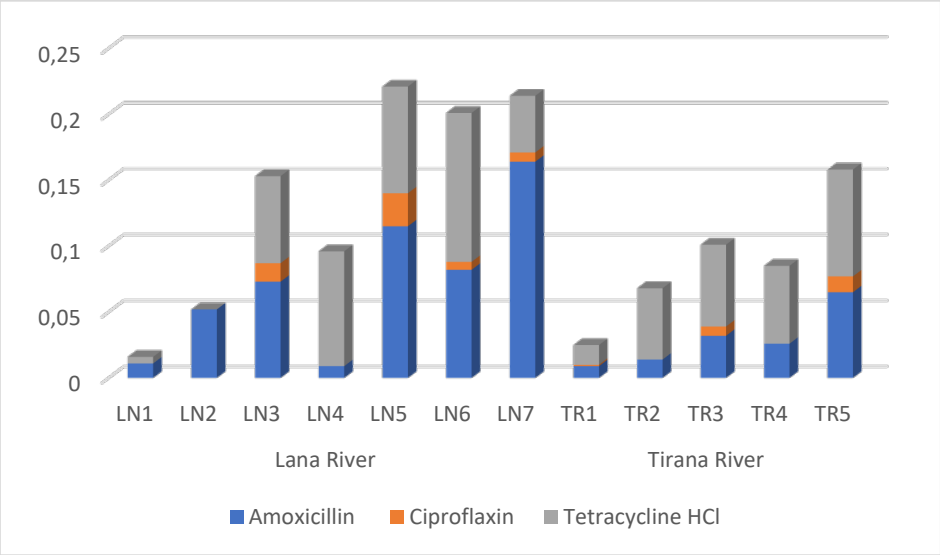

Figure 2. Total of antibiotics (ppm) in water samples of Lana and Tirana rivers

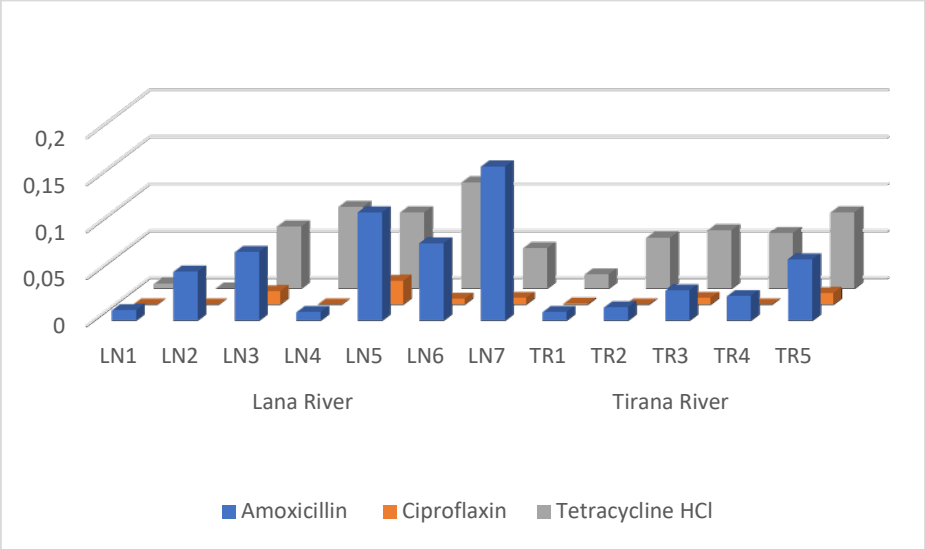

Figure 3. Distribution of antibiotics (ppm) in water samples of Lana and Tirana rivers

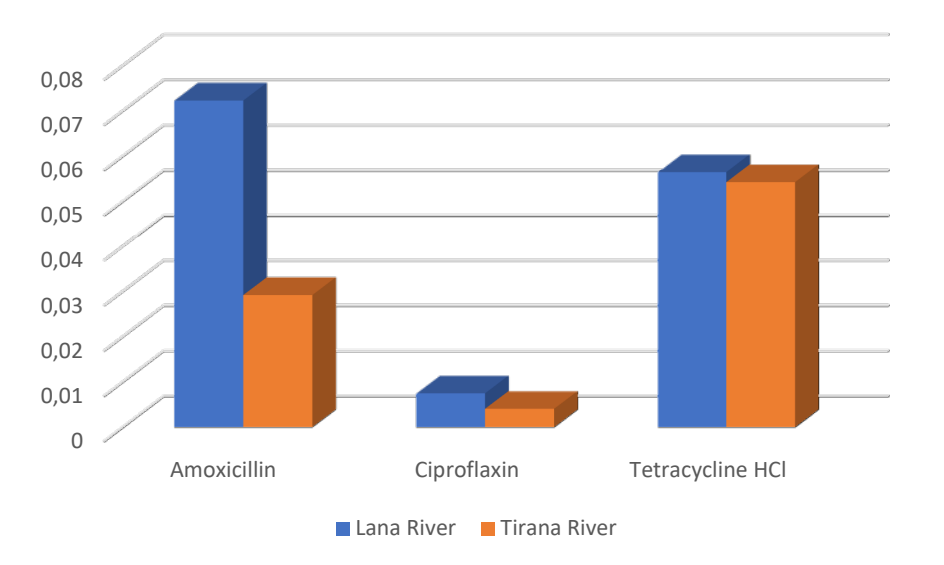

Figure 4. Profile of antibiotics (ppm) in water samples of Lana and Tirana rivers

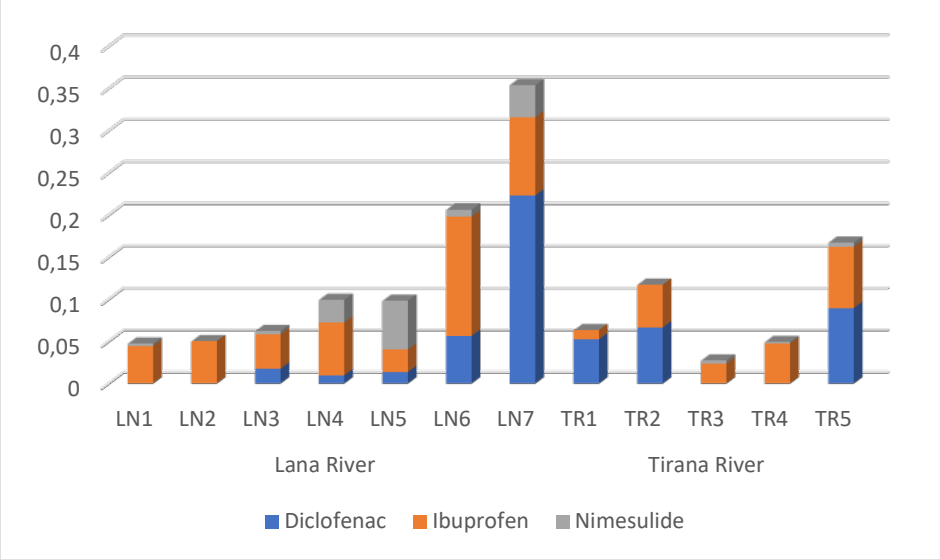

Figure 5. Total of anti-inflammatory medicaments (ppm) in water samples of Lana and Tirana rivers

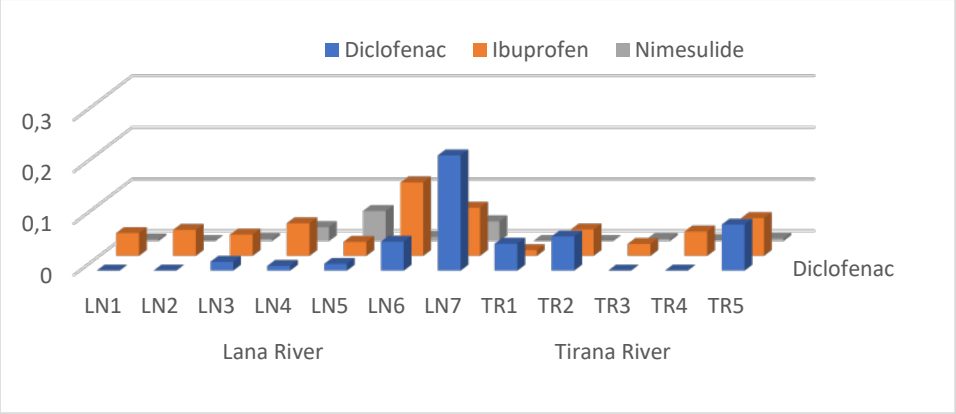

Figure 6. Distribution of anti-inflammatory (ppm) in water samples of Lana and Tirana rivers

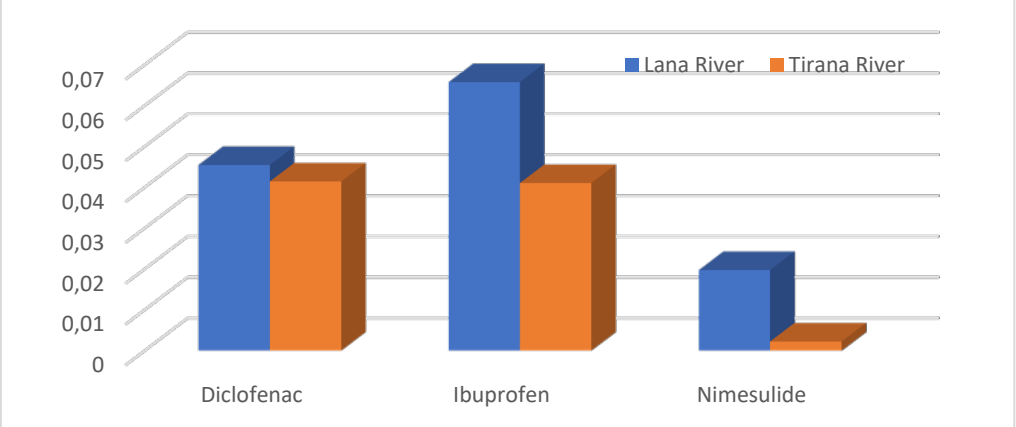

Figure 7. Profile of anti-inflammatories (ppm) in water samples of Lana and Tirana rivers

CONCLUSIONS

The purpose of this study was to determine the level of some antibiotics (amoxicillin, ciprofloxacin and tetracycline) and some anti-inflammatory drugs (diclofenac, ibuprofen and nimesulide) in water samples of Lana and Tirana rivers. Unfortunately, in these rivers discharge without any treatment's hospital, polyclinic and urban wastewaters. These spillages are the main source of pollution with pharmaceutical compounds of these important aquatic ecosystems. The analyzes of PhACs were performed using the

HPLC/DAD technique. The technique is recommended by international and Albanian norms for the analysis of antibiotics and anti-inflammatory drugs in surface waters. From antibiotics and anti-inflammatory drugs were selected those that have wider use in hospital and home medical treatments nowadays in the Tirana city, in this period that coincides with Covid-19 Pandemic. The use of medical drugs in this period has been a significant increase in this period. In the water samples of Lana and Tirana rivers the highest levels of antibiotics belong to amoxicillin while for anti-inflammatory drugs the highest level was found for ibuprofen. This fact must be a consequence of their more frequent uses in the treatment of infections and pain relief during the period.

Analysis of PhACs should be applied regularly by the responsible institutions in the surface water analysis not only for the Lana and Tirana rivers but also for other water ecosystems where it is thought that there is discharge of hospital or urban wastewaters. Also we suggest further work to increase analytical performance using LC/MS/MS analytical techniques for the identification and quantification of pharmaceutical compounds in surface waters.

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