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Occurrence of Pharmaceuticals in the Waters of the Danube Region

Study

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Table of content

1. Introduction

Recently, an increased interest in monitoring of micro-pollutants in various environments occurs in diverse fields of scientific research; especially in analytical chemistry, environmental sciences and environmental engineering. These micro-pollutants include pesticides, pharmaceuticals, drugs and their metabolites, which are predominantly biologically inert and consequently present in the food chain. The prevailing sources of these compounds are mainly households, healthcare facilities, old people´s homes, or agricultural production from where micro-pollutants pass into wastewater and via sewage system to wastewater treatment plants (WWTP) from where part of them outflow to surface streams (Mackuľak et al. 2016, Zhou et al. 2019) – **Fig.1**.

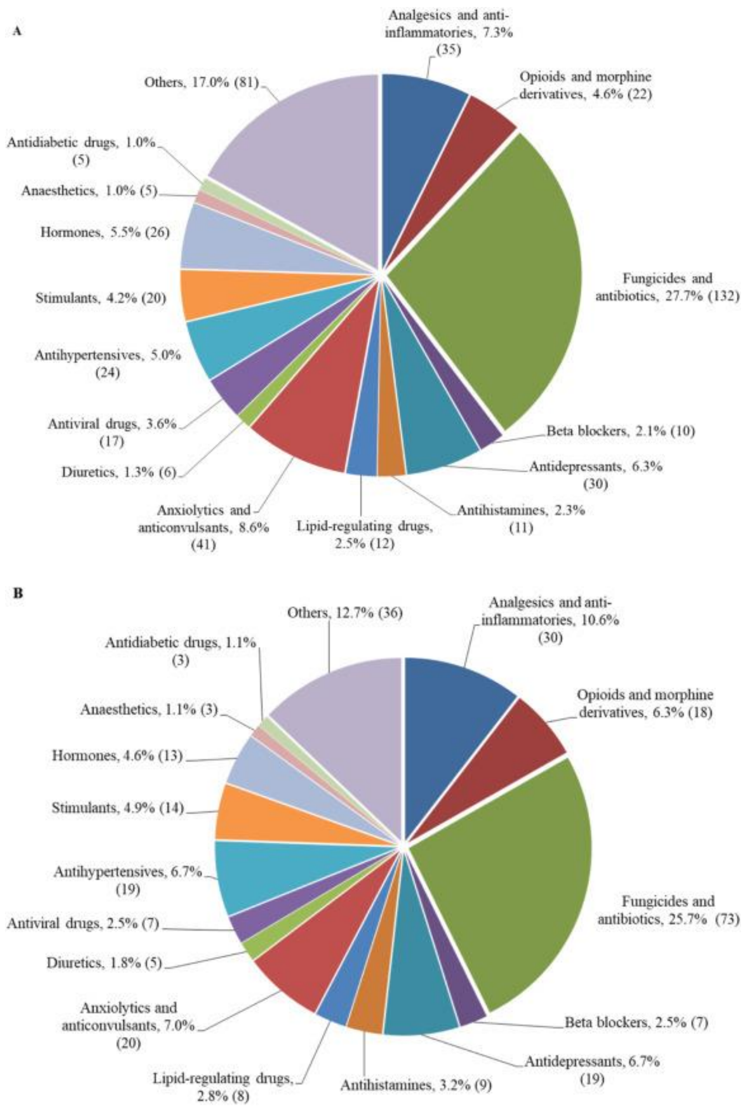

Fig. 1. Individual groups of pharmaceuticals in expert studies conducted in 1996-2019 on the occurrence and ecotoxicology of pharmaceuticals in the environment A – analyzed, B – positively detected (Zhou et al. 2019)

Modern drugs are currently an irreplaceable means of treating and maintaining the quality of life of many patients. However, their unjustified indication or over-consumption leads to a higher penetration of these substances into the environment.

For instance, statistics show that an average Slovak citizen consumes over 17 packages of different medicines per year. Antibiotic consumption in Slovakia has declined over the past five years. In 2018, there were approximately 0,9 packages per capita per year. Overall, 4.2 million prescriptions were prescribed in 2018 and 4,8 million packages of antibiotics were dispensed. As with neighboring countries, there is a gradual increase in consumption of antidepressants. In 2017, 79,89 million daily doses of antidepressants were officially sold in Slovakia. Thus, the number of treatment doses has increased by up to 16 times since 1996. In 2018, around 80 million prescription drug packages were dispensed from pharmacies, which is approximately 0.9 percent less than in 2017. Health insurance companies thus paid EUR 885.3 million in 2018, 0.3 percent less than in 2017. The most commonly prescribed drugs were drugs for the treatment of cardiovascular diseases (26.8 million packages), the nervous system (14 million packages) and digestive tract and metabolism (8.8 million packages). In 2018, patients consumed 40.4 million packages of over-the-counter medicines worth around € 197 million, an 8.3% year-on-year increase. The best-selling over-the-counter medicines were:

- for the treatment of respiratory diseases (10.1 million packages) and the gastrointestinal tract
- for metabolism treatment (8.3 million packages)
- for the treatment of the nervous system (6.9 million packages, most of which are analgesics) (Tesař et al. 2018, NCZISK 2019).

Pharmaceuticals are always developed with a specific goal. The basic prerequisite for their action is a rapid and powerful interaction with the biological system. Active pharmaceutical ingredients undergo a series of clinical trials prior to marketing, but the data on their ecotoxicity are minimal or even completely unknown. There are more than 3000 different active pharmaceutical ingredients, of which more than 200 have been identified in the environment in the form of compounds. This has prompted research into the presence of pharmaceuticals in the environment and a number of studies have been emerging to investigate the presence of pharmaceuticals in waters (Mackuľak et al. 2016, Mackuľak et al. 2017).

Micro-pollutants include a large group of compounds that differ in chemical and physicochemical properties. This affects the possibility of their removal in the sewage system or wastewater treatment plant, but also in the environment. Municipal wastewater treatment plants technologies using biological processes to remove organic contaminants and nutrients are usually unable to remove effectively these compounds. Pharmaceuticals, drugs and their metabolites are thus able to enter and contaminate surface- and consequently, groundwater sources. For example, the presence of antibiotics in the aquatic environment is currently often associated with the occurrence of short- or long-term toxicity or microbial resistance (Birošová et al. 2014). Main sources of pharmaceuticals and drugs in the environment and their possible cycle (Mackuľak et al. 2019) shows **Fig.2**.

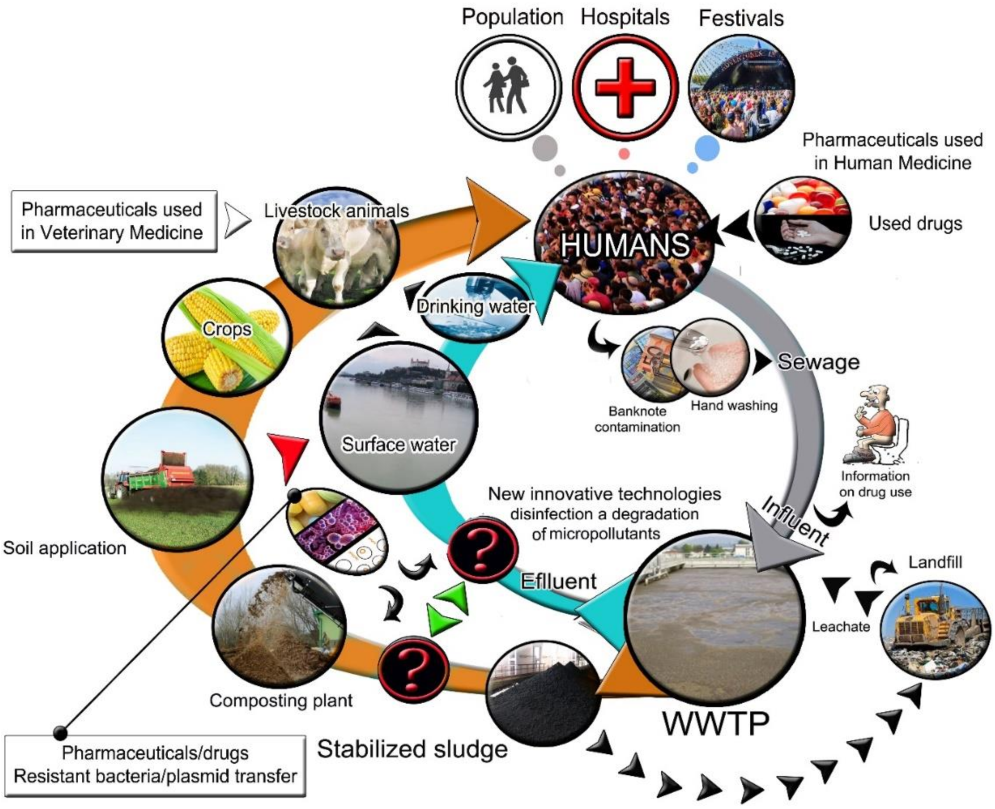

Fig 2. Main sources of pharmaceuticals and drugs in the environment and their possible cycle (Mackuľak et al. 2019).

At present, in the field of analytical chemistry, environmental sciences and environmental engineering it is possible to observe an increased interest in the results obtained by monitoring of selected micro-pollutants in various parts of environment. In general, until now, there are no guidelines or standards for most pharmaceuticals, their metabolites and drugs that set limit values and quality objectives for their discharge into sewage and outlets from municipal wastewater treatment plants. Further research of monitoring and behaviour of micro-pollutants is of particular importance in setting effective regulatory limits for micro-pollutants (Mackuľak et al. 2019, Mackuľak et al. 2020). As it has already been stated, with considerable progress in analytical chemistry, new possibilities emerge to effectively identify compounds (environmental pollutants or micro-pollutants), such as pharmaceuticals, drugs, but also their metabolites with a concentration of only a few $ng.l^{-1}$. This provides us an opportunity to observe the occurrence and behaviour of these micro-pollutants from sewerage through WWTPs to their possible impact on the aquatic ecosystems.

Nowadays, the occurrence of psychoactive drugs, contrast agents and antibiotics in wastewater is particularly studied. Their concentration in water is influenced by several important factors. Firstly, it is the offer of the pharmaceutical industry, the specific consumption of water per capita, the specific consumption of individual medicaments, the season as well as the type and water tightness of the sewage system used. Weather also affects the concentration of pharmaceuticals and drugs in wastewater. For psychoactive drugs, histamines and antibiotics, the seasons must also be taken into account. Especially during the winter months there is an increase in migraines, depressions, inflammatory diseases, there are more injuries, which are reflected in the representation of specific drugs in wastewater. On the other hand, the decrease in concentration during the winter months is mainly observed in allergy medicines - antihistamines. Consequently, this may also affect the occurrence of pharmaceuticals, drugs or their metabolites in surface and groundwater during the year (Mackuľak et al. 2019).

2. Legislation background

European Union legislation provides for measures against chemical pollution of surface waters. There are two components – the selection and regulation of substances of European Union (EU)-wide concern (the priority substances) and the selection by Member States of substances of national or local concern (river basin specific pollutants) for control at the relevant level. The first component constitutes the major part of the Union's strategy against the chemical pollution of surface waters. It is set out in Article 16 of the **Water Framework Directive 2000/60/EC**. This requires the establishment of a list of priority substances, these to be selected from amongst those presenting a significant risk to or via the aquatic environment at EU level. It also requires the designation of a subset of priority hazardous substances, and proposals for controls to reduce the emissions, discharges and losses of all the substances and to phase out the emissions, discharges and losses of the subset of priority hazardous substances.

In order to improve the quality of the monitoring data obtained under the Water Framework Directive, the Commission adopted Directive 2009/90/EC laying down technical specifications for chemical analysis and monitoring of water status.

The regulation of chemical pollutants in water began with Council Directive 76/464/EEC on pollution caused by certain dangerous substances discharged into the aquatic environment. The introduction of provisions under the Water Framework Directive includes transitional elements such that parts of the earlier legislation are applicable until the end of 2012.

Priority Substances legislation

The **Water Framework Directive 2000/60/EC** established provision for a list of Priority Substances (Annex X of the Directive). Decision 2455/2001/EC established the First list, and **Directive 2008/105/EC (the Environmental Quality Standards Directive – EQSD)** set the quality standards as required by Article 16(8) of the Water Framework Directive. Annex II to the EQSD replaced Annex X of the Water Framework Directive.

Member States are required to take actions to meet the quality standards in the EQSD by 2015 as part of chemical status (Water Framework Directive Article 4 and Annex V point 1.4.3). For this purpose a programme of measures (according to Water Framework Directive Article 11) has to become operational by 2012. The river basin specific pollutants are considered as part of ecological status.

Discharge of dangerous substances **(Directive 76/464/EEC)**

Community policy concerning dangerous or hazardous substances in European waters was introduced almost three decades ago by **Council Directive 76/464/EEC on pollution caused by discharges of certain dangerous substance** codified as **2006/11/EC**. Several substances have been regulated in specific directives (also called **"daughter' directives"**) in the 1980s by defining Community-wide emission limit values and quality objectives in the surface and coastal waters:

- **Council Directive 82/176/EEC on limit values and quality objectives for mercury discharges by the chlor-alkali electrolysis industry**
- **Council Directive 83/513/EEC on limit values and quality objectives for cadmium discharges**
- **Council Directive 84/156/EEC on limit values and quality objectives for mercury discharges by sectors other than the chlor-alkali electrolysis industry**
- **Council Directive 84/491/EEC on limit values and quality objectives for the discharges of hexachlorocyclohexane**
- **Council Directive 86/280/EEC as amended by 88/347/EEC and 90/415/EEC on limit values and quality objectives for discharges of certain dangerous substances** included in List I of the Annex to Directive 76/464/EEC

As part of the restructuring of the Community water policy, Directive 76/464/EEC was integrated into the Water Framework Directive 2000/60/EC**.**

Commission Directive 2009/90/EC on technical specifications for chemical analysis and monitoring of water status**,** pursuant to Directive 2000/60/EC of the European Parliament and of the Council, technical specifications for chemical analysis and monitoring of water status entered into force on 21 August 2009. The objective of this Directive is to establish common quality rules for chemical analysis and monitoring of water, sediment and biota carried out by Member States.

Strategic approach to pharmaceuticals in the environment

On 11 March 2019, the Commission adopted a strategic approach to pharmaceuticals in the environment in all member states languages as required by Article 8c of Directive 2008/105/EC on environmental quality standards in the field of water policy as amended by Directive 2013/39/EU. The approach covers all phases of the lifecycle of pharmaceuticals, from design and production through use to disposal. The results of the public consultations done prior to issuing the Strategic approach were used to revise the study report and background document.

This part was elaborated based on information from https://ec.europa.eu/environment/water/water-dangersub/index.htm.

The EC member states (MS) are obliged to transpose the European legislation into national legislation. Alike in the Danube Region, the EC Directives are transposed to the water acts and related acts of the EU MS. Non-member states, which have already signed the Association Agreements, are in the process of transposing the EU legislation into national legislative and binding documents, the others are supposed to face this process soon.

The issue of pharmaceuticals in drugs in environment is highly topical globally.

3. Description of the most commonly occurring substances

The study provides information on occurrence of the selected drugs, illegal drugs and their metabolites (listed in the Table 1) in the waters of the Danube River basin. Although the list of analyzed drugs is quite complex, in the following text is provided the description of the properties, effects and kind of use of the most used/analyzed substances.

Table 1. List of analyzed micro-pollutants in surface- and groundwater of Slovakia

Name of substances in alphabetical order			
2-oxo-3-hydroxy-LSD	Dicycloverine	Miconazole	Terbinafine
6-acethylmorphine	Dihydro CBZ	Mirtazapine	Terbutaline
Alfuzosin	Diltiazem	Morphine	THC-COOH
Alprazolam	Diphenhydramine	N1_Acethylsulphametaxazol	Theophylline
Amitriptyline	Disopyramide	N4_Acethylsulphametaxazol	Tramadol
Amphetamine	Donepezil	N-Desmethylcitalopram	trans-dihydro-dihydroxy CBZ
Atenolol	Epoxy CBZ	Norketamine	Triamterene
Atorvastatin	Erythromycin	Norsertraline	Trimethoprim
Azithromycin	Fenofibrate	O-Desmetylvenlafaxine	Valsartan
Benzoylecgonine	Fexofenadine	Orphenadrine	Venlafaxine
Bezafibrate	Glibenklamid	Oseltamivir carboxylate	Verapamil
Biperiden	Glimepiride	Oxazepam	Trazodone
Bisoprolol	Haloperidol	Oxcarbamazepine	Theophylline_neg
Caffeine	Iopromide	Oxycodone	Lamotrigine
Cannabinol	Irbesartan	Pizotifen	
Carbamazepine	Ketamine	Propranolol	
Cathinone	Loperamide	Ropinirol	
Cetirizine	Maprotiline	Rosuvastatin	
Cilazapril	MDA	Roxithromycin	
Citalopram	MDEA	Sertraline	
Clarithromycin	MDMA	Sotalol	
Clemastine	Meclizine	Sulphadiazine	
Clindamycin_sulfoxide	Memantine	Sulphamerazine	
Clindamycin	Mephedrone	Sulphamethazine	
Clomipramine	Methamphetamine	Sulfamethizole	
Clonazepam	Methadone	Sulphamethoxazole	
Cocaine	Metoprolol	Sulphapyridine	
Codeine	Metoprolol acid	Tamoxifen	
Diclofenac	Mianserin	Telmisartan	

A description of the properties of the most common substances occurring in the environment is given in the following paragraphs:

Methamphetamine (Fig. 3) is a synthetic stimulant drug that is produced predominantly from ephedrine (or currently from pseudoephedrine by reduction with hydrogen iodide in the presence of phosphorus). It has also been used in medicine but is currently mainly used as an illicit drug. Methamphetamine causes severe euphoria and psychological dependence.

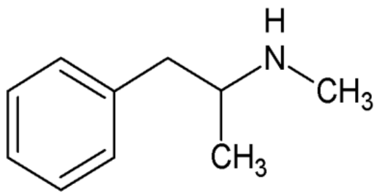

Fig.3. Metamphetamine

Benzoylecgonine (Fig.4) is the dominant metabolite of the drug cocaine (1-9% is excreted in the urine as cocaine, 35-55% as benzoylecgonine, and 32-49% as ecgonine methyl ester). Various studies based on direct monitoring of cocaine in wastewater assume, that 10% of cocaine is excreted from the human body in the form of excrement, followed by degradation of 80% in a wastewater environment (pH = 6 at 20 ° C). Calculations for the direct determination of cocaine give higher results and therefore the indirect method with the metabolite benzoylecgonine is used more frequently.

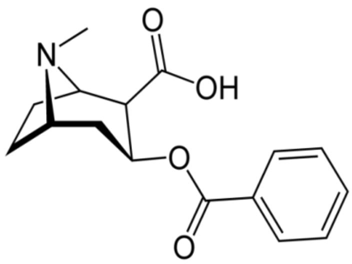

Fig. 4. Benzoylecgonine

Tramadol (Fig.5) is a moderately potent, centrally acting analgesic (acts in the central nervous system - CNS). Inhibition of norepinephrine and serotonin reuptake on nerve synapses contributes to its analgesic effect. At therapeutic doses, it does not cause respiratory depression, affects blood circulation only minimally and causes constipation less frequently. Unlike the morphine-type substances, tramadol has a lower risk of dependence at the recommended therapeutic doses.

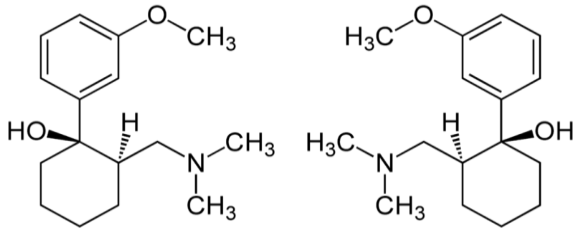

Fig. 5. Tramadol

Oxazepam (Fig. 6) is a short-to-intermediate-acting benzodiazepine with a slow onset of action. It is usually prescribed to individuals who have trouble maintaining uninterrupted sleep. It is also often prescribed for anxiety disorders with associated tension, irritability, and agitation. It is commonly prescribed for drug and alcohol withdrawal; however, it is often abused and mixed with the drugs themselves.

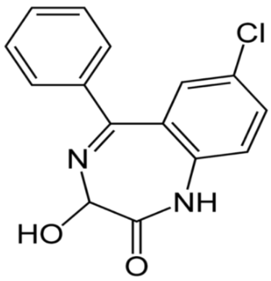

Fig. 6. Oxazepam

Codeine (Fig. 7) (also 3-methoxymorphine) is an opiate used to treat pain, coughing, and diarrhea. It is the most widespread opiate in the world, including Slovakia, and is one of the most effective oral analgesics.

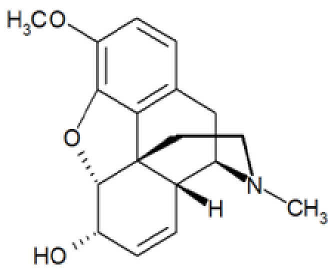

Fig.7. Codeine

Nicotine (Fig.8) is present in all tobacco products, including cigarettes, cigars or nicotine chewing gum. Many young people use tobacco regularly before the age of 15. Nicotine is metabolized to various metabolites in the human body, with **cotinine (Fig.9)** being the most dominant. About 10 to 15% of nicotine is excreted unchanged from the body, 70% is transformed into cotinine. Other tobacco alkaloids such as anatabine and anabasine are also excreted in the urine. In the wastewater, cotinine is generally detected as evidence of nicotine use. The reason is its greater stability.

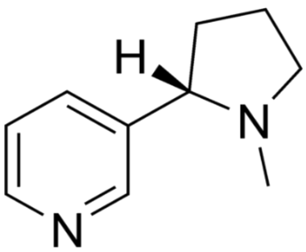

Fig. 8. Nicotine

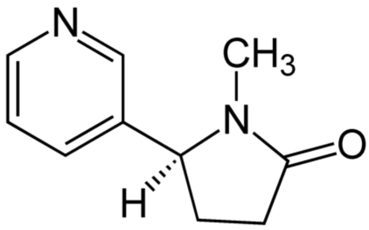

Fig.9. Cotinine

Caffeine (Fig. 10) is an odorless substance. It affects the human body in small concentrations. The effects of codeine include increased heart rate or increased blood pressure. Caffeine can confer a feeling of higher energy, the effects are also manifested by hyperactivity. In some cases, users become addicted. After ingestion, caffeine enters the bloodstream within 30 to 45 minutes. The half-life of degradation is approximately 4 hours (from 2 to 10 hours, depending on metabolic activity). In the human body, caffeine blocks specific adenosine receptors in nerve tissue, thereby increasing alertness and wakefulness. Caffeine is a part of many analgesics as it is able to effectively narrow the blood vessels and thereby dampen migraine conditions.

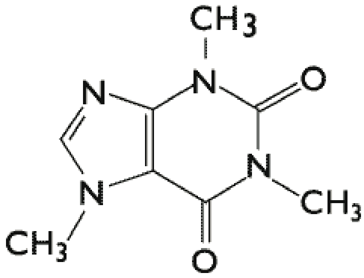

Fig. 10. Caffeine

Amphetamine (Fig. 11), together with methamphetamine, is a stimulant that increases the levels of neuromediators, noradrenaline (norepinephrine), serotonin, and dopamine in the brain. It is used in the treatment of attention deficit hyperactivity disorder (ADHD) in children or in the treatment of chronic fatigue syndrome. It is often abused as a recreational drug, for example under the name Speed. The effects are similar to other amphetamine-based stimulants.

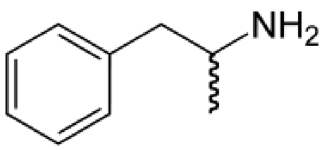

Fig. 11. Amphetamine

Cocaine (Fig.12) is a narcotic, chemically known as an alcaloid of ethylesterbenzoylecgonine. It is a stimulant produced by the South American plant Erythroxylum. Pure cocaine is a crystalline white powder of bitter taste. Cocaine, however, may also be present on the black market, in a combination with another stimulant (amphetamine), with a local anesthetic or the cocaine powder may contain various other ingredients.

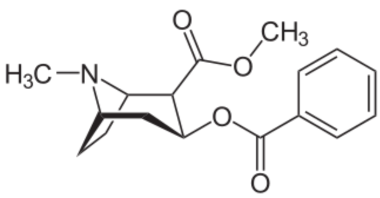

Fig. 12. Cocaine

THC - tetrahydrocannabinol (delta-9-tetrahydrocannabinol) (Fig.13) is one of more than 100 cannabinoids and is the major psychoactive substance found in *Cannabis sativa* and its variants - *Indica*. When in pure form, THC is in the form of translucent crystals, which become sticky upon heating. In wastewater, the compound THC-COOH (11-nor-9-carboxy-delta9-tetrahydrocannabinol) was studied as one of the main products of THC metabolism. Only free THC-COOH is monitored. The expected concentration of THC-COOH in the wastewater is in the range of 10 - 200 ng/L.

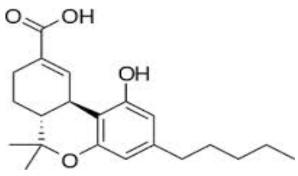

Fig. 13. THC-COOH

MDMA (ecstasy) (Fig.14) is a synthetic drug patented in 1913 by the German company MERCK. Initially, it was sold over the counter as a weight loss medicine. Drug users feel happy, relaxed with increased sociability. It intensifies sensory perception. In the liver, the drug is metabolized to MDA (3,4-methylenedioxyamphetamine - a drug very similar to MDMA). In the urine, 2/3 leaves in the unchanged MDMA structure and 7% as MDA. The biological half-life of MDMA in the body is about 6 hours.

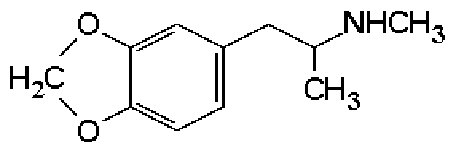

Fig. 14. MDMA

Carbamazepine (Fig. 15) (CBZ) is an anticonvulsant medication used primarily in the treatment of epilepsy and neuropathic pain. It is also used in schizophrenia along with other medications Carbamazepine was discovered in 1953 by Swiss chemist Walter Schindler. It was first marketed in 1962. It is available as a generic medication. In 2016, it was the 197th most likely prescribed medication by doctors in the United States, with more than 2 million prescriptions. Carbamazepine is currently one of the most frequently studied micro-pollutants in the environment. It is only biodegradable to a limited extent and is often identified in surface and groundwater. In addition, several degradation products and metabolites such as dihydro CBZ, epoxy CBZ, trans-dihydro-dihydroxy CBZ or oxcarbazepine are found in the environment. These may exhibit similar or greater ecotoxicity as the parent compound alone (Mackuľak et al. 2019, Mackuľak et al. 2020).

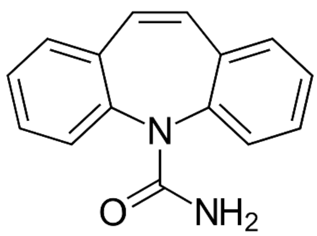

Fig. 15. Carbamazepine (CBZ)

Venlafaxine (Fig. 16), together with **citalopram (Fig. 17)**, is one of the frequently prescribed antidepressants in Slovakia. In addition, **sertraline (Fig.18)** and **trazodone** are also often prescribed. The most abundant metabolites of antidepressants in the environment are desmethyls (e.g.: desmethyl sertraline, norsertraline, N-desmethyl venlafaxine, O-desmethyl venlafaxine, desmethyl citalopram, didesmethyl citalopram,) or hydroxy derivatives. In particular, O-desmethyl venlafaxine occurs at elevated concentrations. Said compounds are the dominant human metabolites. Their concentrations in waste water may exceed 5000 ng/L (Mackuľak et al. 2019, Mackuľak et al. 2020).

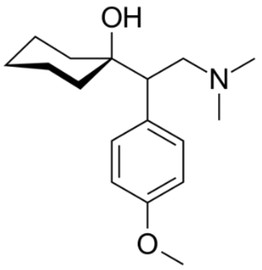

Fig. 16. Venlafaxine

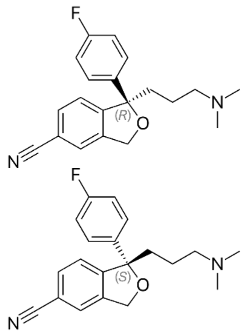

Fig. 17. Citalopram

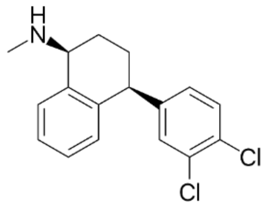

Fig. 18. Sertraline

Tramadol, venlafaxine, diclofenac, carbamazepine, oxazepam and citalopram are most commonly found in purified wastewater from Slovak municipal wastewater treatment plants (the outlet is mainly directed to rivers). Regarding illegal drugs, methamphetamine reaches the highest concentration. The most common metabolites of legal drugs include cotinine, one of the dominant metabolites of nicotine.

Azithromycin (Fig. 19) is an antibiotic belonging to the azalides, a subclass of macrolide antibiotics. It is used for treatment of a number of bacterial infections. In rivers in Slovakia, it is found only to a limited extent at concentrations below 20 ng/L together with clindamycin, ciprofloxacin, erythromycin-H2O, tetracycline, sulfamethoxazole (Mackuľak et al. 2018). In general, antibiotics in surface waters of SR do not exceed concentrations of 50 ng/L. The main sources of these antibiotics are hospitals and households, and thus waste water in which the antibiotic concentrations can often be above 1000 ng/L.

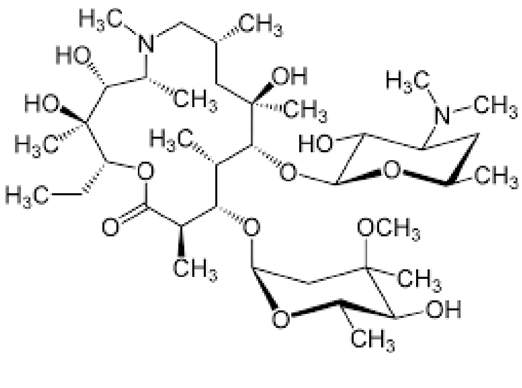

Fig. 19. Azithromycin

Bisoprolol (Fig. 20), sotalol, metoprolol or **atenolol** are among the beta-blockers. For example, bisoprolol is a frequently prescribed drug, among others also under the trade name Concor, which is most commonly used for the treatment of heart disease.

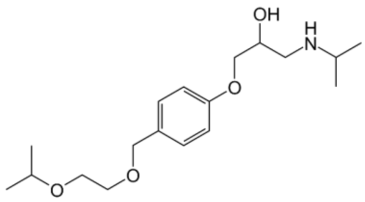

Fig. 20. Bisoprolol

Valsartan (Fig.21), one of the representatives of the angiotensin-2 receptor blockers – ARB (which also include **telmisartan**), is a medication used to treat high blood pressure and heart failure. Valsartan was patented in 1990 and came into medical use in 1996. It is available as a generic medication. Valsartan is a micro-pollutant, with concentrations in wastewater often exceeding 1,000 ng / l. Valsartan, like telmisartan, is able to penetrate various environmental compartments through surface waters and also adsorb to river sediments or, for example, to sewage sludge from sewage treatment plants (subsequently penetrating to fields) (Ivanová et al. 2018, Mackuľak et al. 2020).

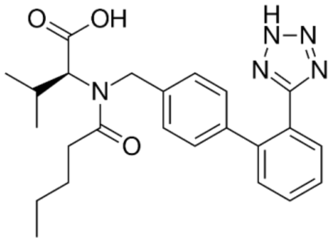

Fig. 21. Valsartan

Verapamil is a medication used for the treatment of high blood pressure, angina pectoris, and supraventricular tachycardia. It may also be used for the prevention of migraines and cluster headaches.

Cetirizine (Fig. 22) and **Fexofenadine** and are antihistamine pharmaceutical drugs. Cetirizine, also marketed under the brand name Zyrtec, is a second-generation antihistamine used to treat allergic symptoms. It was patented in 1981 and came into medical use in 1987. In 2016, it was the 74th most prescribed drug in the United States – with over 10 million prescribed packages. Fexofenadine is also classified as second-generation antihistamine. It was patented in 1979 and came into medical use in 1996. Fexofenadine has been manufactured in generic form since 2011. Fexofenadine and cetirizine are able to be adsorbed to river sediments or sewage sludge (Ivanová et al. 2018, Mackuľak et al. 2020). They can also be identified in surface waters (Mackuľak et al. 2018).

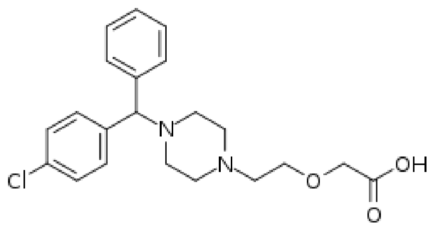

Fig. 22. Cetirizine

Diclofenac (Fig. 23), in Slovakia also known as Voltaren is a non – steroidal anti – inflammatory drug (NSAID) used for treating pain and inflammatory diseases. Diclofenac is one of the most frequently studied micro-pollutant in the environment with considerable toxicity, especially for birds.

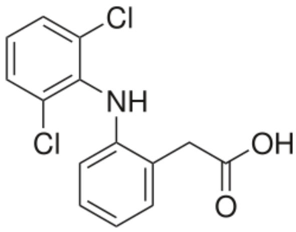

Fig. 23. Diclofenac

It is now known that some pharmaceuticals or drugs adversely affect aquatic organisms at concentrations as low as 10 ng/L (it is not an acute exposure but a continuous exposure to micro-pollutants). Diclofenac and carbamazepine, which may be harmful to the liver, kidneys and gills of fish, even at low concentrations, are among the problematic drugs. At concentrations above 1000 ng/L, compounds such as caffeine, metoprolic acid (a metabolite of the drugs metoprolol and atenolol), telmisartan and theophylline are released from the treatment plant outlet into the streams. These may also have a negative impact on the aquatic ecosystem (Mackuľak et al. 2018, Mackuľak et al. 2019, Mackuľak et al. 2020). In a study by Mackulak et al., 2018, the drugs most commonly found in surface waters are diclofenac, carbamazepine, clindamycin, atenolol, fexofenadine, metoprolol and valsartan; in terms of drugs, especially caffeine and methamphetamine (Mackuľak et al. 2018).

4. Occurrence of selected drugs, pharmaceuticals and their metabolites in the Danube river basin

Danube is the second biggest river in Europe with a total length of 2857 km before flowing out into Black Sea. It is a lifeline for about 111 millions of inhabitants, for many species of animals and a waterway between Central and South Eastern Europe. It connects different cultural and economic areas and landscapes. The River Danube flows through 14 countries. Together with its tributaries, it transports water to the Black Sea.

Pollution from industry, agriculture, municipal wastewater treatment plants is thus drifted either directly to the Danube river or indirectly through its tributaries. Monitoring of all forms of pollution in the Danube and its river basin is particularly important for the protection and sustainable use of the river basin. The pollutants enter the river basin environment by both purified and untreated sewage water, rainwater and sedimentation into the river sediment. Hydrophobic organic contaminants (HOCs), with a low water solubility, have the ability to firmly bind dispersed solids to form aqueous sediments upon settling (Rusina et al. 2019). The currently intensively researched micro-plastics having the ability to be carriers of a wide variety of micro-pollutants can also be included. (Mackuľak et al. 2020).

In recent years, research has focused on the presence of micro-pollutants in waters, which primarily include pharmaceutical products (especially contrast agents), pharmaceuticals, illegal drugs, cosmetics and disinfectants and their metabolites. These substances originate mostly from the outlets of municipal WWTPs and are transferred further into surface streams, water reservoirs and lakes. Micro-pollutants can have a long-term negative impact on surface waters and all components of the aquatic ecosystem (Mackuľak et al. 2019). Some types of micro-pollutants, such as pharmaceuticals, have high water solubility, allowing them to penetrate into groundwater (Radović et al. 2012).

International Commission for the Protection of the Danube River (ICPDR) performed in 2013 Joint Danube Survey 3 (JDS3) (Liška et al., 2015). The general objective of the JDS3 was to undertake an international longitudinal survey that would produce comparable and reliable information on water quality for the whole of the length of the Danube River including the major tributaries on a short-term basis. A part of the JDS3 was the investigation of Chemical and immunochemical analysis of anthropogenic markers and organic contaminants. Different anthropogenic markers indicative of human presence or activity have been selected to track the origin and type of contamination sources - caffeine, carbamazepine and the acesulfame (artificial sweetener) (Buerge, et al. 2003, Buerge, et al. 2009, Clara, et al. 2004 in Liška et al. 2015). Caffeine is efficiently removed (> 99%) in wastewater treatment plants (WWTP), while carbamazepine and acesulfame are not significantly degraded by activated sludge, thus passing almost unchanged to the receiving water bodies. Therefore, caffeine is suitable to indicate the presence of untreated wastewater, while the latter two indicate wastewater in general (treated or untreated). Untreated wastewater may contain very high levels of caffeine up to 500 μg/L, while the concentrations of acesulfame and carbamazepine are usually below 50 and 5 μg/L, respectively. Caffeine was abundantly present in the river Danube and its tributaries, the median concentration found in the Danube was 93 ng/L, while a slightly higher median concentration of 132 ng/L was observed in the tributaries. In Germany and most parts of Austria, caffeine concentrations were below the median of the whole river at 60 and 39 ng/L, respectively. An enormous increase of the caffeine concentration was observed at Hainburg (AT) and Bratislava (SK), which were both taken on the same day during intense rainfall. These high concentrations of caffeine hint at the presence of large amounts of untreated wastewater, which was most likely discharged by an overflow of combined sewers in the upstream regions.

The median concentration of carbamazepine was 30 ng/L in the Danube and 40 ng/L in the tributaries. Along the whole river span, the level of carbamazepine stayed rather constant at concentrations between 20 and 50 ng/L, showing much less variation than the level of caffeine The artificial sweetener, acesulfame, was found at median concentrations of 460 ng/L in the Danube and 470 ng/L in the tributaries.

The JDS4 was performed in 2019, the results, also on pharmaceuticals occurrence in selected sampling sites will be available soon.

So far, several studies have been carried out in the countries of the Danube basin to monitor the distribution of different types of pollution, mainly micro-pollutants (pharmaceuticals, drugs, contrast agents, metabolites, pesticides, or various persistent organic compounds). Water from the outlets of municipal WWTPs, water from the Danube and its tributaries, standing waters of the whole Danube basin region and also river sediments, were the subject of monitoring. One particular study by Škrbić et al. (2018), analyzed the presence of 940 semi-volatile organic compounds including sterols, polycyclic and aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), pesticides and other compounds in 10 river and channel sediments collected in the Northern Serbia region. These compounds were quantified by GC-MS/MS analysis. A total of 117 specimens of compounds were detected. Sterols, for example, were identified in every analyzed sample. Of the 452 pesticide specimens analyzed, 11 specimens with concentrations ranging from 0,564 to 61,6 μg/kg in dry matter were quantified. 47 out of 90 PCB species were quantified in amounts ranging from 0,928 to 32,1 μg/kg in dry matter (Škrbić, et al. 2018).

Study carried out in Serbia dealt with the occurrence of drug residues in the waters of the Danube and its tributaries. In five sampling periods, 70 surface and groundwater samples from 38 sampling sites in Serbia were analyzed by LC-MS/MS. Samples were analyzed for the presence of 14 types of pharmaceuticals, with traces of trimethoprim, carbamazepine, 4-FAA, 4-AAA and lorazepam detected. The penetration of these substances from surface water into groundwater has not been confirmed (Radović et al. 2012).

A study by Loos et al. was engaged in analysis of polar organic contaminants found in samples from the Danube river and its tributaries. The analysis was performed again by LC-MS/MS and a total of 34 kinds of polar organic compounds were analyzed. They focused mainly on the occurrence of drugs (ibuprofen, diclofenac, carbamazepine, sulfamethoxazole), pesticides and their degradation products (bentazone, 2,4-D mecoprop, atrazine, terbuthylazine, tenylterbutylazine), perfused acids and endocrine disruptors (nonylphenol, bisphenol A, estrone). Compounds such as 1H-benzotriazole (average concentration 185 ng/L), caffeine (87 ng/L), tolyltriazole (73 ng/L), nonylphenoxy-acetic acid (49 ng/L), carbamazepine (33 ng/L), 4 - nitrophenol (29 ng/L), 2,4 - dinitrophenol (19 ng/L), sulfamethoxazole (16 ng/L). The highest contamination was recorded in samples originating from Budapest and its countryside, from the tributaries of Arges (Romania), Timok (Bulgaria), Rusenski Lom (Bulgaria) and Velika Morava (Serbia) (Loos et al. 2010).

Another study carried out in Serbia monitored the occurrence of drugs in various samples of water from the Danube basin. Out of 81 analyzed pharmaceuticals, 47 were detected by LC-MS/MS in the municipal wastewater samples. The highest concentrations were achieved for ibuprofen (20.1 μg/L), carbamazepine metabolites: 10.11 - epoxy carbamazepine (16,2 μg/L) and 2 - hydroxycarbamazepine (15,9 μg/L). Pharmaceuticals such as ibuprofen, iopromide contrast agent were detected in concentrations up to 100 ng/L in the Danube surface water samples and in the ground and drinking water samples from the Novi Sad region (Petrović et al. 2014).

The occurrence of ibuprofen, caffeine, carbamazepine and benzotriazole in the surface waters of the Danube was also confirmed by a study by Milić et al. (2018) by LC-MS/MS. During a continuous annual monitoring, they found that carbamazepine and caffeine concentrations fluctuate seasonally in the range of 22 to 621 ng/L. The concentrations of ibuprofen in the analyzed samples ranged around 60 ng/L. Benzotriazole ranged at approximately 27 ng/L and sulfamethoxazole was also detected in trace amounts (approximately 3 ng/L) in some samples (Milić et al. 2018).

In Hungary, the occurrence of steroids and cholic acids in wastewater from two WWTPs and in samples from the Danube was investigated. The analysis was carried out using the GC-MS/MS methodology. The results show that mainly hormones such as androsterone (2,32 and 7,2 μg/L), trans-androsterone (4 and 5,81 μg/L) and estriol are present in the dissolved water phase in the wastewater from the inlet of WWTPs. Hormones such as β-estradiol (0,0049 to 0,032 μg/L), faecal sterols such as coprostanol (9,3 to 488 μg/L), cholesterol (0,79 to 427 μg/L) and phytosterols such as stigmasterol (0,17 to 48,5 μgl) and β-sitosterol (0,42 to 130 μg/L) were detected in the suspended phase at the outlet of WWTPs. Coprostanol (18,6 - 42,1 ng/L), cholesterol (88 - 170 ng/L), β-sitosterol (128 - 379 ng/L), β-estradiol (0,33 - 0,45 ng/L), ethinylestradiol (1,14 - 1,16 ng/L) and stigmasterol (23,3 ng/L) were detected in the dissolved aqueous phase in the Danube samples. The suspended phase contained ethinylestradiol (0,35 - 0,46 ng/L), coprostanol (218 - 263 ng/L), cholesterol (14,5 - 525 ng/L), stigmasterol (18,3 - 179 ng/L) and β-sitosterol (22,3 - 1780 ng/L) (Andrási et al. 2013).

The study of Nagy-Kovács et al. (2018) carried out in Budapest during a half-year period analyzed the occurrence and behaviour of 36 types of micro-pollutants in the surface and leachate of the Danube in the island parts of Csepel and Szentendre of the capital city of Budapest. Of the 36 analyzed substances, 12 were detected in all samples, including both surface water and leachate. Compounds such as bisphenol A, carbamazepine and sulfamethoxazole were also introduced into the leachate (only 20% of their total was removed during natural filtration through the gravel bed). However, 1H-benzotriazole, tolyltriazole, diclofenac, cefepime, iomeprol, metazachlor and acesulfame were detected to a much lesser extent (up to 78% less) in the leachate than in surface water (Nagy-Kovács, et al. 2018).

In the continuous monitoring of the occurrence of pollutants in the river Danube, Novak et al. tested an innovative passive sampling approach. A Dynamic Passive Sampler was employed as a mobile sampler from a ship cruising along 2130 km of the Danube River and sampling was performed in eight subsequent river stretches with two types of complementary passive samplers: silicone rubber sheets suitable for nonpolar compounds and SDR-RPS Empore ™ disks suitable for more hydrophilic compounds. Cross-calibration of the passive samplers enabled a robust estimation of pollutant concentrations with a wide range of physicochemical properties present in water. This type of passive sampler was suitable for sampling contaminants present in water even at concentration levels in pg/L. In total 209 of 267 analyzed compounds were detected in the samples (Novák et al. 2018).

In Romania, a study was carried out to monitor the occurrence of selected drugs in 2014. Samples were taken from tributaries and outlets from selected WWTPs (Brasov, Pitesti, Targu Mures), from surface waters before and after discharge from WWTPs and from selected sampling sites on the Danube and Danube Delta. The analysis was carried out by LC-UV method. As in the above research, ibuprofen, caffeine and acetaminophen dominated Romanian waters. The concentration of ibuprofen and caffeine at the inlet to the wastewater treatment plant was at a level of 20 μg/L, at the outlet approximately 8 μg/L and in surface waters around 5 μg/L. Other analyzed compounds were found in surface waters at concentrations up to 1 μg/L, except for diclofenac, which was detected at 2,12 μg/L (Gheorghe, Petre et al. 2016). Another Romanian study was that of Chitescu et al., which analyzed 67 types of drugs and antifungal agents in the Danube River by LC-MS/MS. A total of 20 samples from the Danube and its three main tributaries were analyzed over a period of 4 months. The analysis confirmed the occurrence of 23 compounds, of which pharmaceuticals such as diclofenac, carbamazepine, sulfamethoxazole, tylosin, indomethacin, ketoprofen, piroxicam and antifungal agents such as thiabendazole or carbendazim were the highest. The highest concentration reached was for diclofenac (166 ng/L) and the average carbamazepine concentration was around 40 ng/L (Chitescu et al. 2015).

Studies in the Danube region have also focused on the occurrence of antibiotic resistance genes, and it has been found that at least one ARG - qnrS (antibiotic resistance gene) correlates with the occurrence of a particular type of antibiotic (quinolones) in waters(Alygizakis et al. 2019).

Monitoring of the occurrence of five types of iodine contrast media was also carried out in the Danube basin and its tributaries in the territory of Hungary, Croatia and Serbia, with the highest concentration being detected in the town of Szekszard in Hungary (**Fig. 24**). This same study also analyzed the occurrence of carbamazepine, N-acetyl-4-aminoantipyrine and N-formyl-4-aminoantipyrine, with the highest concentration also detected in Szekszard (**Fig. 25**) (Storck et al. 2015).

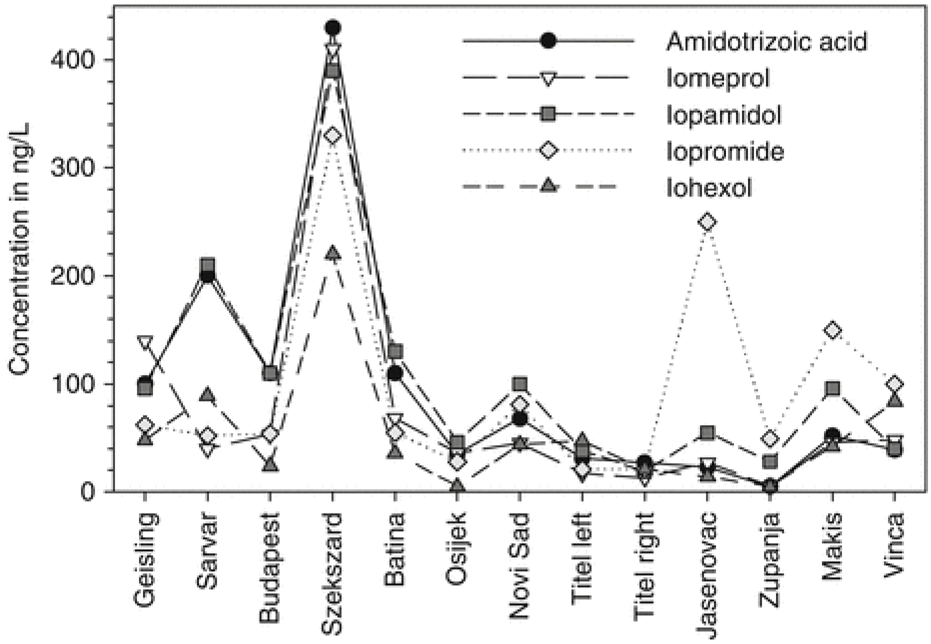

Fig. 24. The measured concentrations of 5 selected iodine contrast agents in sampling sites of Hungary, Serbia and Croatia

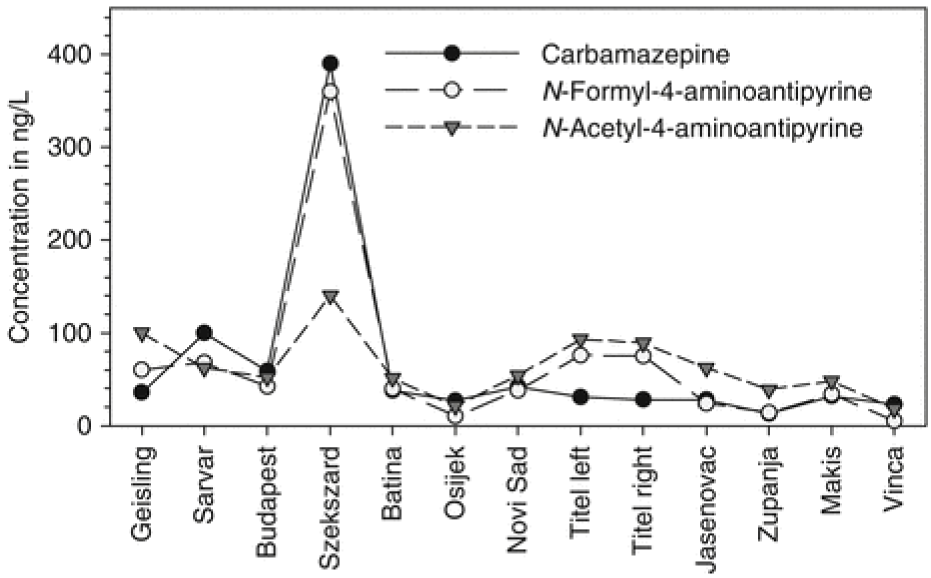

Fig. 25. The measured concentrations of carbamazepine, N – acetyl – 4 – aminoantipyrine a N – formyl – 4 – aminoantipyrine in sampling sites of Hungary, Croatia and Serbia

In Austria, a study was conducted to analyze surface water and sediment samples for the occurrence of 11 types of perfluorinated-alkylated compounds. The most frequently detected compounds in surface waters were perfluorooctanoate (21 ng/L) and perfluorooctane sulfonate (37 ng/L). Perfluoroalkyl carboxylates (about 1.7 μg/kg), perfluorooctanoate, perfluoroohexanoic acid and perfluorooheptanoic acid were detected in sediments originating in the Danube River at concentrations ranging from 0.1 to 5.1 μg/kg (Clara et al. 2009).

Another study conducted in Austria dealt with the occurrence of selected drugs (eg carbamazepine, roxithromycin, iopromide, ibuprofen, diclofenac, bezafibrate, tonalide, galaxolide) in wastewater (at the inlet and outlet of the municipal WWTP) and in groundwater in the Danube basin in the territory of Austria. The results of the study show that the above mentioned drugs were found in all samples from the inlet at the WWTP and drugs like diclofenac, carbamazepine or bezafibrate were also detected in groundwater samples. The analysis was performed by LC-MS / MS (Kreuzinger et al. 2004).

The study carried out in the territory of Slovenia and Croatia dealt with the occurrence of 48 different contaminants on the outflow from 3 selected Slovenian and 3 selected Croatian WWTPs, which flow into the Sava River. The surface water from the river was also analyzed. The analysis was carried out using the GC - MS/MS method and it was observed that, for example, caffeine and 4 - hydroxybenzophenone were found in high concentrations (49,600 ng/L and 28,900 ng/L) in the samples and were most commonly detected. Bisphenol B and E were first detected in samples from WWTP Velika Gorica. Bisphenol AP, CL2, P and Z were also detected in surface water in Europe for the first time (Česen et al. 2019).

The LC-MS/MS method was used in a study conducted in Croatia, which analyzed samples from two municipal WWTPs (Čakovec and Velika Gorica) as well as surface water samples from the Sava, Drava and Danube rivers for the presence of selected antibiotics and antimicrobial agents. The results show that antibiotics such as sulfamethoxazole, ciprofloxacin, erythromycin, clarithromycin or sulfapyridine are found in municipal wastewater samples in concentrations of the order of μg/L, with the highest concentration (4.7 μg/L) reached in the sample from WWTP Velka Gorica. In surface water samples, these substances were also detected but in the order of ng/L. Erythromycin was an exception, reaching similar concentrations in surface waters as in wastewater (Senta et al. 2008).

High concentrations of selected micro-pollutants (eg caffeine, carbamazepine, pentoxyfylline, ibuprofen, diazepam, galaxolide, tonalide and triclosan) were also detected in municipal wastewater samples from the WWTP outlet in Somes, Romania. Caffeine was detected at a concentration of up to 43,000 ng/L. Other micro-pollutants were detected at concentrations of about 800 ng/L. In the water reservoir at the Somes River, these micro-pollutants were detected at concentrations from 10 to 400 ng/L (Moldovan et al. 2008).

Several studies have also been carried out in the Danube Region to analyze the occurrence of selected micro-pollutants in groundwater. The results are summarized in the **Table 2**.

Tab. 2. The occurrence of selected micro-pollutants in groundwater samples from Danube region (Sui et al. 2015).

Compound	Number of samples	Detection frequence (%)	Concentration (ng/L)	Region
Azithromycin	44	5	LOQ - 68	Danube, Serbia
Ibuprofen	6	17	92	Serbia
Naproxen	6	17	27,6	Serbia
Salicylic acid	6	83	LOQ – 2,5	Serbia
carbamazepine	44	23	LOQ - 41	Danube, Serbia
propranolol	6	67	LOQ – 4,5	Serbia

LOQ – limit of quantification

Several studies have also been carried out in Slovakia on the occurrence of micro-pollutants such as pharmaceuticals, drugs or their metabolites, and resistant types of microorganisms in wastewater or surface water especially in the Danube basin. For example, a study conducted by Mackuľak et al. 2016 – 2018 monitored the occurrence of tramadol and other psychoactive pharmaceuticals, drugs and metabolites (citalopram, oxazepam, codeine, methadone, EDDP) in wastewater at the inlet of selected municipal WWTPs in Slovak cities. The results show that, for example, the amount of antidepressants in wastewater fluctuates seasonally, with the highest concentrations in winter (Mackuľak et al. 2016, Mackuľak et al. 2016, Mackuľak et al. 2017, Mackuľak et al. 2018).

In Slovakia, a similar research was carried out in the years 2017 - 2018, when 98 selected drugs, pharmaceuticals and their metabolites were analyzed in surface waters (rivers, reservoirs and tarns) **(Fig. 26a)**. More than 100 samples have been analyzed. The results of the analyzes indicate that the most common pharmaceuticals in surface waters are: diclofenac, carbamazepine, clindamycin, atenolol, fexofenadine, metoprolol and valsartan. Caffeine and methamphetamine are the most frequent drugs **(Fig. 26b).** In addition to monitoring, the ability of the outlet of a treatment plant to influence their occurrence in the recipient and the influence of several municipalities without the treatment plant on the overflowing recipient were examined. It is an important fact, that municipalities without WWTP are a significant source of pharmaceuticals and drugs for surface water. It was also found that caffeine, pharmaceuticals for cardiovascular diseases, depression, anxiety and epilepsy predominate in Slovak tarns and reservoirs (Mackuľak et al. 2018). The study was carried out throughout the year and it was found that the presence of drugs was influenced by the season (increase or decrease in biodegradation and photodegradation) as well as the use of seasonal pharmaceuticals such as antihistamines or antidepressants (Mackuľak et al. 2017).

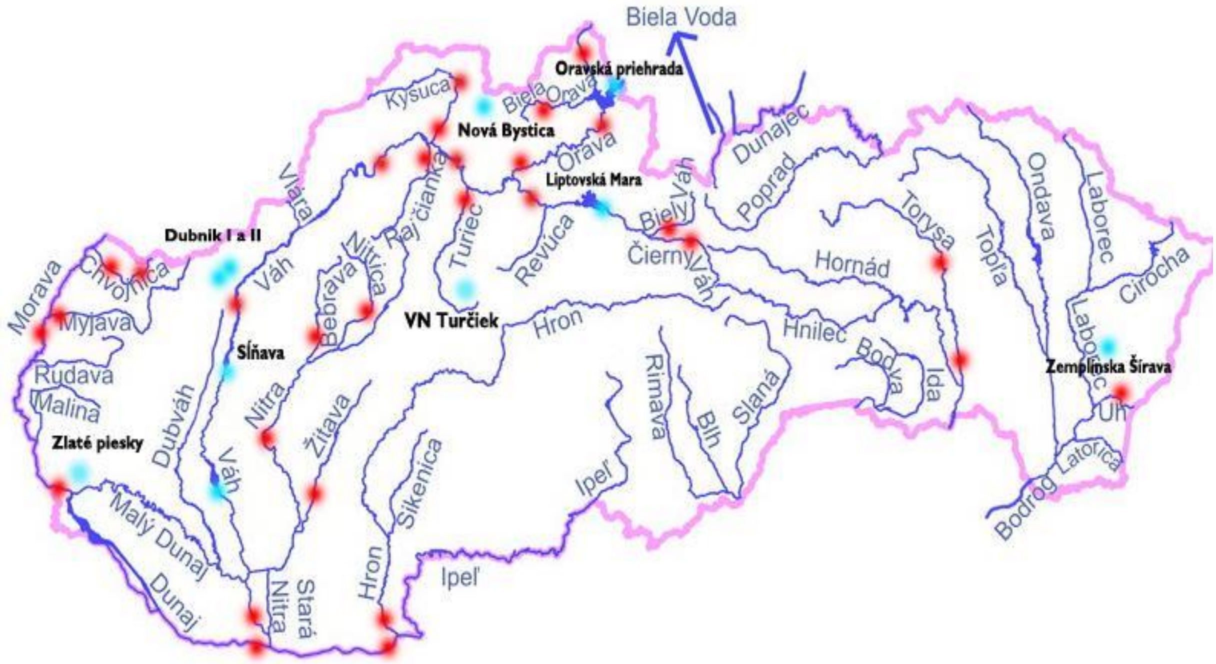

Fig. 26a. Map of selected sampling sites in Slovakia during 2017 – 2018 (Mackuľak et al. 2020).

Fig. 26b. Map of caffeine occurrence in selected Slovak rivers (Mackuľak et al. 2018, Mackuľak et al. 2020)

The study by Mackuľak et al. 2018 describes the presence of psychoactive drugs such as carbamazepine, tramadol or venlafaxine, as well as their metabolites in the river Váh or Danube mostly in concentrations up to 50 ng / l **(Fig.26d)**. Wastewater treatment plants, however, have a limited ability to capture other drugs and their metabolites as well, which is manifested by the presence of pharmaceuticals such as telmisartan, bisoprolol, atenolol, metoprolol and metoprolol acid in surface waters of the Váh and Danube rivers **(Fig. 26c).** Figure 26e describes the occurrence of caffeine in the rivers Váh and Danube. In contrast to psychoactive drugs, caffeine degrades in wastewater treatment plants with an efficiency of over 90%. The problem, however, is the high concentration of this compound at the inlet of the WWTP (Mackuľak et al. 2016), where it can also occur in amounts of micrograms per liter. In general, the occurrence of this compound in water is the evidence of human presence and activity, and its concentration in surface waters of the Váh and Danube rivers can reach concentrations above 300 ng/L **(Fig. 26e).** Antibiotics occur in surface waters of SR at maximum concentrations of tens of ng/L. However, this is mainly due to their behaviour in the environment. They are able to adsorb to sediments and thus concentrate in the aquatic environment, undergo bio or photodegradation, or effectively form complexes, for example in the presence of calcium ions **(Fig. 26f)** (Mackuľak et al. 2018).

Another study conducted by Birošová et al. investigated the occurrence of antibiotic resistant bacteria in sludge from selected Slovak WWTPs, the recipients of which are rivers in the Danube basin. The results show that sewage sludge contains a large number of bacteria resistant mainly to ampicillin or gentamicin, especially in winter. In the summer, the number of resistors decreased, mainly due to the fact that less antibiotics are consumed during the summer. In connection with this research, the occurrence of antibiotics in purified wastewater and outlet was also monitored and the results show that antibiotics that are able to pass into the recipient are mainly ciprofloxacin, azithromycin and clarithromycin (Birošová et al. 2014, Lépesová et al. 2018).

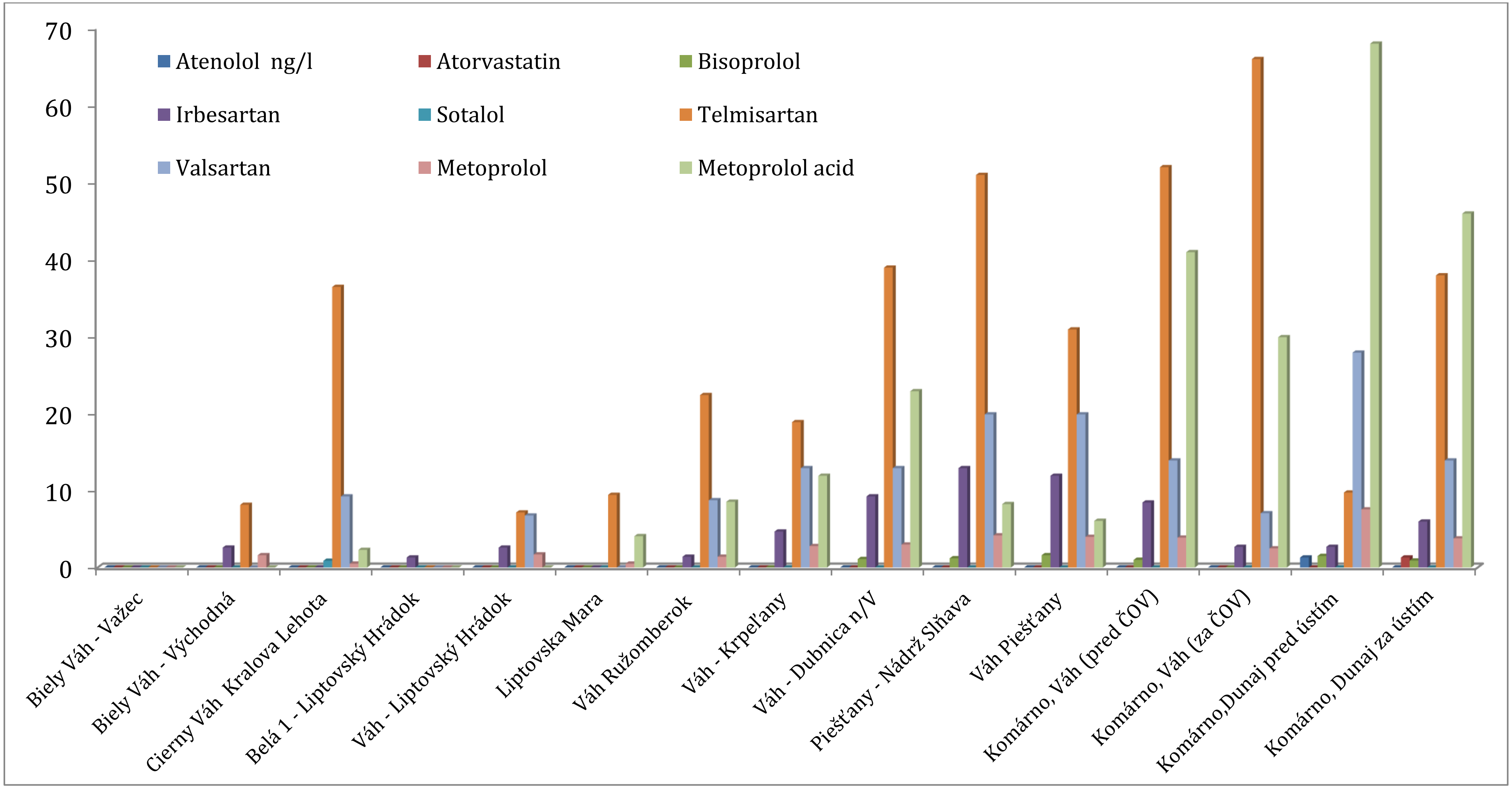

Fig. 26c. Occurence of the most frequently analyzed pharmaceuticals used for cardiovascular diseases in the river Váh and Danube in 2017 (Mackuľak et al. 2018, Mackuľak et al. 2020).

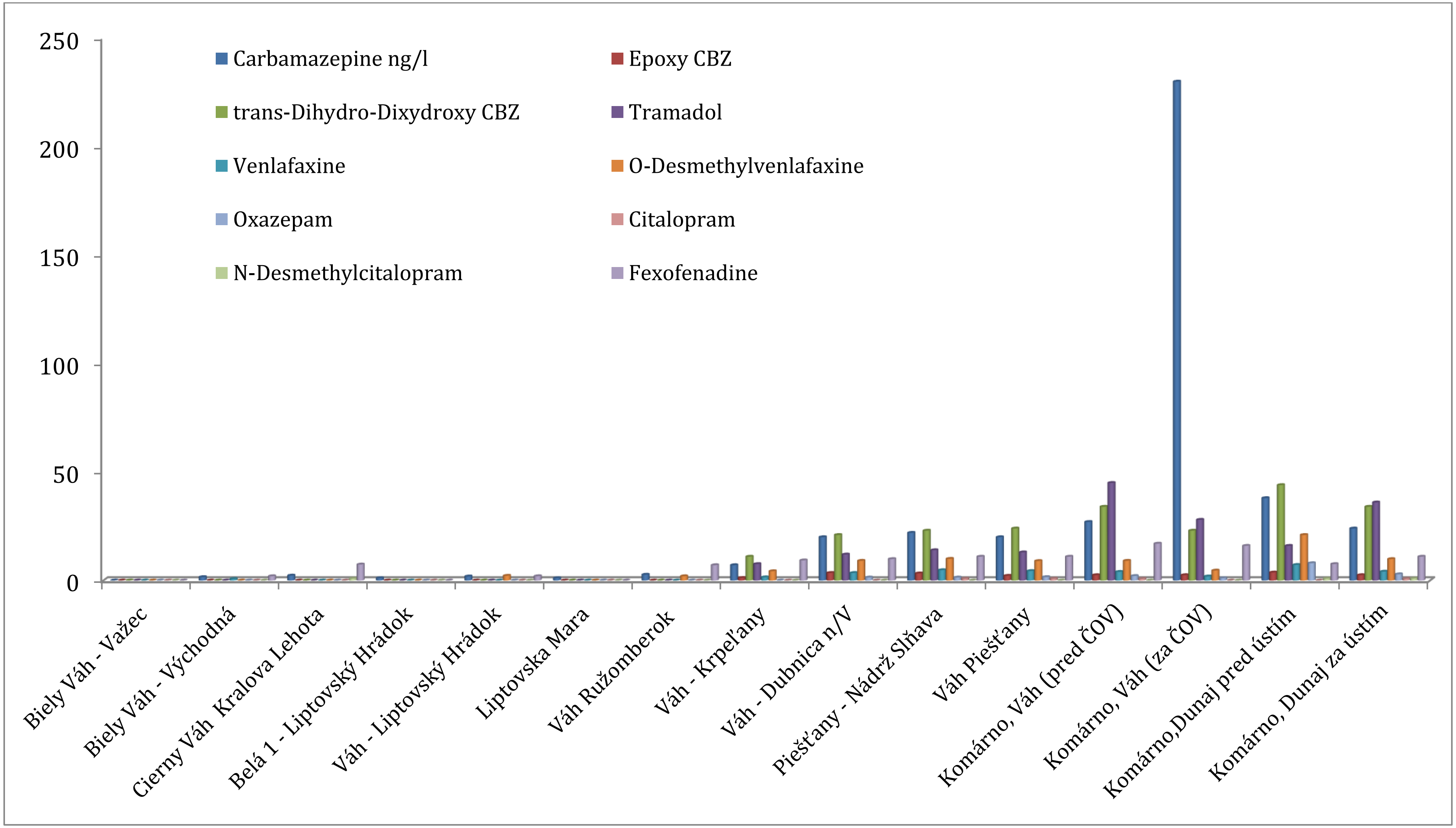

Fig. 26d. Occurence of the most frequently analyzed psychoactive drugs and their metabolites in the river Váh and Danube in 2017 (Mackuľak et al. 2018, Mackuľak et al. 2020).

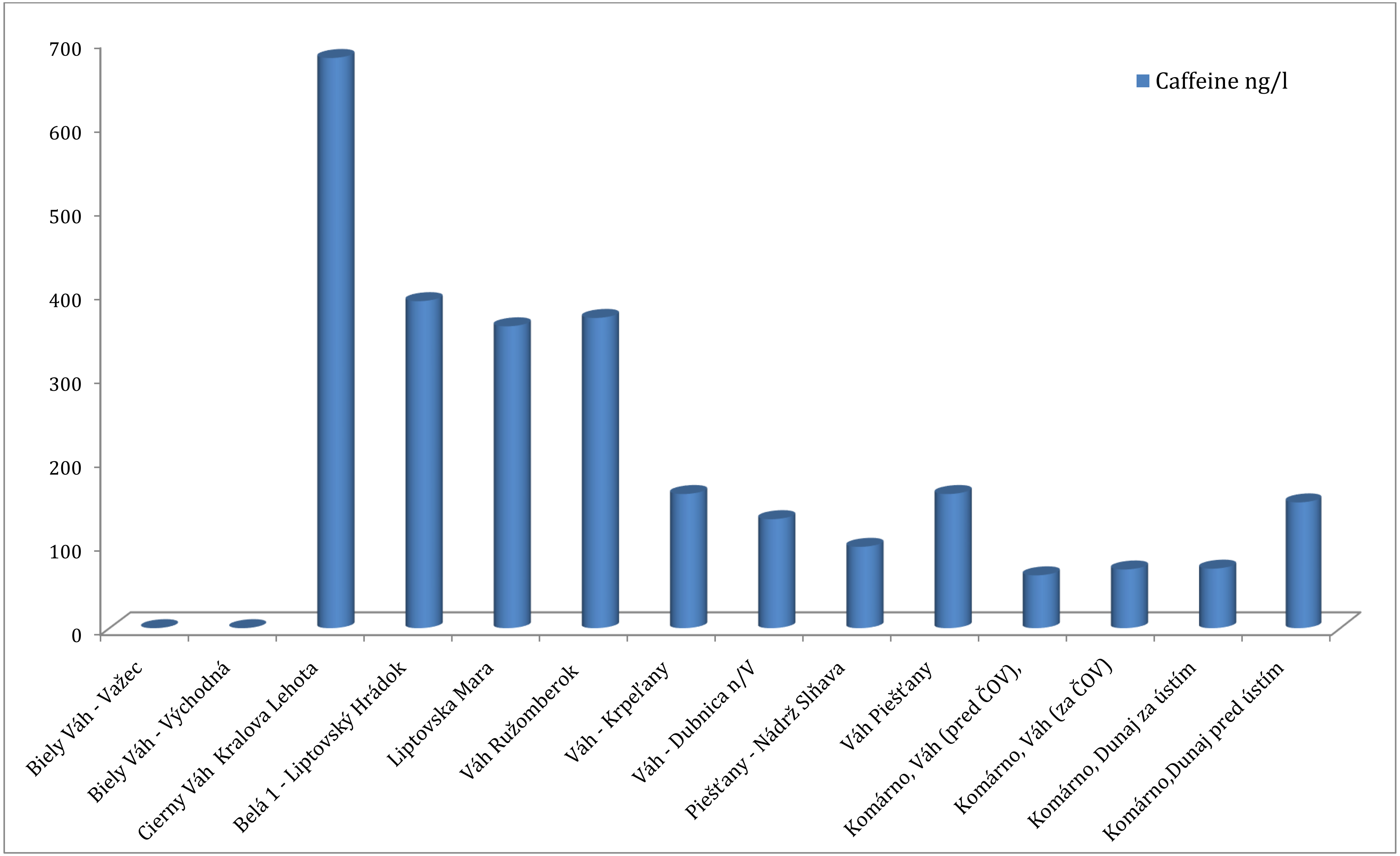

Fig. 26e. Caffeine occurence in river Váh and Danube in 2017 (Mackuľak et al. 2018, Mackuľak et al. 2020).

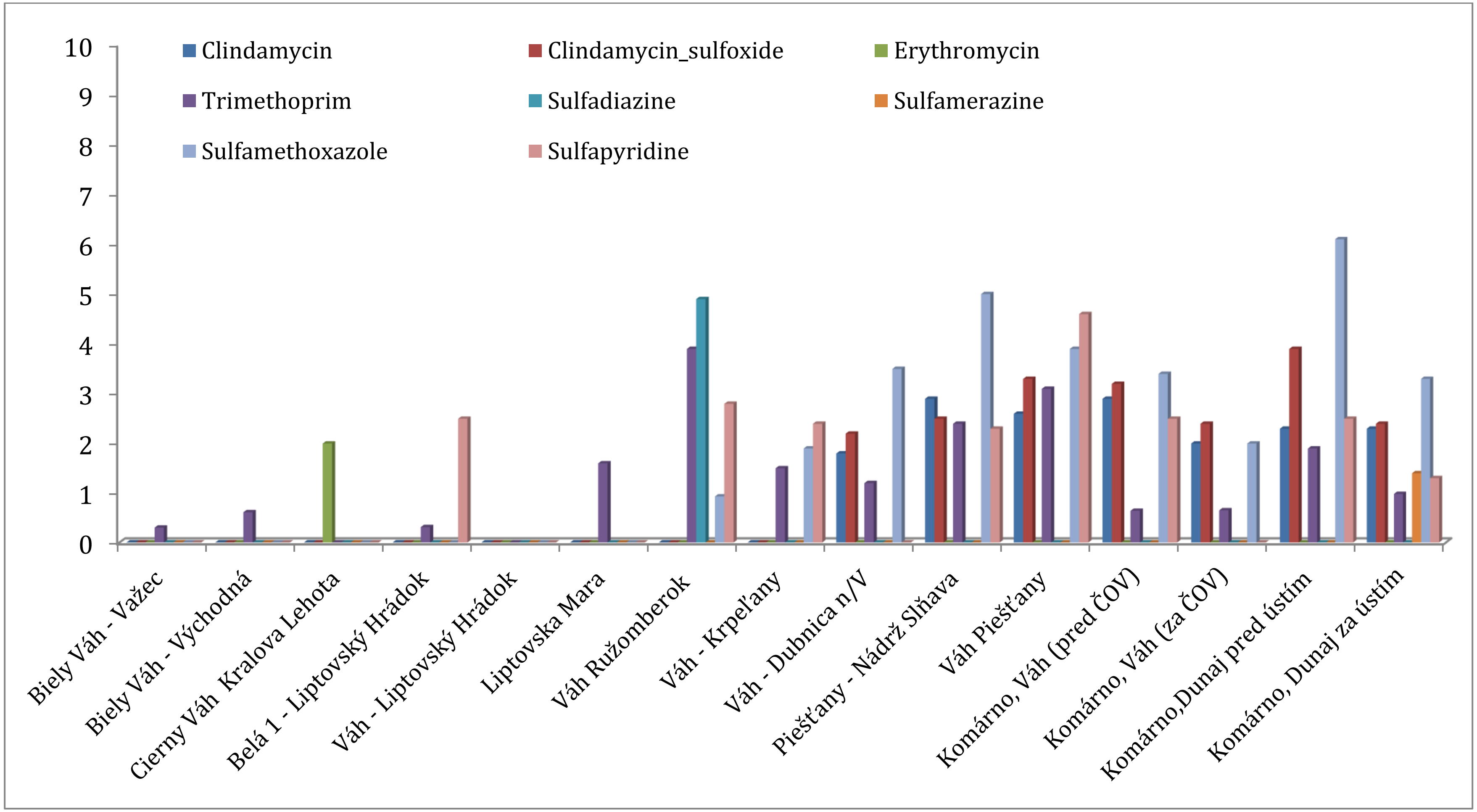

Fig. 26f. Occurence of the most prescribed antibiotics (ng/L) in rivers Váh and Danube in 2017 (Mackuľak et al. 2017, Mackuľak et al. 2020)

5. Monitoring of drugs, pharmaceuticals and their metabolites in wastewater

The households, healthcare services and old people´s homes are the important source of waste water contamination by pharmaceuticals.

With the advances in analytical chemistry, new possibilities are emerging to effectively identify compounds. These include micro-pollutants such as drugs, pharmaceuticals and their metabolites with concentrations in order of ng/L. This gives us the opportunity to monitor the occurrence and behaviour of these compounds from the sewage system, through the wastewater treatment plant to the aquatic ecosystem in the recipient. Nowadays, special attention is paid to the occurrence of psychoactive pharmaceuticals, contrast agents or antibiotics in wastewater. Wastewater monitoring carried out in several EU countries (e.g., England, Netherlands, Italy, Greece, or Spain) pointed out, that some frequently used pharmaceuticals which are not subject to degradation processes in the sewage system or directly at the WWTP, can reach concentrations of several tens of micrograms per litre (μg/L) at the inlet and outlet of a wastewater treatment plant. While most pharmaceuticals and drugs are removed with an efficacy of over 50%, their concentrations are often at levels > 0,1 μg/L (**Table 3**) (Castiglioni, et al. 2006, Roberts and Thomas 2006).

In Slovakia, pharmaceuticals such as tramadol, venlafaxine, diclofenac, carbamazepine, oxazepam and citalopram are the most common drugs found in the treated wastewater from municipal wastewater treatment plants in concentrations above 100 ng/L. In the world, the range of drugs with anti-inflammatory and analgesic effects ranges in concentrations up to 57 μg/L at the outlet from the WWTP. The highest measured concentrations were reported for tramadol (57 μg/L) which is an analgesic, ibuprofen (48 μg/L), carbamazepine (20 μg/L), diazepam (19 μg/L) diclofenac (11 μg/L), trimethoprim (6, 7 μg/L, erythromycin (6.3 μg/L), ciprofloxacin (5,7 μg/L), gemfibrozil (5,2 μg/L), sulfamethoxazole and roxithromycin (5,0 μg/L). However, drug concentrations differ already when comparing neighbouring districts, not to mention different countries or continents. In general the outlet from municipal wastewater treatment plants can be considered as a continuous source of surface water pollution by pharmaceuticals, drugs and metabolites (Mackuľak et al. 2014, Mackuľak et al. 2016, Mackuľak et al. 2016, Mackuľak et al. 2019, Mackuľak et al. 2020).

Tab. 3. Concentrations (ng/L) of selected pharmaceuticals on WWTP effluent in selected countries (Mackuľak et al. 2016).

Pharmaceutical compound	Germany	United Kingdom	Finland	Slovakia	Canada	South Korea
Analgesics						
Acetaminophen	≤ 6000	–	–	–	–	≤ 19
Dextropropoxyphene	–	≤ 585	–	–	–	–
Diclofenac	≤ 2100	≤ 2349	–	≤ 1500	≤ 748	≤ 127
Tramadol	–	–	–	≤ 2035	–	–
Hydrocodone	–	–	–	–	–	≤ 70
Ibuprofen	≤ 3400	≤ 27 256	–	–	≤ 773	≤ 137
Indomethacin	–	–	–	–	≤ 507	–

Pharmaceutical compound	Germany	United Kingdom	Finland	Slovakia	Canada	South Korea
Analgesics						
Ketoprofen	≤ 380	–	–	–	≤ 210	–
Codeine	–	–	–	≤ 200	–	–
Acetylsalicylic acid	≤ 1500	–	–	–	–	–
Naproxen	≤ 520	–	–	–	≤ 1189	≤ 483
Triclosan	–	–	–	≤ 50	≤ 324	≤ 32
***β*-blockers**						
Acebutolol	–	–	≤ 225	–	–	–
Atenolol	–	–	≤ 1180	≤ 300	–	–
Metoprolol	–	–	≤ 1600	≤ 400	–	–
Propanolol	–	≤ 284	–	–	–	–
Sotalol	–	–	≤ 1120	≤ 150	–	–
Antiepileptic drugs						
Carbamazepine	≤ 6300	–	≤ 2440	≤ 2000	–	≤ 729
Antidepressants						
Venlafaxine	–	–	–	≤ 535	–	–
Citalopram	–	–	–	≤ 350	–	–
Hypolipidemic drugs						
Bezafibrate	≤ 4600	–	–	–	–	–
Fenofibrate	≤ 30	–	–	–	–	–
Gemfibrozil	1500	–	–	–	≤ 436	≤ 17
Clofibrate	–	–	–	–	–	–
Contrast agents						
Iopromide	–	–	–	–	–	2630
Illicit drugs						
MDMA	–	–	–	≤ 100	–	–
Cocaine	–	–	–	≤ 180	–	–
Methamphetamine	–	–	–	≤ 420	–	–
Heroin	–	–	–	< 10	–	–
LSD	–	–	–	< 10	–	–
THC - COOH	–	–	–	< 50	–	–

When it comes to illegal drugs, mainly amphetamines (primarily methamphetamine) are dominant in Slovak and Czech wastewater. Methamphetamine concentrations may exceed 1 μg/L, which is a relatively high figure compared to Western European countries. In contrast, cocaine reaches very low concentrations compared to Western European countries. Cocaine concentrations are particularly high in economically developed countries with a high standard of living. Analyzes also show that MDMA (methylenedioxymethamphetamine - the active substance of the drug Ecstasy) is also found in the waters and is one of the most popular dance drugs used in Slovakia, for example during music festivals **(Table 4a,b)** (Mackuľak et al. 2019, Mackuľak et al. 2020).

Tab. 4a. Measured concentrations ranges of illicit drugs in wastewater from selected countries (Pal et al. 2013, Mackuľak et al. 2016).

Drug/Metabolite	Concentration ng/L					
	Slovakia (8)	**Canada (3)**	**Australia (1)**	**Spain (42)**	**England (4)**	**Belgium (41)**
Cocaine	4 – 96	209 – 823	–	4 – 4700	207 – 526	218
Benzoylekgonine	4 – 219	287 – 2624	52	9 – 7500	992 – 1229	603
Methamphetamine	63 – 953	pod 7 – 65	587	3 – 277	–	3,5
MDMA	7 – 73	9 – 35	187	2 – 598	6,4	35
Amphetamine	8 – 257	3 – 25	–	3 – 688	86 – 5236	59 – 206

Note: the number in parentheses represents the number of locations analyzed in the country

Table 4b. Measured concentrations ranges of psychoactive substances in wastewater at influent and effluent of selected WWTPs in Slovakia (Mackuľak et al. 2019).

Compound	Influent (ng/L)	Effluent (ng/L)	Removal efficiency (%)
Cotinine	820 – 13 000	24–230	84<
Codeine	ND-360	ND-150	45-62
Amphetamine	ND-160	ND-34	82<
Methamphetamine	13-1800	ND-420	54-76
MDMA	ND-58	ND-48	<17
Benzoylecgonine	ND-300	ND-130	40-64
Tramadol	180-2400	190-2200	<22
Cocaine	ND-170	ND-87	48-68
Venlafaxine	58-1300	66-1200	<22
Oxazepam	ND-230	ND-210	<26
Citalopram	ND-360	ND-340	<15
EDDP	ND-46	ND-44	<10
Methadone	ND-33	ND-25	<25
THC-COOH	ND-290	ND-47	84<

Several expert studies currently concern themselves with the occurrence of legal stimulants and their metabolites (nicotine, caffeine, cotinine) in wastewater. The main reason is their possible negative impact on various components of the aquatic ecosystem. The results of these studies indicate that during the year, the concentrations of these compounds in water vary. This is mainly because caffeine and nicotine are unstable in the sewage and wastewater treatment processes and are subject to various biological or chemical degradation processes during the year. Therefore, in the case of nicotine, its metabolite *cotinine* is most often analyzed. In wastewater, cotinine achieves a higher concentration than illicit drugs - it often exceeds 2 μg/L. The occurrence of cotinine in wastewater can also be applied to estimate the number of cigarettes consumed at a given site or during certain music festivals. Cotinine concentrations in wastewater in selected European cities are shown in **Table 5**. (Castiglioni,et al. 2015, Mackuľak, et al. 2015, Mackuľak et al. 2015).

Tab. 5. Cotinine concentration in wastewater from selected European cities (Mackuľak et al. 2016).

City/Country	Date of analysis	Sampling type	Concentration on WWTP influent (ng/L)
Banská Bystrica/Slovakia	2.12.2014	*d*	9868
Košice/Slovakia	19.2.2014	*d*	6377
Prešov/Slovakia	14.2.2014	*d*	4005
Lisabon/Portugal	10.10 – 11.11.2014	*m*	2590
Zürich/Switzerland	–	*w*	2650

Note: d – daily collection, w – weekle collection, m – monthly collection

Caffeine is also one of the frequently analyzed micro-pollutants in wastewater. Like other stimulants, caffeine undergoes a decomposition process in the sewage and wastewater treatment and its concentration at the WWTP outlet is often very low. Nevertheless, the interest in monitoring this stimulant in the water is constantly growing, as its increased incidence has been observed in surface water and several lakes. (Baz-Lomba, Salvatore et al. 2016). For example, in a study by Buerge et al., the authors analyzed caffeine at the inlet of selected WWTPs in Swiss cities. They found that the concentration of caffeine in wastewater fluctuated at different times of the year. The values at the inlet ranged from 7 to 73 μg/L. The specific load, calculated on the basis of the flow rate and the number of connected inhabitants, was around 15,8 mg per person per day. The authors also focused on the occurrence of caffeine in the environment. The results show that its occurrence in most Swiss rivers and lakes ranges from 6 to 250 ng/L. Its concentration in alpine lakes was less than 2 ng/L.(Buerge et al. 2003).
The concentrations of caffeine at the outlet of selected Slovak WWTPs are shown in **Table 6**.

Tab. 6. Caffeine concentration on effluent from selected Slovak WWTPs (Mackuľak et al. 2016).

Sampling location	Date of analysis	Caffeine concentration on WWTP effluent (ng/L)
BA – Vrakuňa	18.3.2014	224
Košice	19.2.2014	< 8
Zvolen	13.1.2014	< 9
BA – Petržalka	17.2.2014	11
Malacky	7.3.2014	< 12
Banská Bystrica	2.12.2013	14
Piešťany	21.3.2014	< 9
BA – Devínska Nová Ves	22.2.2014	< 7

5.1 Sludge – a potential source of pharmaceuticals found in agriculture soils

In terms of the penetration of micro-plastics, micro-pollutants and resistant microorganisms or their resistance genes into the environment and soil, it is possible to identify sewage sludge as one of the dominant sources. Sewage sludge mainly contains nutrients and valuable organic substances that improve the growth and yield of agricultural crops, the rheological properties of the soil and so on. At present, about 40% of the sludge produced in the EU countries is placed directly on agricultural land and another about 12% of the sludge is transferred indirectly to the soil as compost. The application of sewage sludge in agriculture varies considerably between the EU-28. In some countries (Denmark, Ireland, France, Spain and the United Kingdom), they use more than half of the sludge production in agriculture; on the other hand, in four countries (Malta, the Netherlands, Slovenia and Slovakia) no sludge is directly applied to agricultural land, and four others (Estonia, Finland, Hungary and Romania) use less than 10% of the total sludge production in agriculture. (Birošová et al. 2014, Ivanová et al. 2018, Mackuľak et al. 2020).

At present, about 640 small (over 5 P.E.) and 2 large WWTPs (Ružomberok - 325 000 PE and Spišská Nová Ves - 48 000 P.E.) operate in Slovakia on the principle of aerobic stabilization. The annual production of aerobically stabilized sludge is approximately 20 - 25% of the total sludge production in Slovakia. Due to the relatively good quality of sludge (WWTPs for small municipalities, cities without industrial pollution), it can be used on agricultural land (indirectly as compost). They carry out the anaerobic sludge digestion process at 51 municipal WWTPs, with approximately 2.6 mil. P.E., where about 75 - 80% of sludge is produced in Slovak WWTPs. Sludge produced in municipal WWTPs is under continuous surveillance (Ivanová et al. 2018, Kozáková 2019).

The annual municipal sludge production has ranged between 53 000 - 59 000 t (CL) in recent years, which represents an average specific sludge production of 10.4 kg/year/resident (Ivanová et al. 2017, Ivanová et al. 2018, Mackuľak et al. 2020). Data on sludge production and its use in the Slovak Republic are summarized in the **(Table 7)** (Ivanová et al. 2018).

Tab. 7. Sludge production in Slovakia and its utilization (Kozáková 2019).

Year	**Amount**	**Sludge production (tons of dry matter)**				
		app. on the soil	**Compost**	**Landfill**	**Energy recovery**	**Others**
2004	53 085	12 067	30 437	4723	0	5858
2006	54 780	0	39 405	9245	0	9400
2008	57 810	0	38 368	8676	0	10 766
2010	54 760	923	47 140	16	0	6681
2012	58 706	1254	46 261	1615	3196	6195
2014	56 883	8	36 524	1073	16 038	3240
2016	53 054	0	34 695	2359	10 975	5025

From the above table it is clear that at present, about 65% of the Slovak sludge gets to agricultural land as compost, direct application to the soil has been completely reduced, some of the sludge is landfilled and some is used for energy production (incineration).

The occurrence of selected pharmaceuticals in sludge is summarised in the **Table 8.**

Table 8. The occurrence of selected pharmaceuticals in sludge (Mackuľak et al. 2020).

Pharmaceuticals	Sludge ng/g DM	OSR ng/g DM
Antibiotics		
Amoxicillin	ND	–
Azithromycin	ND – 666	81 – 1220
Chloramphenicol	ND	ND
Clarithromycin	ND – 537	4,6 – 580
Ciprofloxacin	1400 – 4800	1780 – 160 000
Clindamycin	ND – 6,5	–
Enrofloxacin	7,6 – 11560	ND – 4247
Erythromycin	6 – 79	13 – 50
Erythromycin-H_2O	–	1,6 – 183
Lincomycin	ND – 4967	8,3 – 8,7
Minocycline	1000 – 2000	121 – 2630
Ofloxacin	1480 – 5760	150 – 8140
Oxytetracycline	ND – 3790	8,3 – 114
Sulphamethoxazole	ND – 84	1,5 – 51
Sulphamethazine	ND – 55	ND
Tetracycline	ND – 466	ND – 2790
Tylosin	31 – 139	ND
Vankomycin	ND	ND
Antifungal/antimicrobial		
Miconazole	ND – 2609	1,4 – 1100
Triclocarban	362 – 8460	1200 – 48 100
Triclosan	354 – 15 600	2000 – 19 700
Pharmaceuticals		
Salicylic acid	ND – 13743	–
Diclofenac	ND – 133	ND – 34
Ibuprofen	ND – 3988	ND – 490
Codeine	ND – 79	ND – 110
Fenoprofen	ND	–
Naproxen	ND – 1022	2,9 – 273
Carbamazepine	ND – 50	163 – 238
Beta-blockers		
Atenolol	ND – 86	ND
Metoprolol	ND – 226	ND
Propranolol	ND – 849	ND – 5
Hormones		
Estrone	ND – 18	70 – 280
Estriol	ND – 49	–
17 alfa -etinylestradiol	ND – 17	–
Contrast agents		
Iohexol	–	–
Iopromide	76 – 84	–
Iopamidol	–	–
Stimulants		
Caffeine	ND – 805	18 – 643

Note: ND – not detected, OSR – organic substances recycled from wastewater

A study by Professor Bodík, published in 2018, dealt with the contamination of aerobically and anaerobically stabilized sludge from selected wastewater treatment plants in Slovakia, to which wastewater from approximately 600,000 inhabitants is supplied. The highest total concentration of the investigated pharmaceuticals was in sludge from the treatment plant Devínska Nová Ves and Senec (11,800 and 11,300 ng/g in dry sludge). Fexofenadine (on average 2340 ng/g in dry sludge from WWTP - Devínska Nová Ves) was the most abundant drug in the sludge.
Telmisartan in the sludge from WWTP – Senec, reached an average value of 1170 ng/g in dry matter. The study subsequently estimates that by distribution to agricultural land, pharmaceuticals in quantities exceeding tens of kilograms may enter soil in Slovakia, namely: fexofenadine 120 kg/year and verapamil 29 kg/year. Studies in this area are also gradually examining the possibility of subsequent re-penetration of pharmaceuticals or drugs from the soil into agricultural crops. Testing of various types of salads, potatoes and other vegetables is going on. Similar to aquatic plants, several papers confirm the penetration of pharmaceuticals through the root system and consequently the rapid distribution to various parts. Substances enter the root by diffusion, which depends on their chemical properties (molecular weight below 300, number of H-donors below 3, H-acceptors below 6 or hydrophobicity) (Kodesova et al. 2015, Ivanová et al. 2018).

6. Monitoring of drugs, pharmaceuticals and their metabolites in surface water

From 2014 a drug monitoring has been carried out on several rivers in the Czech Republic. For five years, a team of authors from the University of South Bohemia in České Budějovice closely monitored the presence of various groups of pharmaceuticals in several local streams (Vltava, Labe and others). Currently, in addition to pharmaceuticals, it is possible to identify a number of legal and illegal drugs or different types of their metabolites in surface waters. Analyzes conducted in the Spanish rivers have provided some insight into the concentrations of nicotine, one of the most commonly used legal drugs. Its concentration range varied from 72 to 3875 ng/L. Since nicotine decomposes already in the sewer network, differences in the measured concentration were caused by the sampling site and the distance from the outlet of the treatment plant. This is the reason why most scientific papers concern themselves with the detection of *cotinine*, rather than the parent compound itself.(Castiglioni, et al. 2015, Mackuľak et al. 2015).

Based on the results of several studies **(Table 9)**, it can be concluded that concentrations of drugs and their metabolites in rivers correspond with the drug situation in the countries through which these rivers flow. Except for a secondary marijuana metabolite (THC-COOH), most of the drugs and their metabolites are relatively resistant to degradation processes, as shown mainly in concentrations of cocaine (and its metabolite - benzoylecgonine) in some rivers of Western Europe. Again, it is important to recognize the sampling site or the season of the year. It is also interesting that the presence of illicit drugs and their metabolites in Italian rivers and lakes has triggered research on the identification of sources of these substances, as well as a study on their environmental impacts.(Zuccato et al. 2010).

Tab. 9. The occurrence of psychoactive compounds (ng/L) in surface waters of selected European rivers (Kotyza et al. 2009, Pal et al. 2013, Mackuľak et al. 2017, Mackuľak et al. 2018).

River (country/year)	**BE**	**Cocaine**	**MDMA**	**MF**
Ebro (Spain/ 2007 – 2008)	11,4	1,4	1,0	0,4
Llobregat (Spain/ 2007)	77	6 – 23	3 – 14	1,3
Sar (Spain/-)	316	30	–	–
Olona (Italy/2006)	183	44	1,7	1,7
Po (Italy/2006)	3,7 – 25	0,5 – 12	0,2	–
Thames (England/2005)	11,2	4,7	3,6	–
22 rivers (Switzerland/2010)	1,6	0,4	–	–
Váh (Slovakia/2016)	20	–	7	2
Trnávka (Slovakia/2016)	21	2	14	107
Danube (Slovakia/2017)	ND	ND	ND	ND
Morava (Slovakia/2017)	ND	ND	ND	ND
Hornád (Slovakia/2017)	ND	ND	ND	ND
Torysa (Slovakia/2018)	ND	ND	ND	ND
Nitra (Slovakia/2017)	ND	ND	ND	5,4
Uh (Slovakia/2017)	ND	ND	ND	4,8
Myjava (Slovakia/2017)	1,4	ND	ND	ND

ND – not detected (under detection limit), BE – benzoylecgonine, MDMA – 3,4-methylendioxy-N-metylamphetamine, MF – methamphetamine

Besides psychoactive compounds, other kinds of pharmaceuticals occur in European surface waters (Zhou et al. 2019) – see **Table 10**.

Tab. 10. The occurrence of selected pharmaceuticals in European surface waters (Zhou et al. 2019).

Pharmaceuticals	Mean concentration (ng/L)
Ciprofloxacin	657
Diclofenac	247
Carbamazepine	183
Caffeine	885
Ibuprofen	337
Tramadol	1127
Telmisartan	110
Valsartan	1507
Amoxicillin	201

Currently, research focuses on monitoring the occurrence of psychoactive pharmaceuticals and drugs in drinking water **(Table 11)**. The data indicate that the concentration limit of 100 ng/L is only exceeded in isolated cases. However, carbamazepine, which may reach higher values, is an exception. Recent studies in this area have shown the presence of three pharmaceuticals in particular (diclofenac, clofibric acid and carbamazepine) (Bexfield, Toccalino et al. 2019, Peng, Gautam et al. 2019). Research in the field of possible contamination of drinking water sources by pharmaceuticals, drugs and their metabolites is also being carried out in Slovakia (more than 120 compounds are analyzed). Over the period from 2016 to 2019, more than 60 samples of drinking water (also from water mains) were taken. Illegal drugs or their metabolites in concentrations above the limit of detection were not found in any of the collected samples. The analysis of legal drugs (such as caffeine) and pharmaceuticals or their metabolites in drinking water in the Slovak Republic still carries on (results of the analyzes are available on the website - https://analyzuj-slovensko.webnode.sk).

Table 11. The occurrence of illicit drugs and their metabolites (ng/L) in groundwater and Public water networks in selected countries (Pal et al. 2013, Mackuľak et al. 2016).

City / year	Country	Morphine	Cocaine	MDMA	BE	KK	EDDP	Methadone
Barcelona / 2010	ESP	1,4	3,8	3,9	1,5	0,1	0,7	7,4
43 cities / 2008 – 2009	ESP	–	0,4	–	–	–	0,4	0,2
Toledo / 2010	ESP	–	–	–	–	–	–	0,99
–	JPN	–	–	–	–	–	0,1	–
–	Europe *	–	0,1	–	0,2	–	0,4	0,1
–	Latin America †	–	0,6	–	4,5	–	0,4	0,2

ESP – Spain, JPN – Japan *countries: France, Germany, Slovakia, Switzerland, Austria, United Kingdom; †countries: Brazil, Chile, Columbia, Panama, Peru, Uruguay; MDMA – 3,4-methylendioxy-N-methylamphetamin, BE – benzoylecgonine, KK – cocaethylene (cocaine metabolites), EDDP - 2-ethyliden-l,5-dimethyl-3,3-diphenylpyrolidine (methadone metabolite)

Clofibric acid was first detected in drinking water over 20 years ago in Lower Saxony (Germany), where they had used contaminated surface water (~ 1 μg/L) for field irrigation for nearly 40 years. Sludge from surrounding WWTPs was also applied to agricultural land. Later, the whole area was subjected to groundwater analyzes searching for more than 50 compounds. The results of the study confirmed the presence of two pharmaceuticals (carbamazepine and sulfamethoxazole) in drinking water and two contrast agents used for X-ray examinations. These compounds are stable in the environment; they are not subject to degradation or sorption processes, which helps them to penetrate into groundwater (Storck et al. 2015, Sui et al. 2015). Sources of contamination that can induce the presence of drugs in drinking water are generally considered as point sources. In many cases, we have witnessed that contaminated surface water was used to irrigate agricultural land. Along with leakages from landfills and sewers, this gradually leads to contamination of the groundwater by various pharmaceuticals. However, such groundwater contamination is mostly local and it is not possible to describe the distribution of individual drugs on a global scale on its basis (Sui et al. 2015).

In terms of contamination of drinking water sources, it is important to determine whether it is a surface or groundwater source. Surface sources are generally more susceptible to possible contamination. In Slovakia, more than 84% of drinking water comes from underground sources. In the Czech Republic, this figure is only around 47%. The rest of the drinking water - that is 53% - comes from surface water sources. This is one of the reasons why analyzes of some sources of drinking water have already been carried out in the Czech Republic (in Slovakia, analyzes concerning the occurrence of pharmaceuticals or drugs in drinking water are not being systematically carried out). In addition to pharmaceuticals, the performed analyzes also focused on hormonal substances. For example, they found oestrogens in the Želivka reservoir, which is the main source of drinking water for Prague.(Morteani et al. 2006).

6.1 The impact of drugs, pharmaceuticals and their metabolites on the environment

In addition to water, micro-pollutants also enter soil. In this case, the micro-pollutants originate from various compounds feed for the treatment and prevention of livestock diseases. These veterinary preparations contain various types of pharmaceuticals, especially antibiotics, which are used to increase the weight gain of livestock. Wastewater from these facilities are mostly not directed to the sewage system, but are trapped in reservoirs and subsequently applied to fields as fertilizer. In this way, the non-metabolized pharmaceuticals enter the soil, where their further fate is often unknown, but they may penetrate into agricultural crops (Mackuľak et al. 2016).

In addition to surface waters, micro-pollutants also occur in groundwater. Besides these sources, this contamination can also originate from landfill leaks and residues from production of pharmaceuticals, leaks from septic tanks and the pollution interacts with surface water and groundwater. Surface water contamination depends on several factors. These include, for example, the wastewater treatment technology used in the wastewater treatment plant, the volume of water discharged from the wastewater treatment plant, the size and type of the recipient, the season of the year, the water temperature, the intensity of sunlight or the type of sediment found in the recipient. Because of the dilution effect, the concentration of drugs, pharmaceuticals and their metabolites is much lower than that at the outlet of WWTPs (Sui et al. 2015).

The degradation of these compounds may be affected by the action of microorganisms and their communities directly in water or in river sediment, by abiotic routes or by a combination of these processes. In addition to biodegradation, direct or indirect photodegradation can occur in surface waters. While direct photodegradation is caused by the absorption of sunlight in the region of 290 nm, indirect photodegradation occurs in the presence of photosensitizers and is mainly affected by UV-A radiation (315-400 nm)) and UV-B (280-315 nm). Direct photolysis of drugs and pharmaceuticals depends on the ability of the molecules to absorb sunlight. The decomposition of compounds is also influenced by the intensity of solar radiation, but also by the location of the molecule in the river (Kotyza et al. 2009, Mackuľak et al. 2016). Simple schematic representation of the behaviour and penetration of pharmaceuticals, drugs and their metabolites in various parts of the environment is shown in **Fig. 27.**

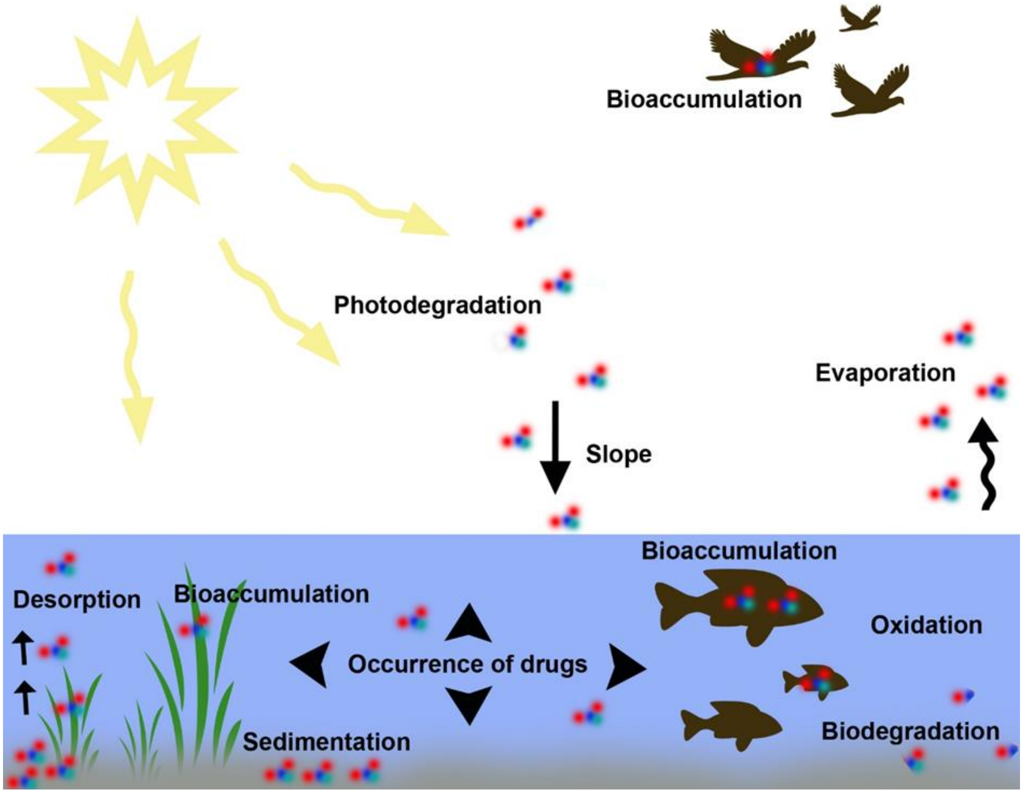

Fig. 27. Simple schematic representation of the behaviour and penetration of pharmaceuticals, drugs and their metabolites in various parts of the environment

A study by Trixier et al. investigated the occurrence and degradation of pharmaceuticals (carbamazepine, diclofenac and ibuprofen) in surface waters. Carbamazepine has been found to be largely able to withstand the degradation processes - as a result of which it reaches groundwater. Photodegradation was the dominant degradation method for diclofenac. Sedimentation was the dominant method for the removal of ibuprofen. Ibuprofen is one of the most commonly used pharmaceuticals in Europe. Other pharmaceuticals and drugs that have been identified in surface waters are codeine, fenoprofen, hydrocodone, ketoprofen, naproxen, phenazone, tramadol, methamphetamine and others. The presence of various psychoactive drugs and their metabolites in surface waters such as tramadol (25-381 ng/L) or venlafaxine (18-122 ng/L) and their dominant metabolites (o-desmethyltramadol, o-desmethylvenlafaxine) in the water of German rivers is also a worrying finding (Tixier et al. 2003).

Research suggests that certain concentrations of pharmaceuticals are constantly present in surface streams. The most commonly identified pharmaceuticals include ibuprofen, carbamazepine, naproxen or diclofenac. The occurrence of caffeine in the surface waters of Costa Rica, where they grow coffee in large quantities is also interesting. In production areas, caffeine concentrations in surface waters reached values up to about 1,1 mg/L (Spongberg et al. 2011). Hormones of interest (estradiol and estrone) have not been identified in surface waters as they are subject to degradation, biodegradation and sorption processes (Mackuľak et al. 2016). Of the frequently used antibacterial substances for disinfection, triclosan also occurs in surface waters. This compound has come to the attention of researchers in connection with antibiotic monitoring and the emergence of resistant microorganisms in the environment (Birošová and Mikulášová 2009). The incidence of pharmaceuticals in surface waters in selected countries is shown in **Table 12**.

Table 12. The occurrence of pharmaceuticals (ng/L) in surface waters from selected countries (Pal et al. 2013, Mackuľak et al. 2016, Mackuľak et al. 2020).

Compound	**Country**						
	CAN	CHN	CRC	FRA	GER	GRE	GBR
Ibuprofen	0,9	ND – 1417	5	ND – 8	–	1 – 67	0,3 – 100
Naproxen	1	ND – 328	–	ND – 6,4	–	3 – 322	0,3 – 149
Ketoprofen	–	–	7	ND – 22,0	–	0,4 – 39,5	0,5 – 14
Diclofenac	–	–	14	ND – 35,0	–	0,8 – 1043	0,5 – 261
Mefenamic acid	–	–	–	–	–	–	0,3 – 169
Carbamazepine	3	–	1	ND – 31,6	10 – 1194	–	0,5 – 684
Gemfibrozil	–	–	41	–	–	–	
Atenolol	–	–	–	ND – 34	–	–	1 – 560
Trimethoprim	–	–	–	–	–	–	7 – 122
Triclosan	0,4	35 – 1023	11	–	124 – 220	3 – 39	5 – 95
Galaxolide	–	–	–	–	35 – 1814	–	–
Tonalide	–	–	–	–	5 – 273	–	–
Estrone	–	ND – 65	–	–	–	–	–
Estradiol	–	ND – 2	–	–	–	–	–
Estriol	–	ND – 1	–	–	–	–	–
Caffeine	–	–	24	–	–	–	–

Note: ND – not detected (under detection limit), CAN – Canada, CHN – China, CRC – Costa Rica, FRA – France, GER – Germany, GRE – Greece, GBR – Great Britain

7. Pilot study

The pilot study area for investigation of the occurrence of selected pharmaceuticals in the surface water, groundwater and sediments was selected in Slovakia. The sampling sites distribution covers the whole area of Slovakia including the rivers (also close to WWTP), dams and groundwater wells in order to obtain the representative overview of the current situation related to selected pollutants concentrations.

7.1 Sampling

During the year 2019 fifty three water samplings were taken from which 32 surface water samples **(Table 12)**, 15 groundwater samples **(Table 13),** 6 sediments **(Table 14)**. The sampling was performed by Water Research Institute, Bratislava. In these samples about 100 chemical compounds were analysed **(see Table 1** in Chapter 3. – page 8**).**

Tab. 12. List of sampling sites – surface waters

Surface water	Sampling site	Sampling date	
Bodrog	Streda n/Bodrogom	10.4.2019	8.10.2019
Dunaj	Szob, stred	3.4.2019	16.10.2019
Hornád	Hidasnémeti	9.4.2019	7.10.2019
Ipeľ	Salka	12.4.2019	14.10.2019
Morava	Devín	8.4.2019	21.10.2019
Poprad	Piwniczna	11.4.2019	1.10.2019
Hron	Kamenica	9.4.2019	14.10.2019
Vajskovský potok	Dolná Lehota nad Chatou	11.4.2019	2.10.2019
Slaná	Sajopüspöki	9.4.2019	7.10.2019
Váh	Komárno	9.4.2019	14.10.2019
VN Sĺňava		21.10.2019	
VN Hriňová		22.10.2019	
VN Klenovec		22.10.2019	
VN Teplý vrch		23.10.2019	
VN Málinec		23.10.2019	
VN Kráľová		24.10.2019	
VN Budmerice		28.10.2019	
VN Môťová		29.10.2019	
VN Ružiná		29.10.2019	
VN Petrovce		30.10.2019	
VN Ľuboreč		30.10.2019	
VN Kunov		31.10.2019	

Tab. 13. List of sampling sites - groundwaters

Sampling site	Sampling date
Holíč	7.9.2019
Senica n/Myjavou	7.9.2019
Jarovce – Hrádza	6.9.2019
Bratislava – Vrakuňa	6.9.2019
Komárno	6.9.2019
Štúrovo	4.9.2019
Krásno n/Kysucou	5.9.2019
Hrabové	5.9.2019
Považany	5.9.2019
Nitrianska Streda	6.9.2019
Sliač	4.9.2019
Rimavská Sobota	4.9.2019
Prešov – Haniska	3.9.2019
Košice – Krásna	3.9.2019
Michalovce - Betlenovce	3.9.2019

Tab. 14. List of sampling sites - sediments

Sampling site
Oravská priehrada
Veľké Kozmálovce
Liptovská Mara
Zemplínska Šírava
Veľká Domaša
Sĺňava

Description of groundwater sampling

Groundwater sampling was carried out according to the following standards:
STN ISO 5667-11: Water quality. Sampling. Part 11: Groundwater sampling guidelines
STN EN ISO 5667-14: Water quality. Sampling. Part 14: Guidelines for quality assurance and quality management in environmental water sampling and handling
STN EN ISO 5667-1: Water quality. Sampling. Part 1: Guidance on the design of sampling programs and sampling techniques
STN EN ISO 5667-3: Water quality. Sampling. Part 3: Instructions for preserving and handling water samples
ČSN ISO 17289 Water quality. Determination of dissolved oxygen. Optical sensor method
STN EN 27888: Water quality. Determination of electrolytic conductivity
STN EN ISO 10523: Water quality. Determination of pH
STN 757375: Water quality. Determination of temperature

Description of surface water sampling
The sampling of surface water was carried out in accordance with STN EN ISO 5667
Description of sediments sampling

The sediment sampling itself was governed by the requirements of ISO 5667 Parts 1, 4, 12, 14 and 15 and Guidance document No. 25 on Chemical monitoring of sediment and biota under the Water Framework Directive. The monitoring of sediment quality was carried out in accordance with Article 3 (1). 2 of the Directive 2008/105 / EC on environmental quality standards in the area of water policy transposed into our legislation by the Government Regulation no. 270/2010 Coll. and Decree of the Ministry of Agriculture, Environment and Regional Development of the Slovak Republic No. 418/2010 Coll., Section 4, Para. 5, point f.

For the purposes of monitoring sediments in water reservoirs, one mixed sample was taken from a site located as close as possible to the dam wall (usually at its centre level). The mixed sample was formed from four simple samples from the top 10 cm of the sediment layer. For the sampling of sediments, we used the sampling device CORER 90 from the company UWITEC **(Figure 28)** in accordance with STN EN 5667-12: 2001 Water quality. Sampling. Part 12: Guidance for collection of bottom sediments.

Fig. 28 Sampling device CORER 90 from the company UWITEC

7.2 Analysing of samples by LC – MS/MS methodology

Analyses were provided on TSQ Quantiva triple – stage quadrupole mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) (water and sediment analysis), both of which were coupled with Accela 1250 LC and Accela 600 LC pumps (Thermo Fisher Scientific, San Jose, CA, USA) and HTS XT – CTC autosamplers (CTC Analytics AG, Zwingen, Switzerland) were used to separate and detect the target compound. Samples of surface and groundwater were analyzed by methodology from study Mackuľak et al. (Mackuľak, Medvecká et al. 2020). Samples of sediments were pre – treated by extraction methods and analyzed by methodolody from studies Golovko et al. and Ivanová et al. (Golovko et al. 2014, Ivanová et al. 2018).

7.3 Results and discussion

In the present report, over 100 selected pharmaceutical, drugs, contrast agents and their metabolites were analyzed in more than 50 samples of groundwater, surface waters and sediments collected from all over Slovakia analyzed by LC-MS/MS methodology. Individual sample sets were divided into 32 surface water samples, 15 groundwater samples and 6 sediment samples. The results obtained from surface water samples indicate that caffeine, which was identified in the range of 12 to 290 ng/L, is the dominant of all analyzed micro-pollutants (Slaná Sajopüspöki 7.10.2019). Caffeine was subsequently identified in concentrations above 200 ng/L also in the samples "Váh Komárno 9.4.2019" or in the sample "water reservoir Môťová 29.10.2019". The lowest concentrations were reached in the sample "Vajskovský p. Pod Chatou 11.4.2019" - 12 ng/L or in the sample "water reservoir Klenovec 22.10.2019". Mackuľak et al. 2018 also observed similar results of caffeine occurrence in Slovak surface waters in the study, where caffeine was the dominant micro-pollutant among the 98 pharmaceuticals and drugs analyzed. A study by Loos et al. 2010 was also engaged in analysis of polar organic contaminants found in samples from the Danube river and its tributaries. The most commonly identified compounds also included caffeine (87 ng/L). In the samples from the Danube (samples - Danube Szob Stred 3.4 and 16.10.2019), the caffeine concentrations were 25 and 160 ng/L. This seasonal variation in caffeine concentration in the river is also confirmed by the study Milic et al.2018. During continuous annual monitoring, they found that carbamazepine and caffeine concentrations fluctuate seasonally in the range of 22 to 621 ng/L (Milić, et al. 2018). However, the presence of caffeine can also be influenced by the occurrence of plants in the riverbed that have the ability to accumulate it significantly (Mackuľak, et al. 2018).

In the groundwater examined in this report, caffeine was also at concentrations that exceeded the other analyzed micro-pollutants (range from 2.7 to 160 ng/L - Komárno In sediments, caffeine was identified in four samples out of six. The maximum concentration in the sediment sample of Velké Kozmálovce was 21 ng/g. In addition to caffeine, the contrast substance iopromide (max. 280 ng/L - "Poprad Piwniczma 1.10.2019") (Annex 1 Graph 15) was identified in surface waters above 250 ng/L. It is commonly used in radiographic studies such as intravenous urograms, cerebral computed tomography (CT) and CT pulmonary angiograms (CTPA). In contrast to caffeine, however, we have often observed that the concentration of the contrast agent is below the detection limit in some samples. It is necessary to realize that mainly medical facilities are the source of this compound and from there it reaches the wastewater, although the patient may excrete the compound in the form of urine or excrement for several hours, even in at home. At present, we have limited information on dominant sources, on the spread of contrast media in sewage, wastewater treatment plants and the environment, and on the mechanisms that may affect their behaviour or decomposition – i.e. fluctuations in surface waters (Mackuľak et al. 2020). In the Danube basin and its tributaries on the territory of Hungary, Croatia and Serbia, a similar monitoring of occurrence of selected types of iodine contrast substances was performed, including iopromide. The concentration of this compound in the Danube varies considerably. For example, in the town of Jasenovac, a value of about 300 ng/L was measured (Storck et al. 2015). In the Danube samples taken for this report, the concentration of contrast agent iopromide ranged from 41 to 43 ng/L - Danube Szob Stred

(Annex 1 Graph 15). In the sample from the Morava River, concentrations of up to 190 ng/L (Morava Devin 8.4.2019) were measured, in Hron 150 - 220 ng/L (Hron Kamenica) and in the Poprad River 280 ng/L (Poprad Piwniczma 1.10. 2019) (Annex 1 Graph 15). The results obtained from groundwater in the Slovak Republic indicate in this report that iopromide does not have a significant ability to contaminate groundwater. Similarly, it was not found in sediment samples.

In addition to illegal drugs and their metabolites, MDMA, cocaine, THC and their metabolites or even methamphetamine itself are found in the surface waters of European rivers (Pal, et al. 2013, Mackuľak, et al. 2016, Mackuľak, et al. 2019). This is largely dependent on the national drug scene of the individual states through which rivers flow and the ability of wastewater treatment plants to remove these micro-pollutants. Cocaine and its metabolites are therefore often identified in Western Europe. In the Czech or Slovak rivers it is methamphetamine (Golovko et al. 2014, Mackuľak et al. 2020). However, concentrations of drugs or their metabolites are in trace amounts below 50 ng/L (Mackuľak, et al. 2018). These results are also confirmed by surface and groundwater samples. The surface waters analyzed in this report indicate that e.g. methamphetamine concentrations reached a maximum of 2 ng/L (Annex 1 graph 17). Amphetamine and cocaine have not been identified; MDMA identified in only one sample had a concentration close to the limit of detection - 0.92 ng/L (Hornád Hidasnémeti 9.4.2019) (Annex 1 graph 17). Metabolite of heroin (6-acetylmorphine) was also successfully identified only once - sample Ipeľ Salka 9.4.2019 (concentration 1.1 ng/L). The metabolite benzoylecgonine was analyzed in several surface water samples at concentrations up to 1.3 ng / l. However, the highest concentrations were observed for the secondary metabolite of THC (THC is the active ingredient of the marijuana drug). The maximum concentration was reached in the sample of VN Málinec 23.10.2019 (20 ng/L) and Danube Szob Stred 3.4.2019 (19 ng/L) (Annex 1 graph 17). In groundwater, methamphetamine was identified in two samples at a concentration of about 1 ng/L (Nitrianska Streda and Komárno) and in one sample cocaine (Nitrianska Streda) was identified (Annex 1 graph 6). With the exception of amphetamine, there were not found any illegal drugs and their metabolites in the sediments collected for this report. Amphetamine, however, was identified in five samples out of six, ranging from 9 to 32 ng/g - Veľké Kozmálovce (Annex 1 graph 1).

Antibiotics occur in surface waters of SR only at concentrations of tens of ng/L. This is mainly due to their behaviour in the environment. Antibiotics are a wide range of compounds that are able to withstand various degradation processes in the environment. Some also have the ability to adsorb to sediments and thus concentrate in the aquatic environment, undergo bio- or photodegradation, or effectively form complexes - for example in the presence of calcium ions (Mackuľak et al. 2016, Mackuľak et al. 2018). Their presence in the environment is currently being studied intensively, not only by chemists, but also by biochemists and microbiologists. The reason is the ability of these compounds to induce resistance in microorganisms at concentrations below 1000 ng/L (Birošová, et al. 2014). The results of the analysis carried out in this report indicate that antibiotics and their metabolites are found in surface waters at a significantly lower concentration compared to waste water. Their occurrence is at most tens of ng/L. In surface waters (Annex 1 Graph 13), only three (clarithromycin, clindamycine and erythromycine) were successfully identified among several of the antibiotics and their metabolites analyzed. One metabolite of the antibiotic clindamycin has also been successfully identified (Annex 1 Graph 13). In groundwater, two antibiotics (clarithromycin and erythromycin) were identified at concentrations below 12 ng/L, with clarithromycin being identified in each sample and erythromycin in only one sample (Annex 1 Graph 9). This suggests the ability of these two antibiotics to penetrate into groundwater in Slovakia. In the sediments, the occurrence of antibiotics was observed in all samples only in the case of azithromycin (maximum concentration in the sample "Sĺňava" 7.6 ng/g) (Annex 1 graph 5).

Psychoactive pharmaceuticals are another currently intensively investigated group of drugs. This group of substances is chemically quite diverse, resulting in considerably different environmental behaviour. There are studies which, in particular for antidepressants (eg venlafaxine), show their ability to influence the behaviour of aquatic animals at concentrations below 500 ng/L (Mackuľak, Černanský et al. 2019). In particular, carbamazepine, tramadol, venlafaxine, citalopram, oxazepam and their metabolites are most commonly studied. Metabolites often have higher concentrations in surface or wastewater compared to parent compounds. Several psychoactive drugs and their metabolites in surface water, groundwater and sediments were analyzed in the present report (Annex 1 graph 2, 7, 12). Their concentrations in surface waters were found to vary considerably. E.g., tramadol was identified in all surface water samples ranging from 0.56 ng/L (Vajskovský p. pod Chatou 11.4.2019) to 160 ng/L (Váh Komárno 14.10.2019). Carbamazepine was identified in 26 out of 32 samples ranging from 0.65 ng/L (VN Teplý vrch 23/10/2019) to 86 ng/L (Ipeľ Salka 9.4.2019). Other frequently identified drugs were: venlafaxine (22 times at a concentration of up to 17 ng/L), oxazepam (22 times at a concentration of up to 14 ng/L), citalopram (13 times at a concentration of up to 1.1 ng/L), but also their metabolites such as trans-dihydro-dihydroxy CBZ (24 times at a concentration of up to 140 ng/L VN Môťová 29.10.2019), oxcarbazepine, O-Desmethylvenlafaxine, Epoxy CBZ and norsertraline (Annex 1 graph 12). Similar results, in particular for carbamazepine, have been measured in several scientific studies carried out on the Danube (Loos, et al. 2010, Radović, et al. 2012, Milić, et al. 2018, Nagy-Kovács, et al. 2018) The presence of metabolites in the Danube has also been confirmed by several studies (Petrović, et al. 2014).

In Slovakia, a study conducted by Mackuľak et al. 2018, monitored the occurrence of tramadol and other psychoactive drugs, drugs and metabolites (citalopram, oxazepam, codeine, methadone, EDDP) in wastewater for inlet and outlet from selected municipal wastewater treatment plants in Slovak cities, also connected to the Váh and Danube basins. The results show that the amount of antidepressants (venlafaxine, citalopram) in wastewater fluctuates seasonally, with the highest in the waters during the winter (Mackuľak, et al. 2016, Mackuľak, et al. 2016, Mackuľak, et al. 2017, Mackuľak, et al. 2018), which thus also affects their occurrence in surface and groundwater.

Psychoactive drugs have also been successfully identified in groundwater. The highest concentrations were achieved by carbamazepine, which was identified in 12 of 15 samples (from 0.86 to 31 ng/L BA - Vrakuňa). In addition to carbamazepine, its trans-dihydro-dihydroxy carbamazepine (5 out of 15 samples) and Epoxy carbamazepine (7 out of 15 samples) metabolites were also identified. Furthermore, tramadol and oxazepam were indetified. Their concentrations were below 5 ng/L in all samples (Annex 1 graph 7). Similarly, results confirming the penetration of psychoactive drugs into groundwater were also observed in a study (Nagy-Kovács et al. 2018), suggesting a limited ability of natural filtration to retain carbamazepine in particular. In the sediments, the active substances citalopram (in 5 samples of 6 in concentrations up to 14 ng/g - Veľké Kozmálovce), carbamazepine (up to 1.4 ng/g - Veľké Kozmálovce), venlafaxine (up to 4.5 ng/L Liptovská Mara) or sertraline were found (up to 3.1 ng/g - Veľké Kozmálovce). It has been found that the metabolites of these pharmaceuticals are better adsorbed into the sediments. For example, N-Desmethylcitalopram (in 5 samples of 6 at a concentration of up to 11 ng/g - Veľké Kozmálovce), O-Desmethylvenlafaxine (at a concentration of up to 3.6 ng/g - Veľké Kozmálovce), Oxcarbazepine at a concentration of up to 52 ng/g - Sĺňava (Annex 1 graph 2).

Antihistamines are another group of pharmaceuticals under investigation. It is now known that, as with antibiotics or antidepressants, their concentrations may vary over the year, not only in wastewater but also in surface water (Pal, et al. 2013, Golovko, et al. 2014, Mackuľak, et al. 2014, Mackuľak, et al. 2020). Only a limited number of antihistamines were analyzed in samples taken from several Slovak sites. Two drugs - cetirizine and fexofenadine - have been successfully identified in surface waters. Their concentrations fluctuate throughout the year, which can be observed, for example, on samples taken from a single site but at different times of the year (Annex 1 graph 19). Fexofenadine was identified in 24 out of a total of 32 surface water samples. In these samples the concentration ranged from 1.4 ng/L to 61 ng/L - Hornád Hidasnémeti 9.4.2019. Cetirizine was identified similarly, in 23 samples out of a total of 32 surface water samples. In these samples, its concentration ranged from 1.2 ng/L to 47 ng/L - Hornád Hidasnémeti 9.4.2019 (Annex 1 graph 19). The occurrence of antihistamines in surface waters may be due to the limited ability of WWTPs to remove them, but may also be washed up by precipitation from soil to which sewage sludge has previously been applied (Ivanová, et al. 2018, Mackuľak, et al. 2019). Their ability to adsorb may be one of the reasons why they have not been identified in groundwater. Identification in sediments confirms this claim by their occurrence in all samples. The incidence of fexofenadine ranged from 0.48 to 9.5 ng/g - Veľké Kozmálovce, in cetirizine the value in sediments was from 0.2 to 3.5 ng/g - Veľké Kozmálovce (Annex 1 graph 4).

Antihypertensive drugs and their metabolites are another group that are being studied for their incidence, behaviour and environmental impact. This includes a chemically heterogeneous group of compounds that are removed from wastewater with varying efficacy on WWTPs (Mackuľak et al. 2016). For this reason, some drugs are able to exceed concentrations of 50 ng/L in surface waters. From the measured values for these drugs in surface waters, it can be observed that telmisartan reaches the highest concentration, with values ranging from 2.5 ng/L to 270 ng/L - Morava Devín 21.10.2019. Valsartan, which belongs to the same category of drugs, was present in concentrations from 1.2 ng/L to 40 ng/L Slaná Sajopuspoky 7.10.2019 (Annex 1 graph 16). Ivanova et al. 2018 describes one of the possible ways in which these drugs can reach agricultural land and consequently into surface, groundwater and sediments. This study points to the ability of telmisartan to adsorb to sewage sludge (the drug concentration in sewage sludge from the Senec treatment plant had an average value of 1170 ng/g sludge dry matter). Ivanova et al. 2018 estimates that by application to Slovak soils, pharmaceuticals in quantities exceeding tens of kilograms can be introduced into it, namely e.g. for fexofenadine 120 kg/year and verapamil 29 kg/year. Studies in this area are also gradually investigating the possibility of subsequent, re-penetration of pharmaceuticals or drugs from soil into agricultural crops (Kodesova, et al. 2015, Ivanová, et al. 2018, Mackuľak, et al. 2018). Irbesartan, metoprolol and its metabolite - metoprolic acid (Mackuľak, et al. 2018) can often be identified in surface waters of Slovakia. The results obtained in this study also point to the possible occurrence of sotalol and atenolol in surface waters of Slovakia. However, their concentrations do not exceed 50 ng/L (e.g. metoprolol was identified in 23 of 32 samples ranging from 1.1 to 49 ng/L Hornád Hidasnémeti 9.4.2019). The study Mackuľak et al. 2018, which carried out monitoring of selected types of drugs in surface waters of the Váh and Danube rivers points to the metabolite of the drugs metoprolol and atenolol - metoprolol acid - as one of the frequently occurring micro-pollutants in surface waters with a concentration even above 50 ng/L (Mackuľak, et al. 2018). This can also be seen in the results of this report, where this metabolite was identified up to 27 times out of 32 surface water samples. Its concentrations ranged from 2.8 to 91 ng/L - VN Môťová 29.10.2019 (Annex 1 graph 16).

In groundwater, antihypertensive drugs and their metabolites may also occur. This depends on their physicochemical properties (their ability to adsorb to soil and resist biodegradation and other degradation processes). Analyzes of groundwater sampling in this report indicate that, in particular, the metabolite metoprolol acid, bisoprolol, metoprolol, valsartan and ibersartan are able to penetrate into groundwater. However, their occurrence is in trace amounts, the highest concentration was measured for the metabolite metoprolol acid - 8.5 ng/L BA - Vrakuňa (Annex 1 graph 10). In samples of sediments, antihypertensive drugs and their metabolites point to the abovementioned ability of these types of drugs to adsorb to solid particles, in this case to surface water sediment (Annex 1 graph 3). In addition to valsartan, all drugs in this group that occur in surface waters have also been identified in sediments. Telmisartan was the only one identified in all sediment samples ranging from 1.1 to 27 ng/g - Veľké Kozmálovce; bisoprolol, verapamil and metoprolol were identified in 5 sediment samples out of 6 (Annex 1 graph 3). Diclofenac, like carbamazepine, is one of the most commonly studied drugs in the environment. This drug is often identified in both surface water and drinking water, and is therefore often the subject of various studies. Diclofenac is highly resistant to biological and chemical processes in sewage treatment plants (its removal efficiency ranges mostly between 20-90% - influenced by many factors such as season, water temperature or the treatment technology itself). It has also been found that diclofenac can already have toxic effects at low concentrations, especially on aquatic animals - it can damage the liver, kidneys and gills of fish (Mackuľak, et al. 2016, Mackuľak, et al. 2020). Diclofenac is one of the frequently used analgesics in Slovakia. Tramadol and venlafaxine are most commonly found in purified wastewater from Slovak municipal WWTPs in concentrations above 100 ng/L (diclofenac) (Mackuľak, et al. 2016).

Diclofenac was found in 25 samples out of 32 surface water samples. Its concentration range was from 0.87 to 72 ng/L - Hornád Hidasnémeti 9.4.2019 (Annex 1 graph 14). In groundwater, diclofenac occurred in 3 samples out of 15 samples and only in trace concentrations from 0.57 to 1.7 ng/L - Nitrianska Streda (Annex 1 graph 8).

In addition to the drugs already described, other drugs have been identified in surface, groundwater and sediments. In surface waters, these include sulfamethoxazole (an antibiotic from the sulfoamide family), sulfapyridine (an antibacterial drug), trimethoprim (an antibacterial drug), lamotrigine (an antiepileptic drug similar to carbamazepine), memantine (belongs to a class of anti-dementia drugs).

In surface waters, lamotrigine occurs most frequently (22 samples) and in the highest concentrations (90 ng/L Morava Devín 21.10.2019) from among these drugs. Similarly, sulphamethoxazole was identified 22 times out of 32 samples, the highest concentration was 52 ng/L - Hornád Hidasnémeti 9.4.2019. Other drugs did not exceed 30 ng/L in any of the samples (Annex 1 graph 18). In groundwater, these compounds were also identified but their concentrations did not exceed 10 ng/L. The most concentrated drug was sulfamethazine at a concentration of 6.1 ng/L - Holíč and sulfamethoxazole 4.2 ng/L Považany (Annex 1 graph 8).

In the sediments, two drugs were identified last. These are miconazole (an antimicotic) and alfuzosin (a remedy for prostate problems). Miconazole was identified in all sedimen samples. However, its concentrations were below 0.5 ng/g. Alfuzosin was identified in 3 sediment samples below 0.35 ng/g (Annex 1 graph 5).

7.4. Conclusion of pilot study

Resulting from the work on pilot area, 101 selected pharmaceuticals, drugs and their metabolites were analyzed by LC-MS / MS methodology in 53 samples of groundwater, surface water and sediments collected from all over Slovakia (sampling done by WRI) – see **Table 1,** page 8. Individual sample sets were divided into 32 surface water samples, 15 groundwater samples and 6 sediment samples **(Table 12, 13, 14** – pages 40,41**).**

Results achieved in individual points:

- Of the 101 micro-pollutants analyzed, up to 50 pharmaceuticals, drugs and their metabolites were identified in 32 surface water samples.
- Iopromide – a contrast agent, was identified 21 times in concentrations up to 280 ng/L in surface water.
- The drug with the highest concentration in surface waters was caffeine, with a concentration ranging from 12 to 290 ng/L. Among the drug metabolites, the highest concentration was reached by the secondary metabolite of compound THC (marijuana drug) of 20 ng/L.
- Pharmaceuticals with the highest concentrations in 32 surface water samples of Slovakia were telmisartan (concentration up to 270 ng/L), tramadol (up to 160 ng/L), lamotrigine (up to 90 ng/L), carbamazepine (up to 86 ng/L), diclofenac (up to 72 ng/L), fexofenadine (up to 61 ng/L), sulfamethoxazole (up to 52 ng/L), metoprolol (up to 49 ng/L), cetirizine (up to 47 ng/L), valsartan (up to 40 ng/L). 6-acetylmophine (heroin metabolite), alprazolam, amitryptyline, atenolol, benzoylecgonine (cocaine metabolite), bezafibrate, citalopram, codeine, dihydro CBZ, oxcarbazepine and epoxy CBZ (metabolites of carbamazepine), erythromycin, MDMA (drug ecstasy drug), MDEA (metabolite MDMA), memantine, metamphetamine, mirtazapine, norsertraline, rosuvastatin, sulfamethazine, sulfamethizole, trimethoprim, trazodone have been identified up to a concentration of 10 ng/L at least once.
- The following pharmaceuticals, drugs and metabolites have not been identified in surface waters: 2-oxo-3-hydroxy-LSD (LSD metabolite), amphetamine, atorvastatin, azithromycin, cannabinol, catinone, cilazapril, clemastine, clomipramine, clonazepam, cocaine, dicyclover disopyramide, diltiazem, diphenhydramine, diltiazem,

diphenhydramine, disopyramide, donepezil, fenofibrate, glibenclamide, glimepiride, haloperidol, ketamine, MDA (drug and MDMA metabolite), meclozine mephedrone, methadone, mianserine, nacetamine, nonazole, morphine , oseltamivir carboxylate, oxycodone, pizotifen, propranolol, roxythromycin, sertraline, sulfamerazine, terbinafine, terbutaline, triamterene, verapamil, vortioxetine (a total of 51 unidentified micropolutants).

- Drug metabolites reaching the highest concentrations in surface waters of Slovakia were trans-dihydro-dihydroxy CBZ (up to 140 ng/L), metoprolol acid (up to 91 ng/L), N4_acetylsufamethaxazole (up to 27 ng/L).
- The most commonly identified pharmaceuticals, drugs and metabolites in 32 surface water samples were tramadol and caffeine (32 times identified), metoprolol acid (27 times identified), telmisartan (26 times identified), carbamazepine (26 times identified), diclofenac (25 times identified).), trans-dihydro-dihydroxy CBZ (24 times identified), fexofenadine (24 times identified), metoprolol (23 times identified), cetirizine (23 times identified), lamotrigine, clindamycin_sulfoxide, clindamycine, venlafaxine, and oxazepam (22 times identified), valsartan (19 times identified), memantine (18 times identified), bisoprolol (15 times identified).
- Out of a total of 101 micro-pollutants, 27 pharmaceuticals, drugs and their metabolites were identified at least once, of which 21 had concentrations below 10 ng/L in groundwater
- Pharmaceuticals, drugs and their metabolites that reached the highest concentrations in sediments included Oxcarbazepine (up to 52 ng/g), amphetamine (up to 32 ng/g), telmisartan (up to 27 ng/g), caffeine (up to 21 ng/g) g), citalopram (up to 14 ng/g), N-Desmethylcitalopram (up to 11 ng/g), fexofenadine (up to 9.5 ng/g).
- The most commonly identified pharmaceuticals, drugs and metabolites in six sediment samples were telmisartan, fexofenadine, cetirizine and miconazole (identified 6 times), amphetamine, citalopram, N-Desmethylcitalopram, bisoprolol, verapamil and metoprolol (identified 5 times) and caffeine (identified 4 times)

8. Conclusions

The data of many investigations summarised in reported studies and new outcomes from pilot area show that the pharmaceuticals´ occurrence in various components of environment is gradually increasing, in some cases it is alarming. Till now, the research and results of the studies are not so complex and the knowledge base is not so deep to predict all the consequences of pharmaceuticals and their metabolites occurrence onto fauna, flora and environment, and of course, simultaneously the impact on mankind.

The dominant pollution sources of pharmaceuticals, drugs and their metabolites are mainly our households, healthcare facilities, old people´s homes, and agricultural production. The micro-pollutants are spread mainly through the wastewater via sewage systems to wastewater treatment plants and outflowing to the aquatic ecosystem. Water, as the transportation agent, distributes these compounds to all components of environment.

Pharmaceuticals and drugs are a wide group of compounds that can differ significantly in their chemical and physicochemical properties. **Many of these micro-pollutants are biologically inert what results in their subsequent presence in the nature.** They can reach surface water and eventually contaminate groundwater sources. The occurrence of some; drugs and their metabolites in groundwater, although in trace amounts (units of tens of ng/l), is caused mainly due to their physicochemical properties (mostly poorly adsorbed into the soil and resistant to degradation processes), which subsequently confirms their absence in surface water sediments. Their presence in groundwater in units up to tens of ng/l has been described by several studies abroad. Although the presence of antibiotics in surface and groundwater is not significantly alarming, antibiotics can also have an undesirable secondary effect associated with the development of antibiotic resistance of microorganisms. At present, it is not expected that the occurrence of pharmaceuticals in the water could have a negative impact on human health in quantities not exceeding tens of ng/l. However, further research is needed, especially for the possibility of their synergetic effect.

Very limited data on the use of veterinary medicines mean that we do not have a comprehensive overview of their contribution to surface water contamination and the impact on various environmental compartments (not only in the Central European region). In the future, contrast agents, especially those based on iodine, can pose a significant environmental problem. Compared to drugs, they are highly biologically inert and investigated only to a limited extent.

Although recently in the field of analytical chemistry significant progress has been observed in the possibilities of analysing large groups of micro-pollutants in various components of environment, until now, **in general, there are no guidelines or standards for many of pharmaceuticals, their metabolites and drugs that set limit values and quality objectives for discharges into sewage and outlets from municipal wastewater treatment plants.** To set up effective regulatory limits for micro-pollutants, further research on monitoring and behaviour of micro-pollutants is of particular importance.

Moreover, the monitoring of pharmaceuticals, drugs and their metabolites in surface or drinking water is often timely and financially limited – i.e. non-systematic. It is only regularly carried out in some regions of Europe, and therefore the ability of scientists to compare regions in terms of the presence of these micro-pollutants is inadequate. In addition, the analytical procedures used to monitor these types of micro-pollutants can often vary, which may ultimately be reflected in an inaccurate description of the current state. In the framework of systematic collection, it would be interesting to obtain results directly from WWTP (influent and effluent). However, placing analytical equipment (mostly LC MS/MS) on wastewater treatment plants seems to be unrealistic in the future for several reasons - the price of the equipment, its operation and the need for a specially trained analytical chemist. Therefore, **establishing the network of specialised laboratories in different regions of Europe to collect and analyse water samples on regular micro-pollutant´s monitoring might be a proper solution.**

The occurrence of drugs in the surface waters of the Danube and its tributaries points to the need **to apply new types of technologies (which may include different combinations such as membrane technologies and oxidation processes or sorption materials) that**

would be able to remove micro-pollutants and resistant micro-organisms from waste water and from sludge (usually applied to soil). The results of identification of some selected micro-pollutants in the surface waters prove the limited ability of wastewater treatment plants to remove them. The fourth step of wastewater treatment aimed at treatment of the micro-pollutants is economically demanding due to high wastewater flowrate during the day. Better solution is **to focus on the cleaning of dominant point sources of this type of pollution (mainly medical facilities) where the wastewater flow is usually up to 5000 m^3 per day and concentration of contrast agents, drugs and their metabolites are significantly higher.**

A number of drugs which are found in the environment are used by patients preferably in order to improve the quality of life in particular. In general, it is unrealistic to anticipate a decline in the production or use of some dominant groups of medicines (especially antidepressants, antibiotics, or medicines to treat cardiovascular diseases). From a pharmaceutical standpoint, the findings of a significant number of pharmaceuticals and their metabolites in the environment suggest improper use of therapeutic doses of medication. Whether due to low drug metabolism, over-prescription, drug overuse or drug abuse, the active substances are excluded from the body in quantities in which they are able to further significantly influence various components of the environment or even re-enter the food chain through agricultural crops. Therefore, increased emphasis should be placed on responsible, appropriate and rational pharmacotherapy and new drug delivery systems. In other words, giving the right medicine to the right patient at the right time, and with the right dosage. **The new drug delivery systems should target diseases more effectively and precisely taking into account the individual characteristics of the patient in order to maximize the effect and safety of the medicine and minimize the amount of drug that leaves the human body unchanged.**

One of the ways to decrease the amount of pharmaceuticals released in environment is to improve diagnostics. The wider use of CRP tests by general practitioners that is currently insufficient in several regions of Europe, could reduce consumption of antibiotics to some extent. Next challenging issue is improving the life cycle of unused medicines which in many cases end up in the toilet and subsequently in the sewage system. An example of good practice is motivation of consumers by forms of discounts in pharmacies when returning the unused medicines.

The future research concentrates on new types of drugs and nanotechnologies which are less persistent in environment, like technologies based on the use of various nano-materials with antibacterial activity as carbon, titanium, silver or gold. The possibility of modifying conventional plastics to obtain good antibacterial properties and their possible subsequent use in medical facilities such as waiting room wall surfaces is also under investigation.

Perhaps nanotechnology, targeted intracellular delivery and other new drug delivery systems will be able to reliably deliver treatment to targeted cells, to the correct structures inside cells or transport drugs to targeted intracellular sites, and release the drugs in response to specific molecular signals. This could result in increased therapeutic effect at lower drug doses and thus lower quantities of unutilised drugs leaving the human body.

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2019

Annex I Graphs

Occurrence of Pharmaceuticals in the Waters of the Danube Region

Study

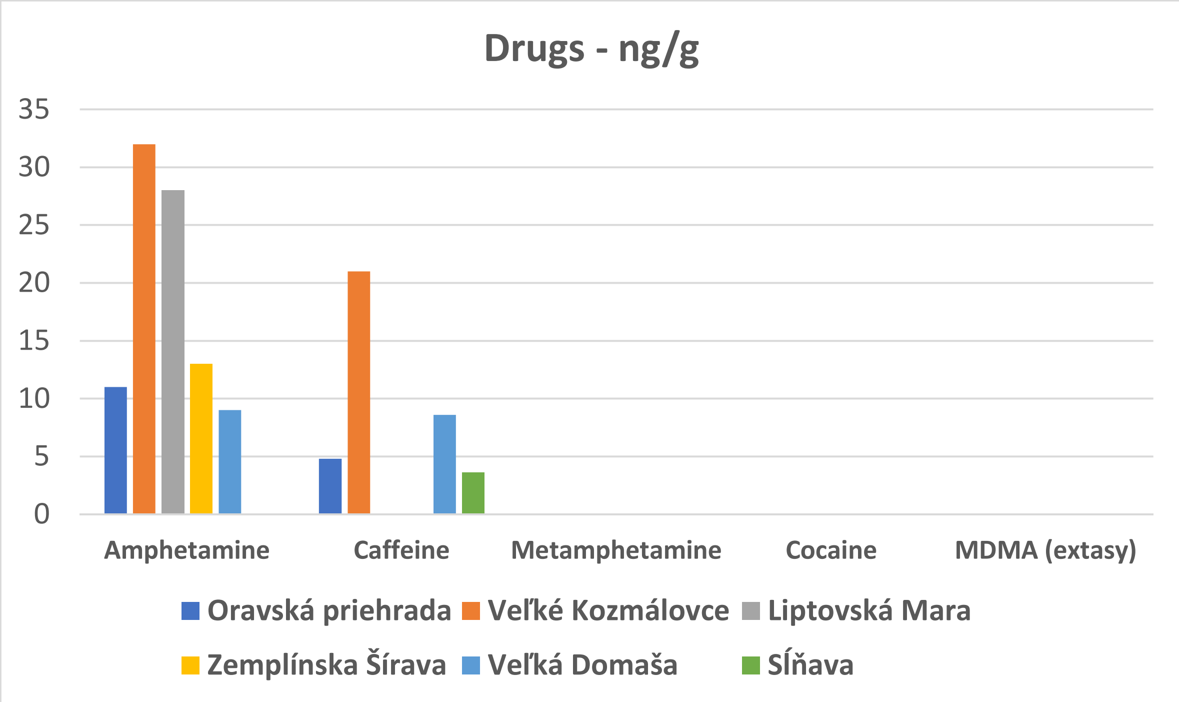
Interreg
Danube Transnational Programme

DANUBE REGION strategy
Water Quality

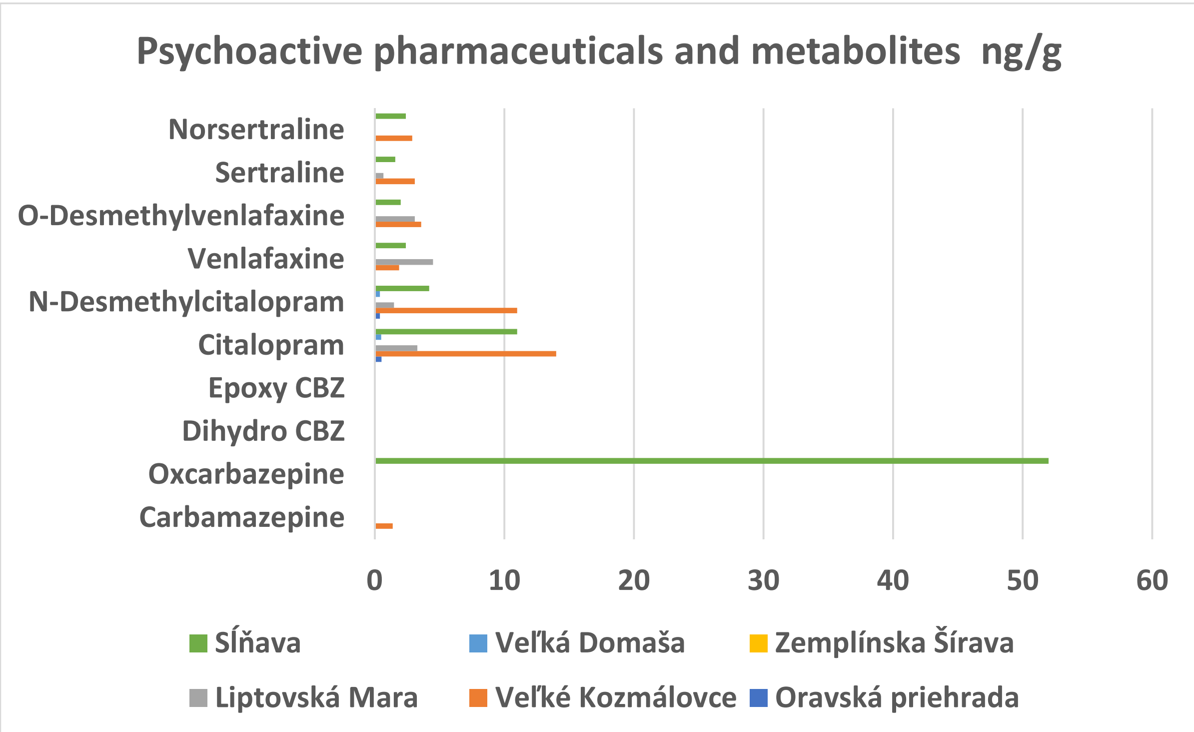
Content

STU FCHPT
Slovak Technical University, Bratislava
Faculty of Chemical and Food Technology

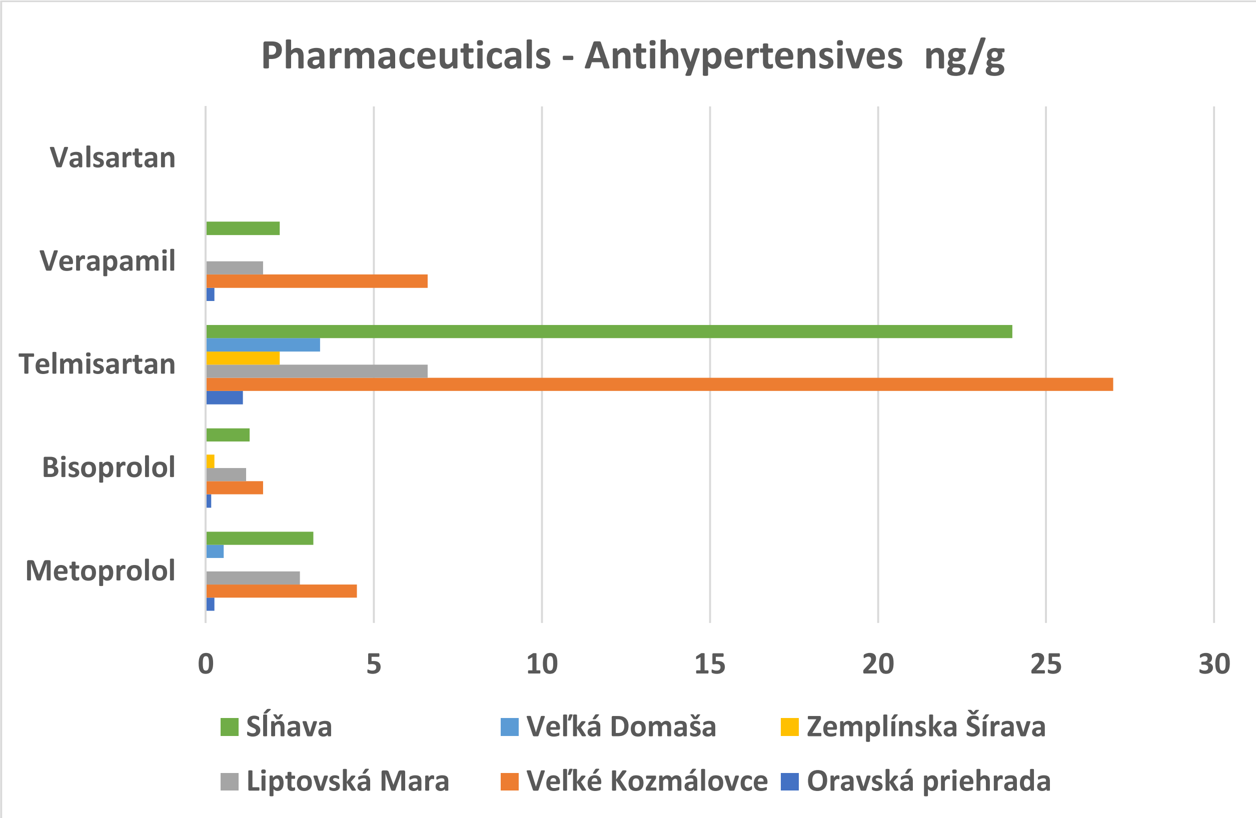
Results – sediments and selected micropolutants



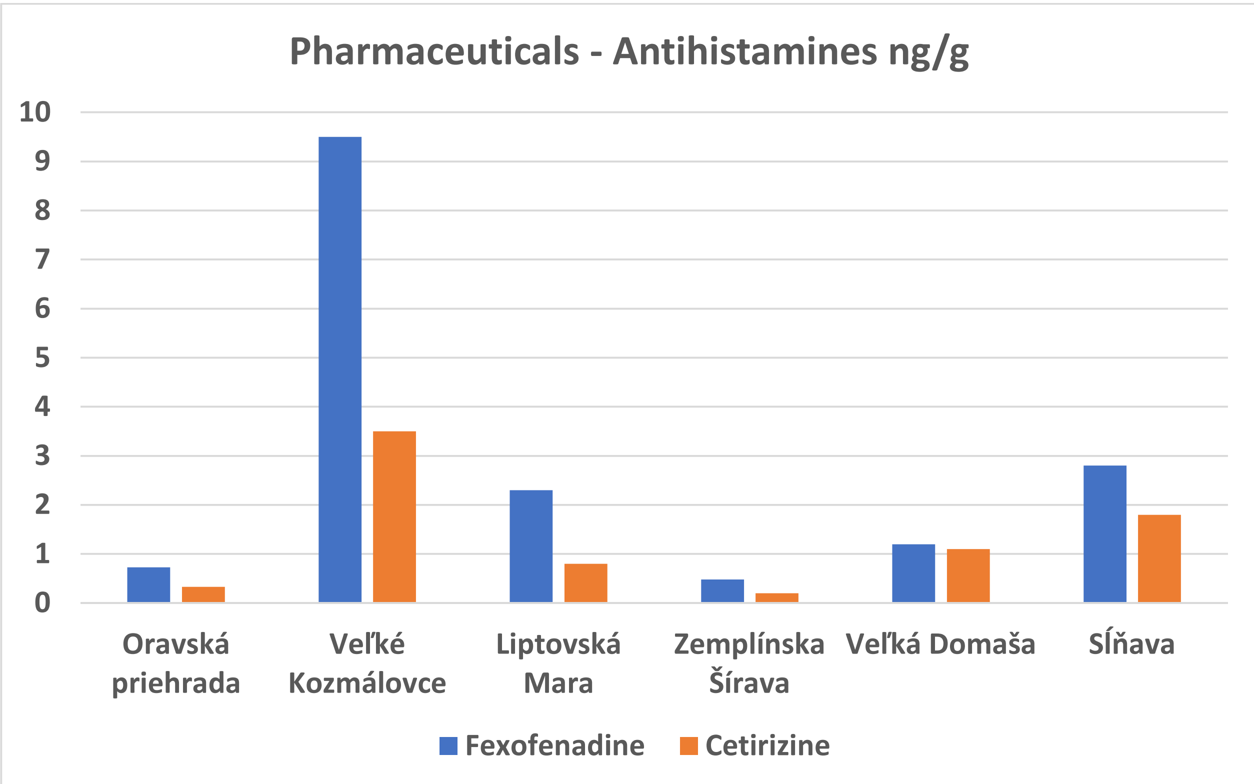
Graph 1. Occurrence of selected drugs in sediments



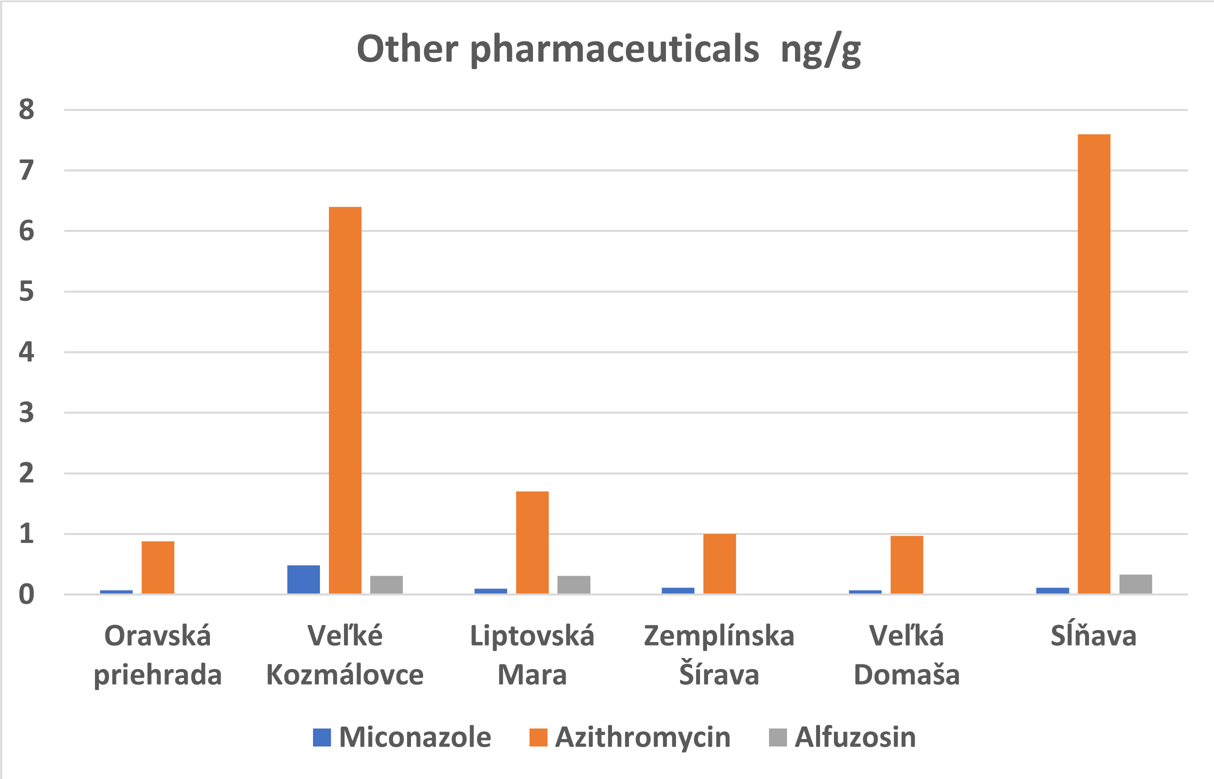
Graph 2. Occurrence of a selected group of pharmaceuticals in sediments



Graph 3. Occurrence of a selected group of pharmaceuticals in sediments

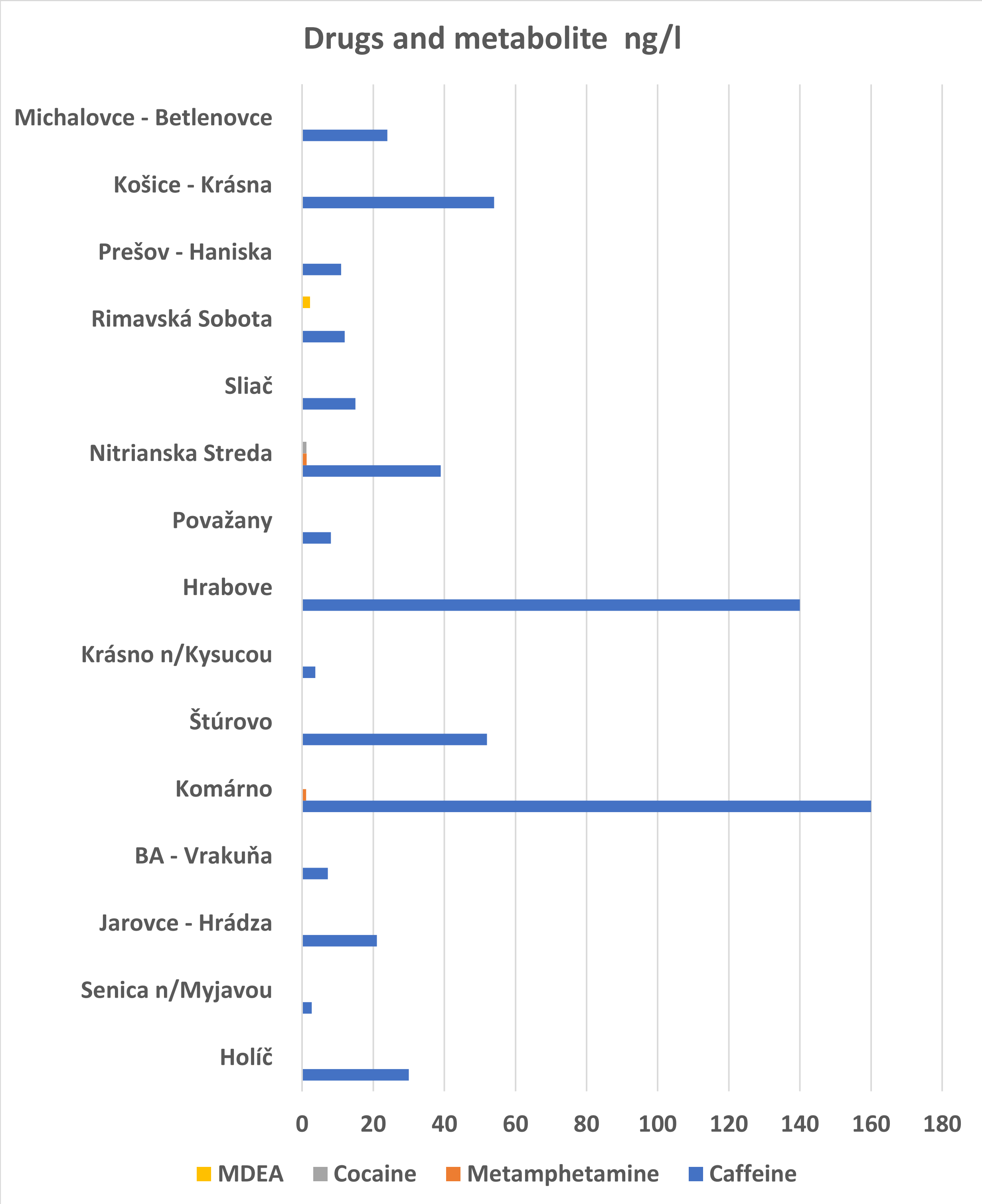


Graph 4. Occurrence of a selected group of pharmaceuticals in sediments

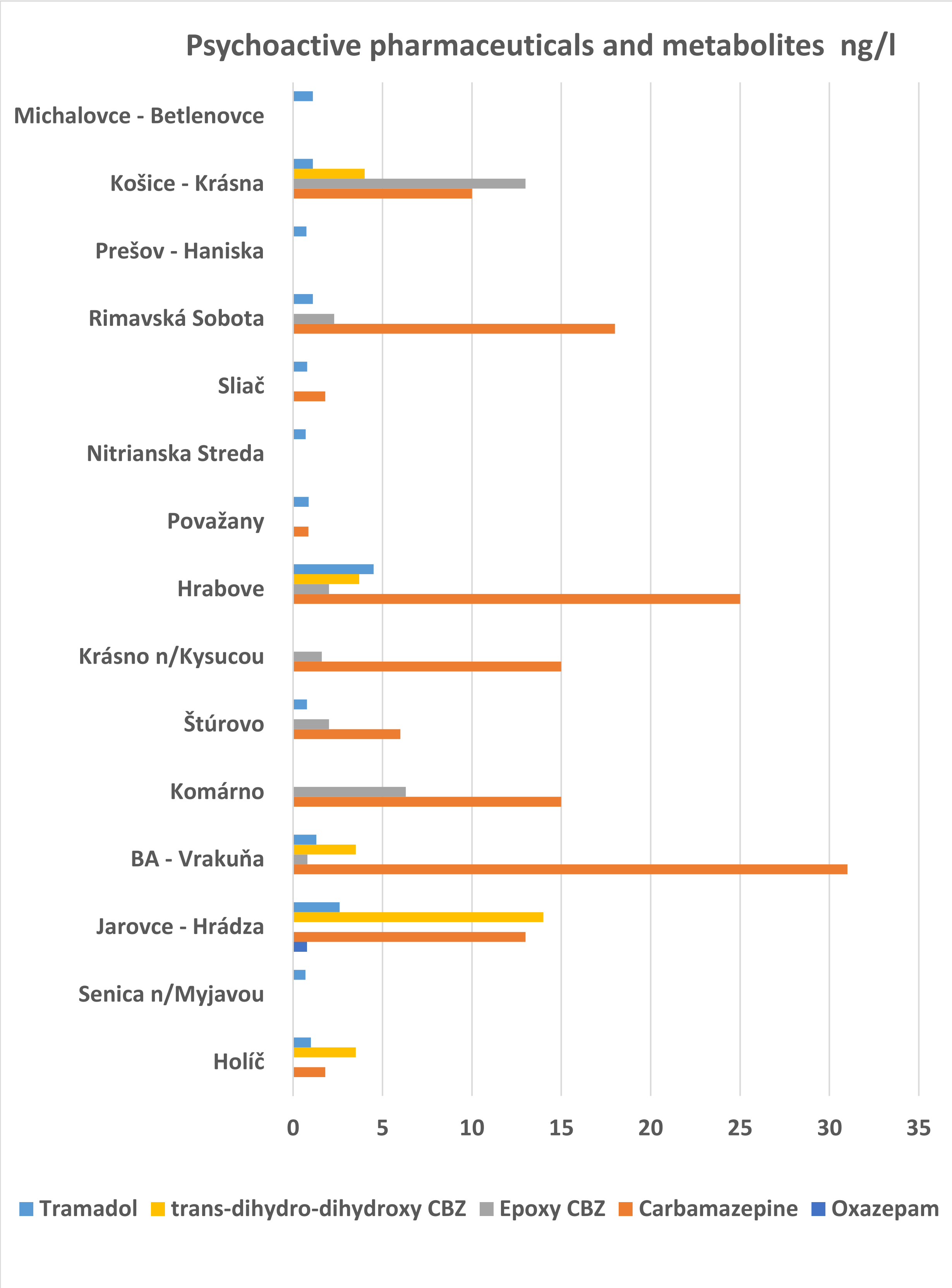


Graph 5. Occurrence of selected pharmaceuticals in sediments

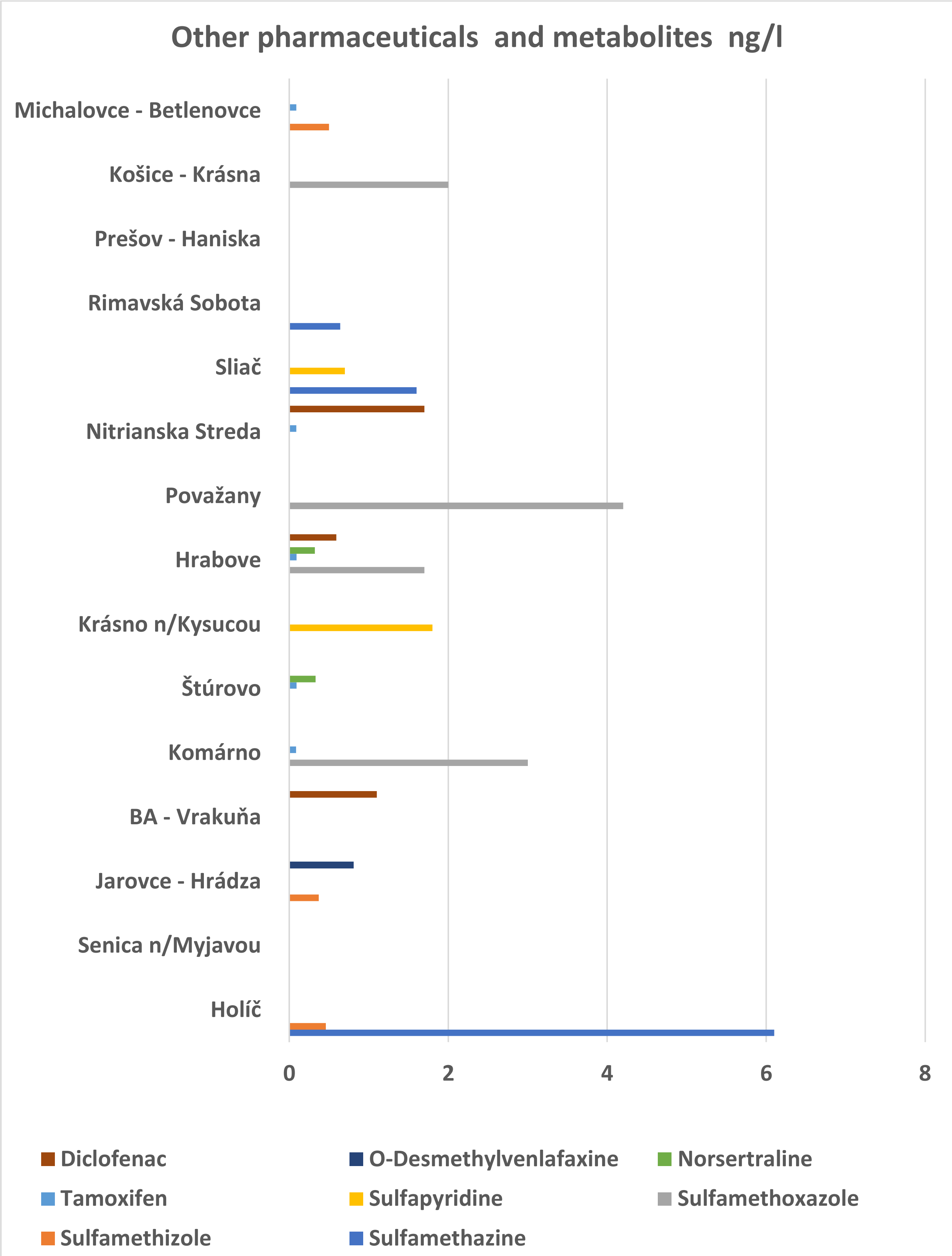
Results - groundwater and selected micropolutants



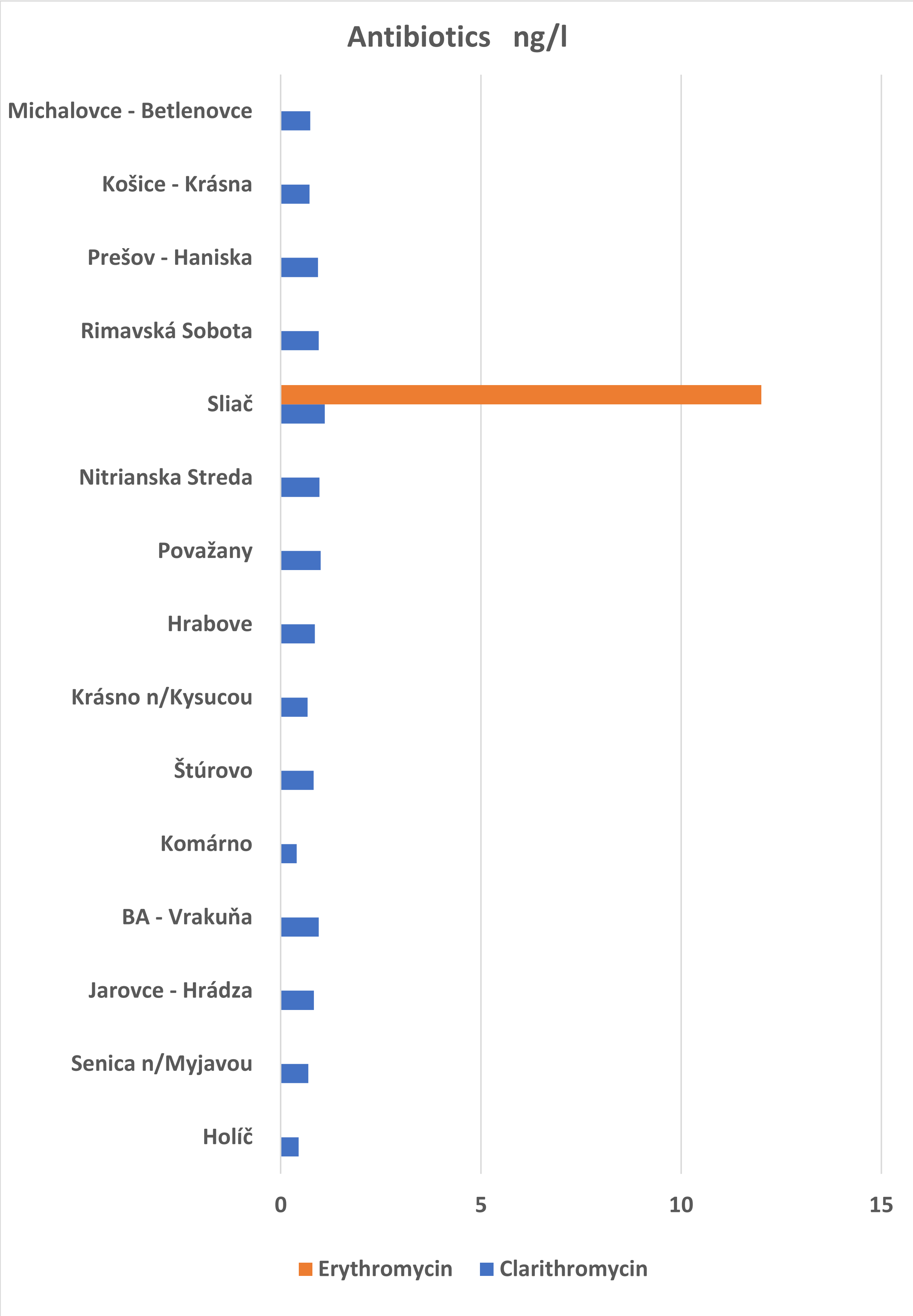
Graph 6. Occurrence of selected drugs and metabolite in groundwater



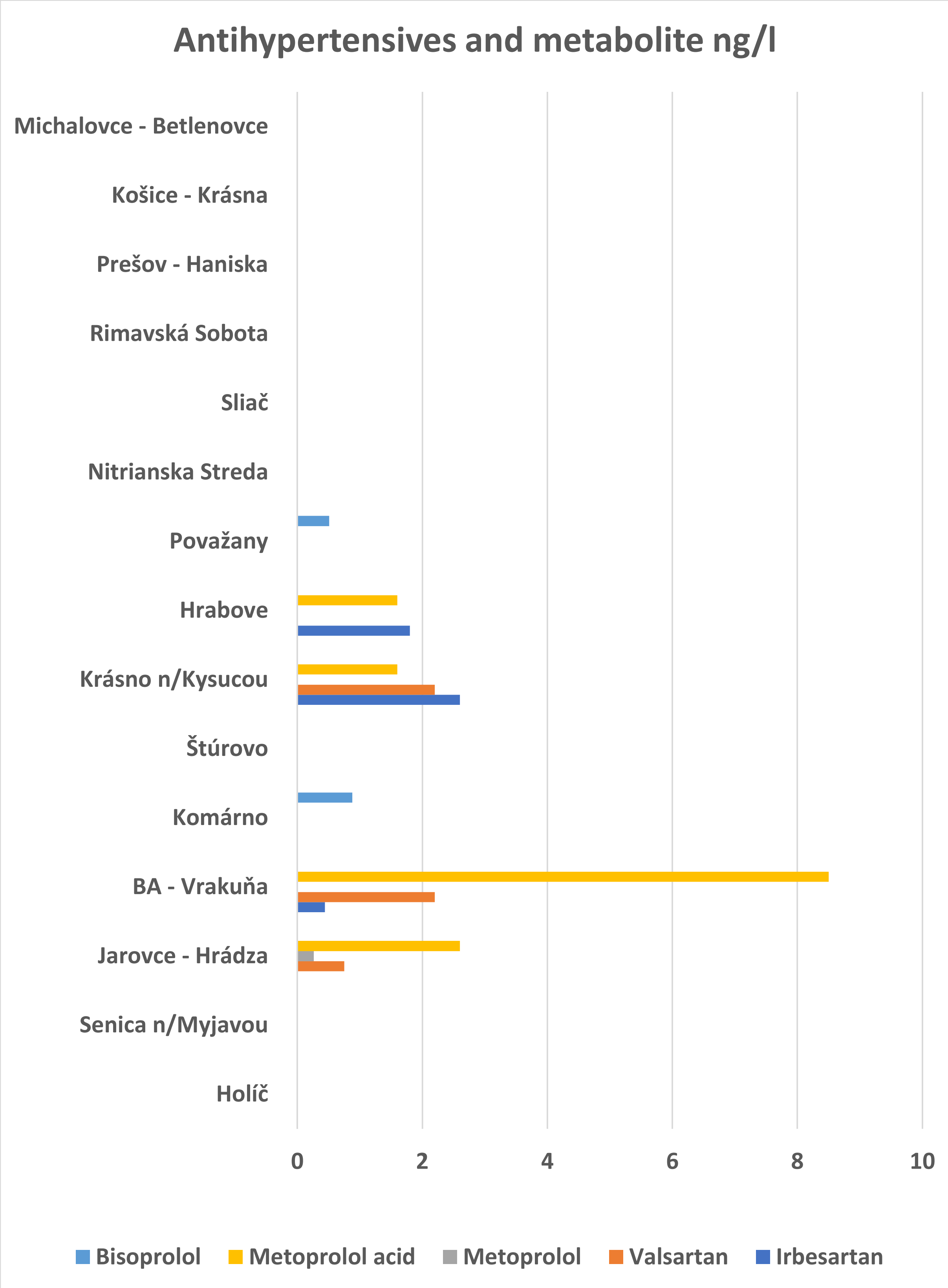
Graph 7. Occurrence of selected pharmaceuticals and metabolites in groundwater



Graph 8. Occurrence of selected pharmaceuticals and metabolites in groundwater

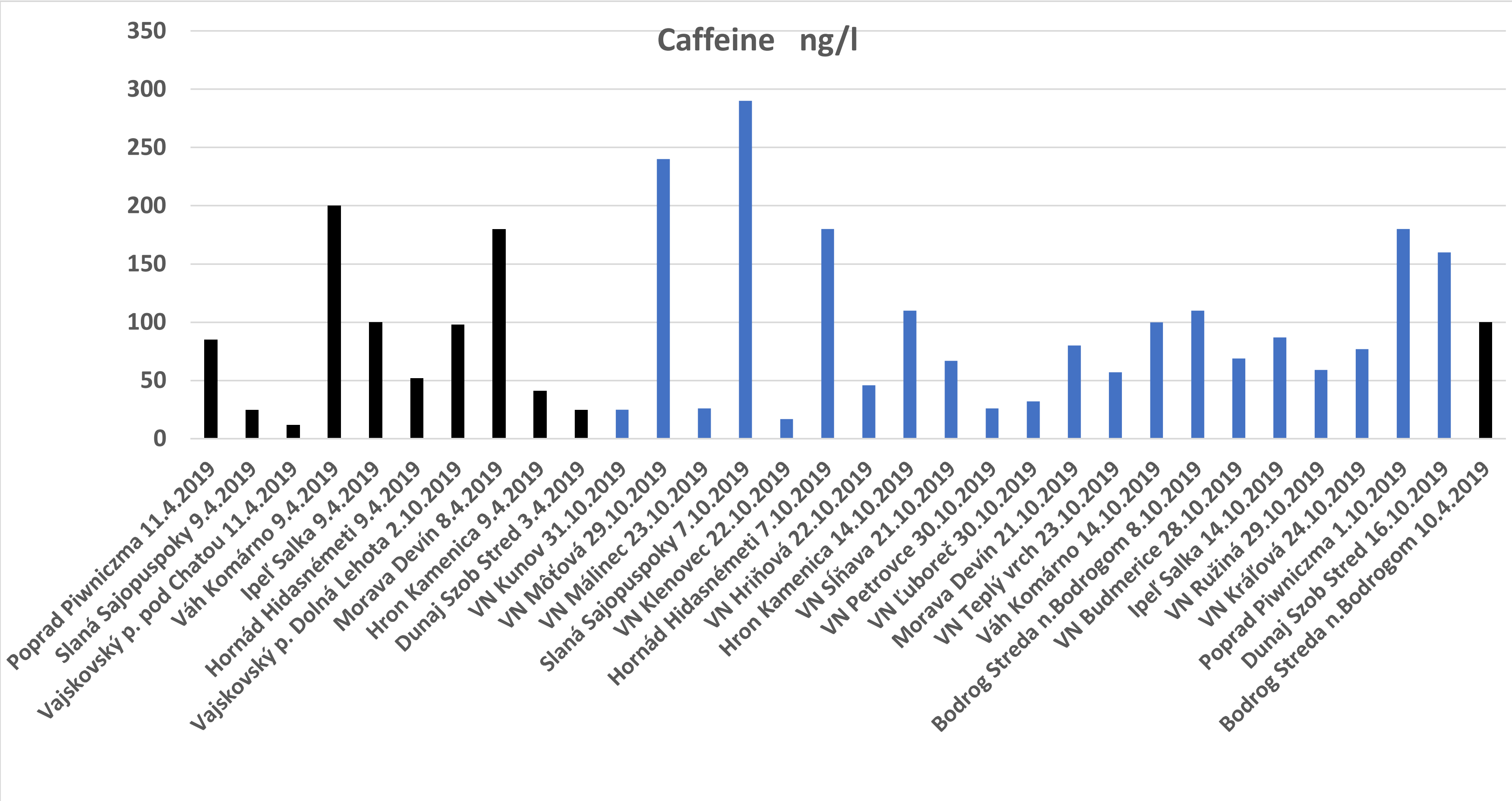


Graph 9. Occurrence of selected pharmaceuticals in groundwater

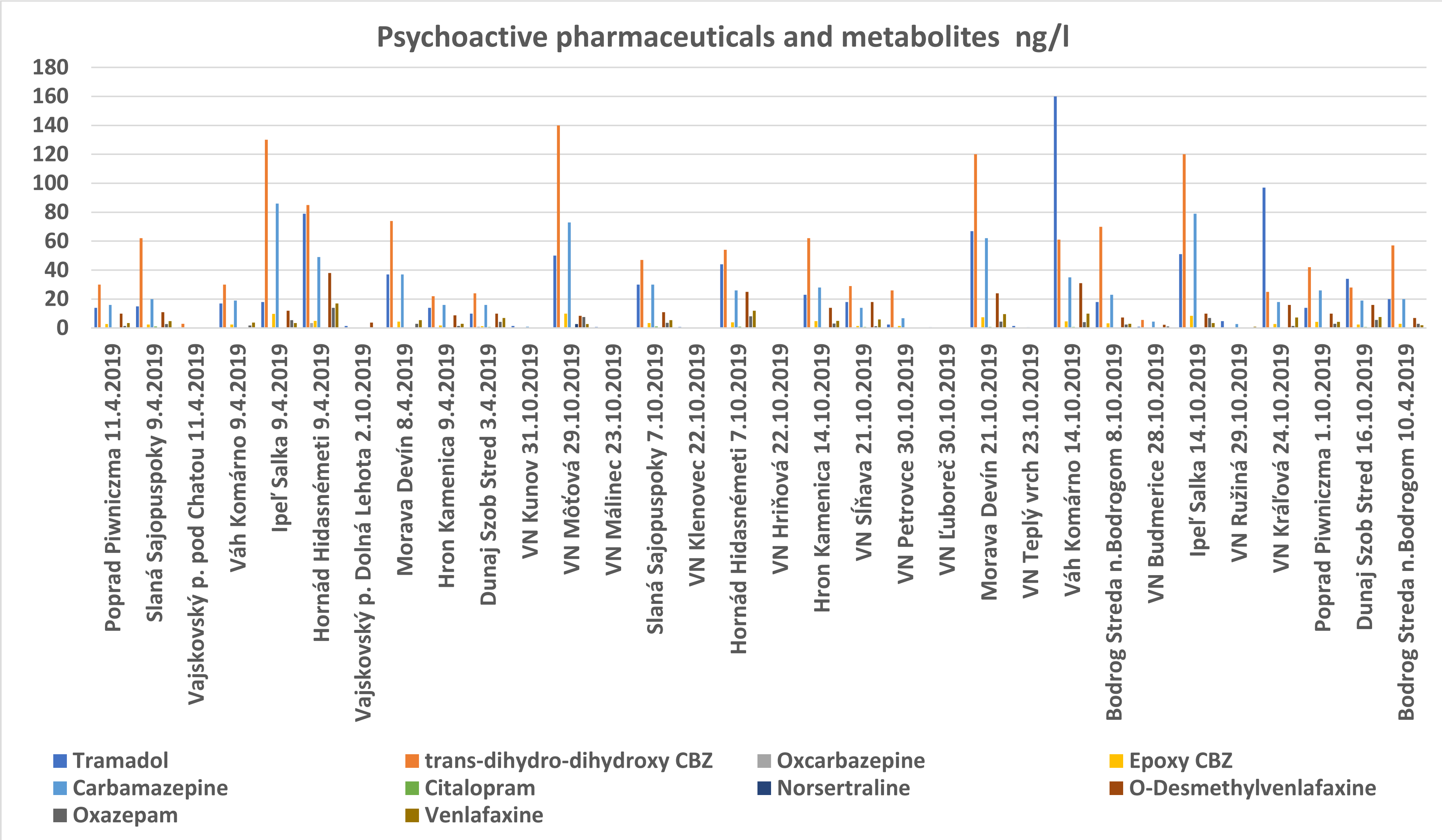


Graph 10. Occurrence of selected pharmaceuticals and metabolite in groundwater

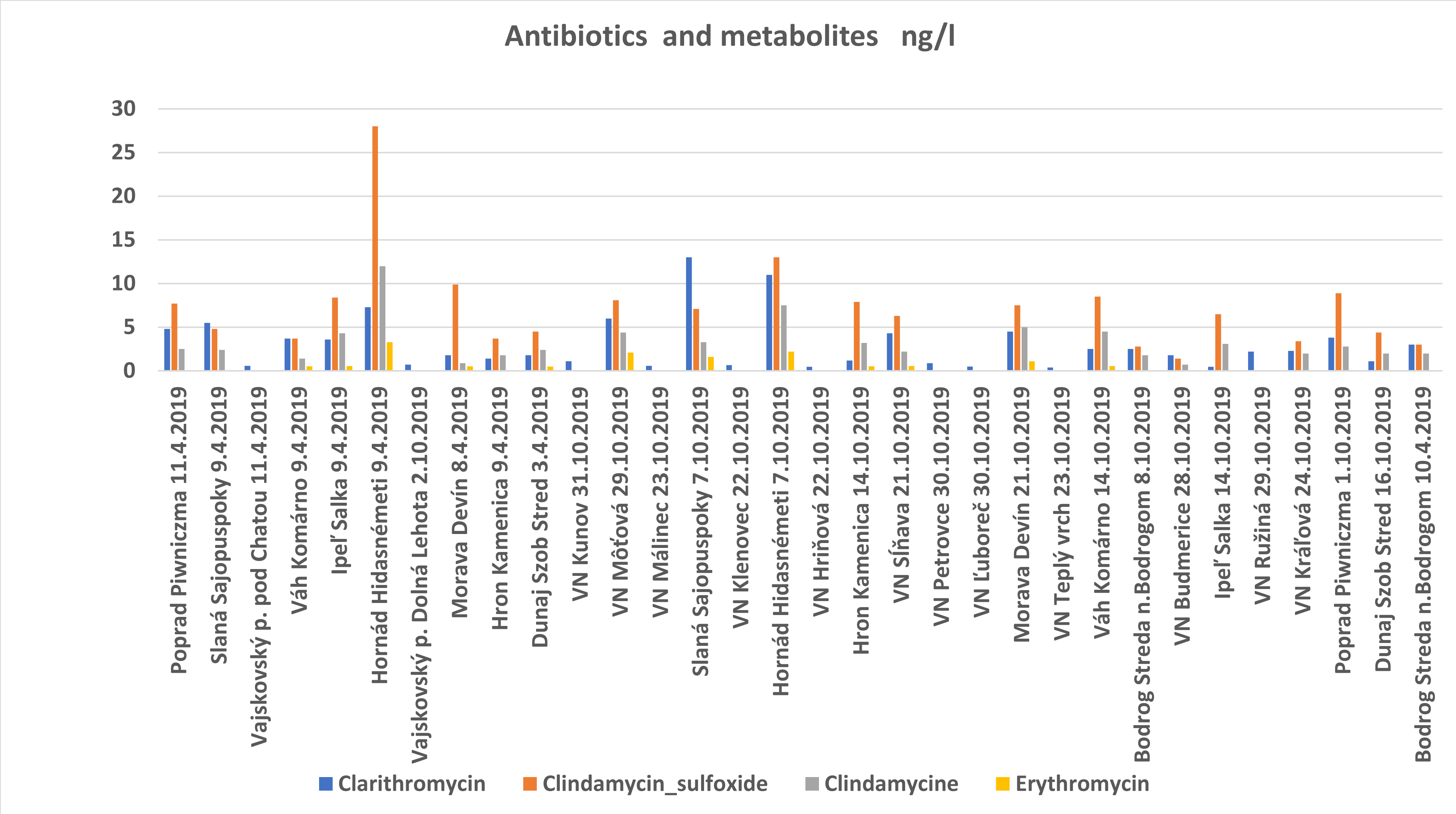
Results - surface waters and selected micropolutants



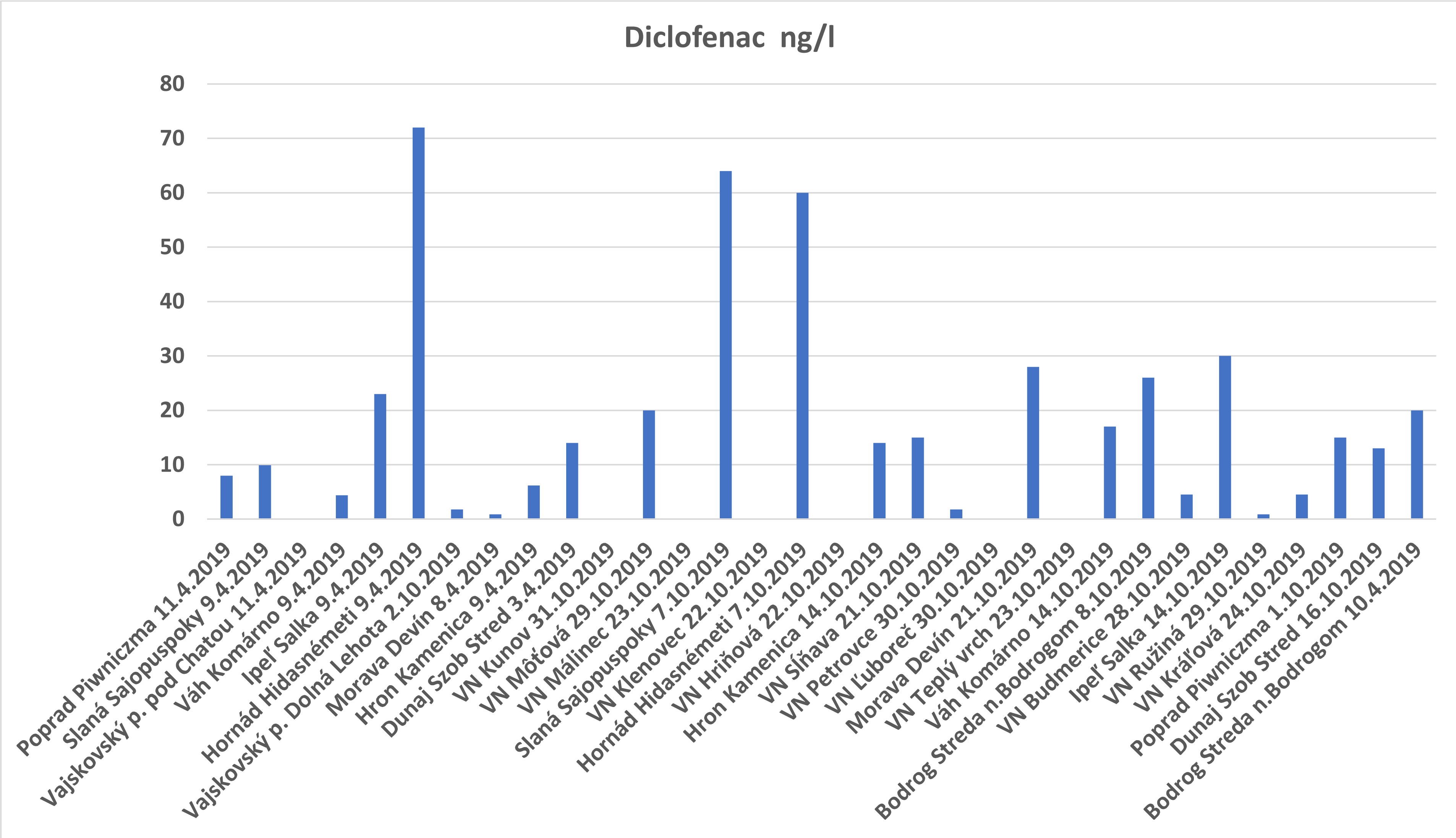
Graph 11. Occurrence of the drug caffeine in surface waters



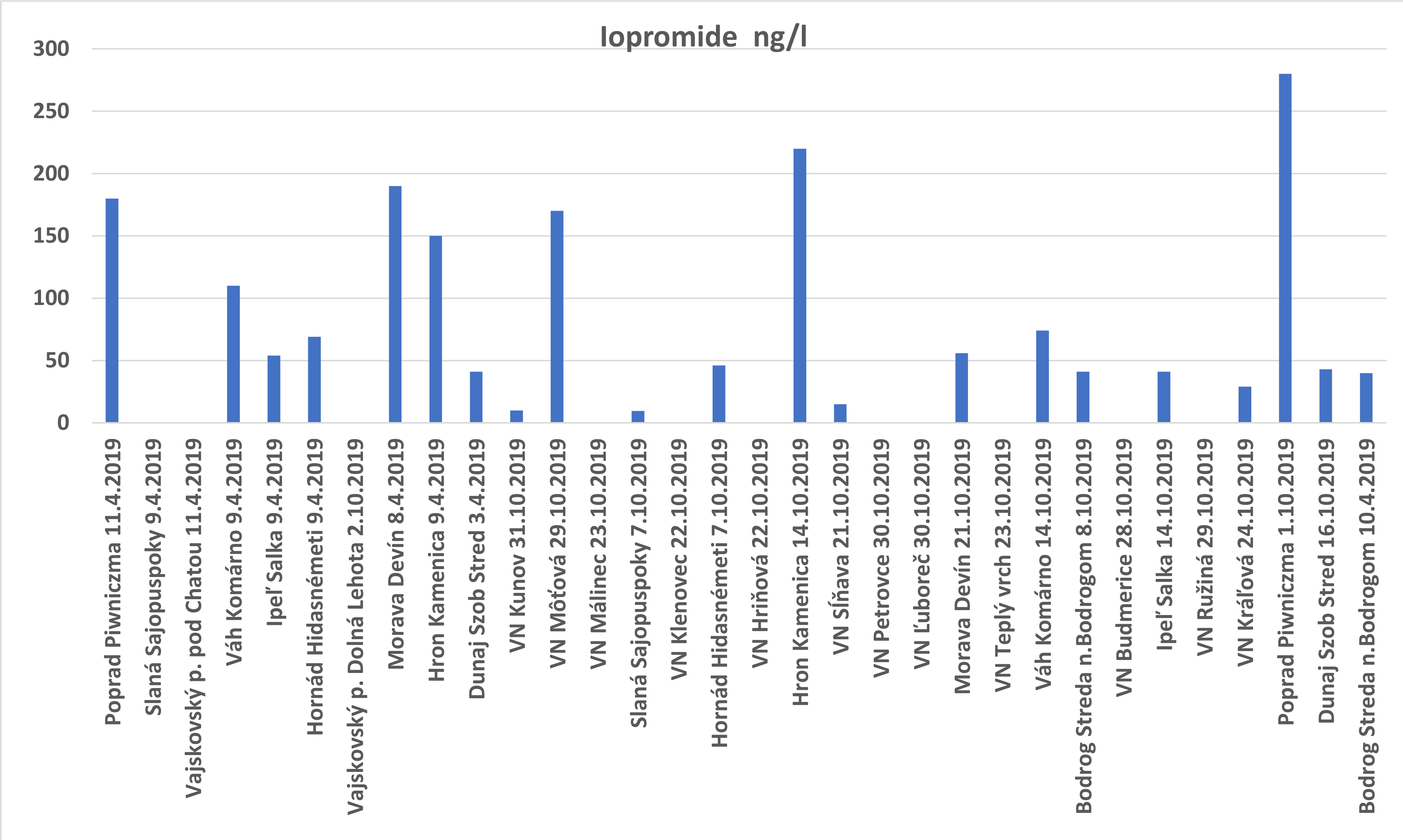
Graph 12. Occurrence of selected pharmaceuticalss and metabolites in surface waters



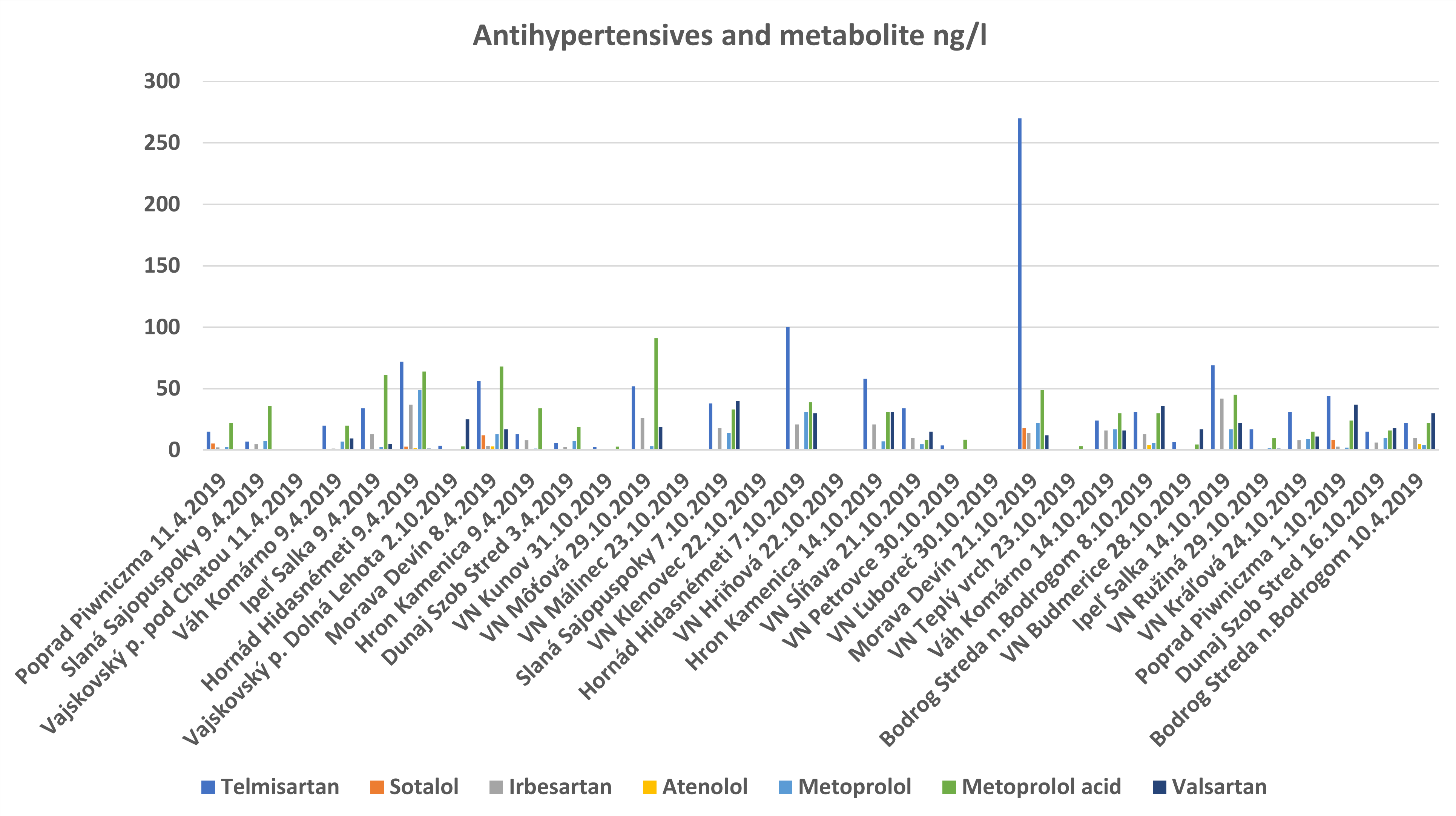
Graph 13. Occurrence of selected pharmaceuticalss and metabolites in surface waters



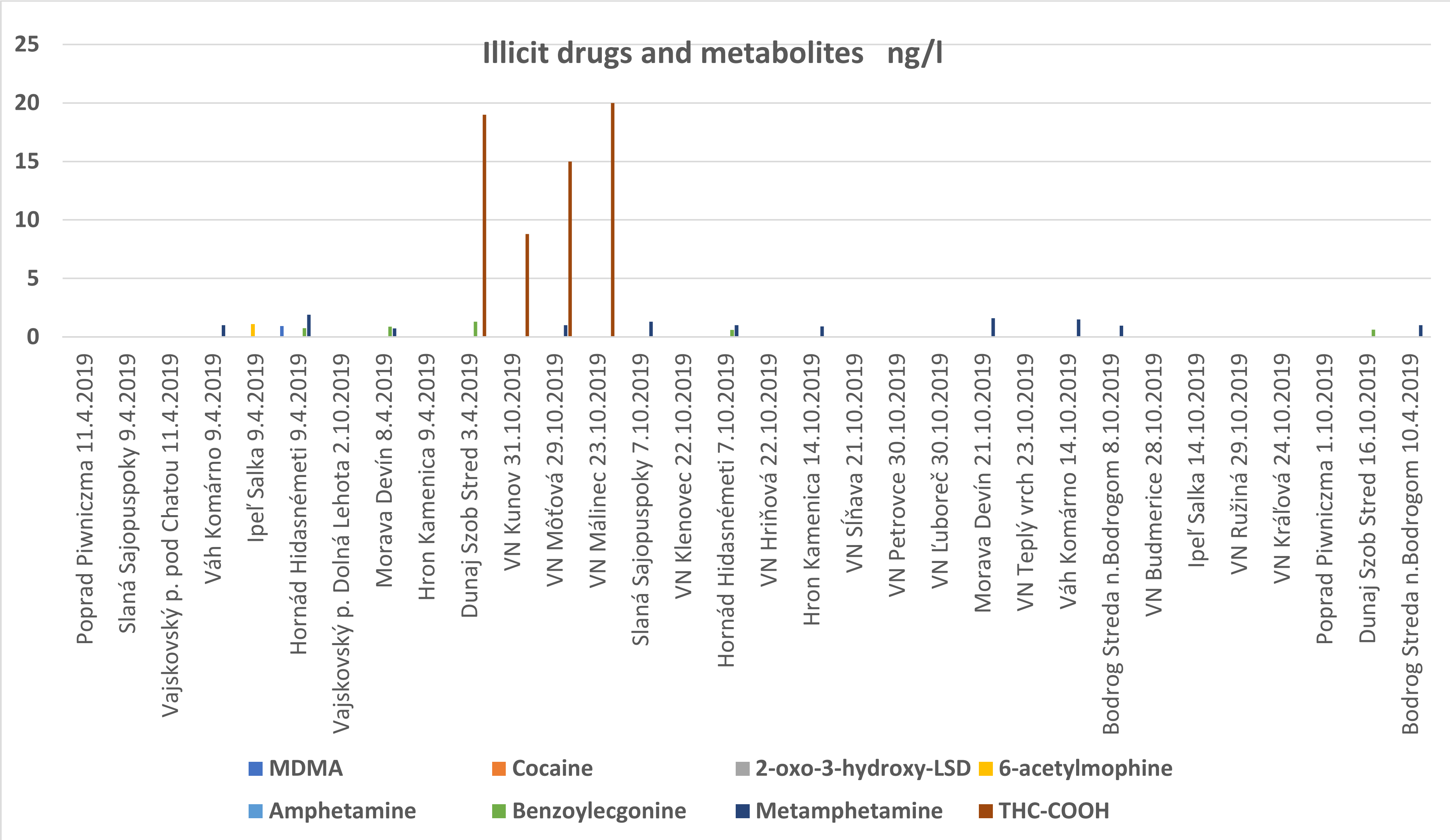
Graph 14. Occurrence of selected pharmaceutical in surface waters



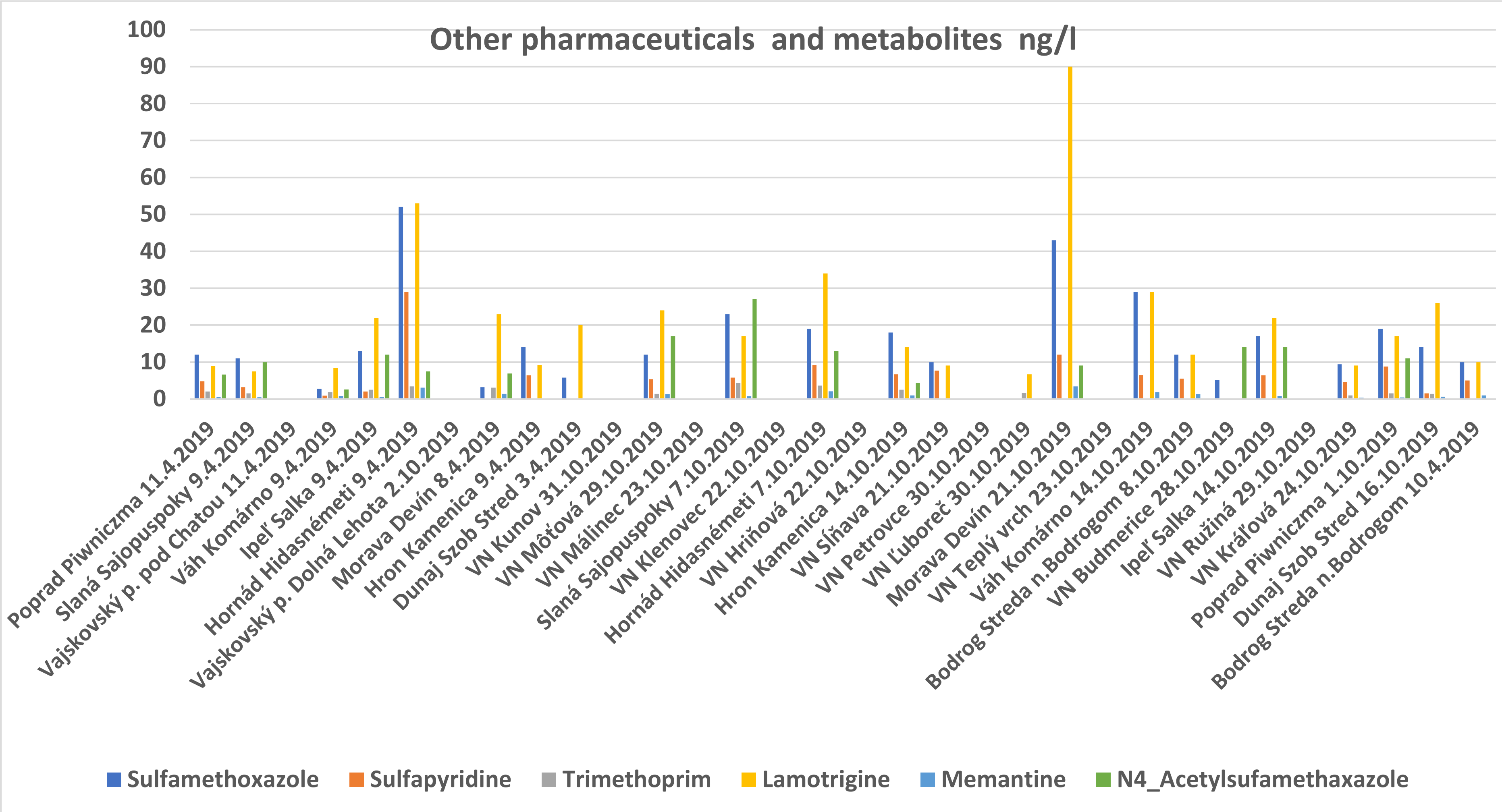
Graph 15. Occurrence of selected contrast medium in surface waters



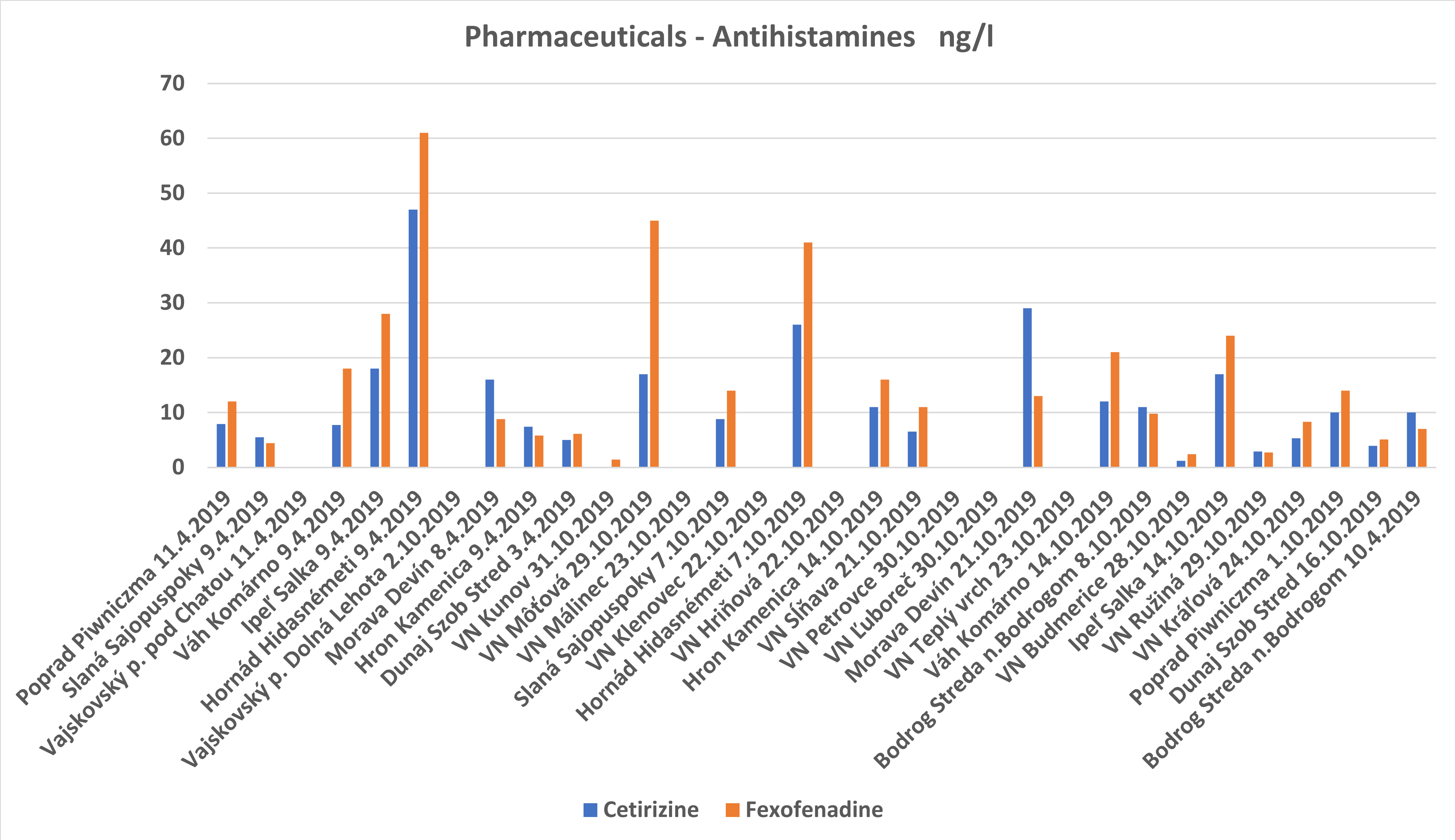
Graph 16. Occurrence of a selected group of pharmaceuticals and metabolite in surface waters



Graph 17. Occurrence of a selected of illicit drugs and metabolites in surface waters



Graph 18. Occurrence of a selected group of pharmaceuticals and metabolite in surface waters



Graph 19. Occurrence of a selected group of pharmaceuticals in surface waters