

# Selected licit and illicit drugs in surface water in sampling profiles near wastewater treatment plant outlets

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**Keywords:** surface water – illicit substances – THC – methamphetamine – amphetamine – MDMA – cocaine – nicotine – cotinine – *trans*-3-hydroxycotinine

## ABSTRACT

The majority of the population (85 %) in the Czech Republic is connected to the public sewerage network of almost 3,000 wastewater treatment plants (WWTPs). Municipal wastewater contains a number of substances providing information on the state of the population. This information is evaluated by Wastewater-Based Epidemiology (WBE). A WWTP does not remove all contaminants that are discharged into the recipient. In this study the loading of a recipient with selected licit and illicit drugs was monitored. Concentrations of the following drugs were monitored: tetrahydrocannabinol (THC), methamphetamine,

ecstasy (MDMA), cocaine, and selected metabolites, i.e. amphetamine and benzoylecgonine, methadone and EDDP and nicotine, including its metabolite cotinine and *trans*-3-hydroxycotinine. The monitored locations were Vltava – Trojská lávka (control profile), Vltava – Podbaba, Dražanský brook, Podmoráňský brook, and Únětický brook. All samples were positive; therefore, it depends on the ability to remove the monitored substances in the given WWTP. In the recipient, the treated waters are diluted, yet the residues of the monitored substances have an impact on the environment. Therefore, it is desirable to continue monitoring these substances in surface waters.

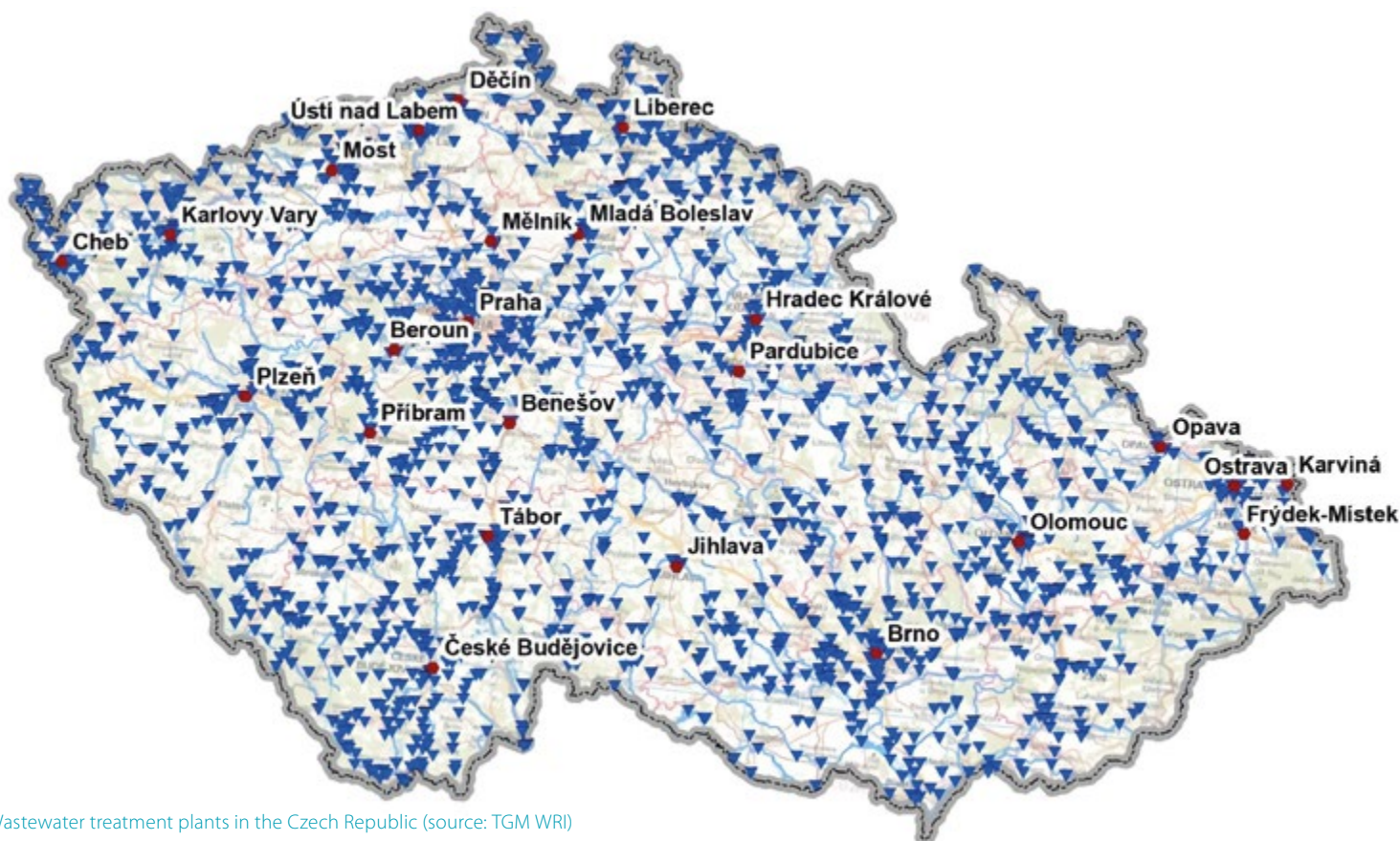


Fig. 1. Wastewater treatment plants in the Czech Republic (source: TGM WRI)



## INTRODUCTION

85 % of the population in the Czech Republic is connected to almost 3,000 wastewater treatment plants (WWTPs; Fig. 1). These figures rank the country among the most advanced countries in the EU in terms of water management, as even many original EU member states do not reach these figures [1]. Municipal wastewater contains a number of substances that, when analysed, provide very significant information about the state of the population. This is being exploited by the recently rapidly developing multidisciplinary scientific discipline of Wastewater-Based Epidemiology (WBE). The hypothesis that wastewater could be treated as a very diluted urine sample led to the emergence of this field [2, 3]. This approach was first applied in the Po River basin to detect cocaine consumption [4]. The WWTP does not remove all contaminants contained in municipal wastewater; with the treated wastewater, residues of illegal substances – drugs – enter surface waters as well.

### Monitored streams and characteristics of the relevant WWTPS

For this article, we selected several profiles on smaller streams that flow into the Vltava in Prague and below Prague and which are affected by WWTP outlets into these streams. A sampling point above Prague Central Wastewater Treatment Plant (CWWTP) was chosen as the control profile; the sampling was carried out from Trojská lávka (Fig. 2). Another sampling point was below both outlets from Prague CWWTP in Podbaba (Fig. 3).



Fig. 2. Vltava control profile – Trojská lávka (source: Mapy.cz)

Treated wastewater from Dolní Chabry WWTP is discharged into Drahanský brook, a right-hand tributary of the Vltava. The sampling point was approximately 1 km from its mouth into the Vltava. Drahanský brook (Fig. 4) is 3.3 km long, and its catchment area is 6.7 km<sup>2</sup>. The average flow rate is 7.7 m<sup>3</sup>/s. The long-term average flow rate  $Q_a$  at the outlet of Dolní Chabry WWTP (at river km 3) is 83 l/s.  $Q_{355}$  (the average daily flow rate reached or exceeded during 355 days of the year) is 12.0 l/s. The average amount of treated wastewater discharged into the recipient is 10.6 l/s. In the immediate vicinity of Chabry there are several protected areas, such as Drahanské údolí, in the lower part also called Drahanská rokle. The data are taken from the *Sewerage Rules of the WWTP-Dolní Chabry* [5].



Fig. 3. Output from the Prague CWWTP to the Vltava (source: ŠJů / Wikimedia Commons. This file is licensed under the Creative Commons Attribution-Share Alike 4.0 International license)



Fig. 4. Drahanský brook (source: ŠJů / Wikimedia Commons. This file is licensed under the Creative Commons Attribution-Share Alike 4.0 International license)





Fig. 5. Podmoráňský brook (photo: Horakvlado / Wikimedia Commons. This file is licensed under the Creative Commons Attribution-Share Alike 4.0 International license)

The left-hand tributary of the Vltava, the Podmoráňský brook (Fig. 5), is affected by treated wastewater from Velké Přílepy WWTP discharged into the recipient at river km 2.8; the sampling profile was before the stream's influx into the Vltava. The length of the stream is 4.1 km, and the average flow rate is 24 l/s. The catchment area is 9.6 km<sup>2</sup>. The average amount of wastewater discharged is 11.8 l/s. The data are taken from the *Sewerage Rules of the Velké Přílepy WWTP* [6].

The next left-hand tributary, the Únětický brook (Figs. 6 and 7), receives treated wastewater from Horoměřice and Tuchoměřice WWTPs; in this case, too, the sampling profile was before the stream influx into the Vltava. The Únětický brook originates in Kněževěs, flows through Tuchoměřice, Státnice, Černý Vůl, and Únětice, and then flows into Prague, where it forms its border. This part is home to Údolí Únětického potoka (Únětický Brook Valley) natural monument and Tiché údolí and Roztocký háj nature reserves. In Roztoky, Únětický brook flows into the Vltava. The stream is 4.1 km long; the catchment area is 19 km<sup>2</sup>. The average flow rate is 100 l/s.



Fig. 6. Únětický brook in Tuchoměřice (source: Aktron / Wikimedia Commons. This file is licensed under the Creative Commons Attribution-Share Alike 3.0 Unported license)



Fig. 7. Únětický Brook Valley Nature Reserve (source: Meruzalka / Wikimedia Commons. This file is under Creative Commons license)

## METHODOLOGY

The sampling points are marked in Fig. 8 and described in Tab. 1; the characteristics of individual WWTPs on the monitored streams are given in Tab. 2. The data are taken from the publication by Zvěřinová Mlejnková et al., focused on microbial contamination of the Vltava below Prague [7].

Illicit substances and their metabolites are not routinely monitored in wastewater or surface waters and are not subject to relevant legislation. In surface waters, these substances can have an impact on the environment, as shown, for example, by studies on fish behaviour [8–11].

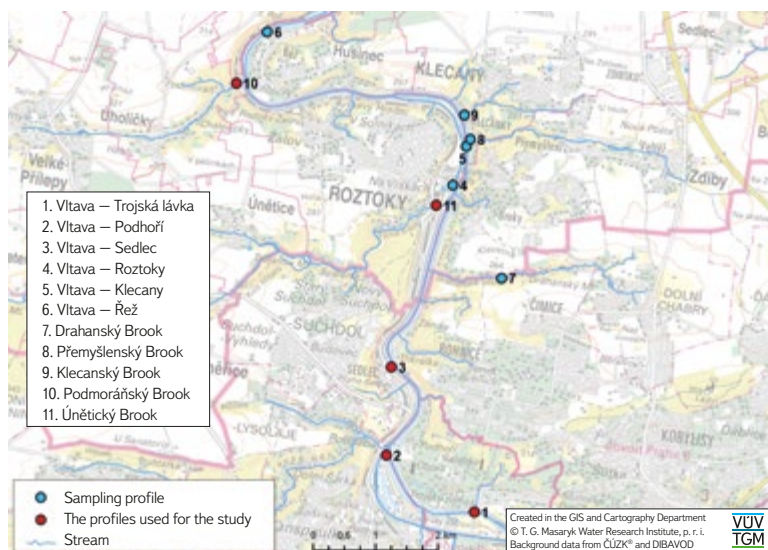


Fig. 8. Map with marked sampling profiles; the profiles used for this study are marked with a red dot (source: H. Zvěřinová Mlejnková [7])

Tab. 1. Sampling place description (source: H. Zvěřinová Mlejnková [7])

Profil No.	Name of sampling profile	Description of sampling profile
1	Vltava – Trojská lávka	Control profile above CWWTP Prague, sampling from Trojská lávka
2	Vltava – Podbaba	Sampling below both outlets from CWWTP Prague, from the left bank at the end of Císařský ostrov. Water below outlets is not sufficiently mixed
7	Drahanský brook	Right-hand tributary of the Vltava, outlet of the Prague – Čimice WWTP, sampling is carried out about 1 km before the mouth to the Vltava
10	Podmoráňský brook	Left-hand tributary of the Vltava, outlet of the Velké Přílepy WWTP, sampling is carried out before the mouth to the Vltava
11	Únětický brook	Left-hand tributary of the Vltava, outlets of Horoměřice and Tuchoměřice WWTPs, sampling is carried out from the road bridge about 150 m before the mouth to the Vltava

Tab. 2. Characteristics of WWTPs on monitored streams (source: H. Zvěřinová Mlejnková [7])

Name of WWTP	Category according to PE	Recipient	Number of persons connected to WWTP (2021)	Annual volume of treated water [thous. m <sup>3</sup> /year]
CWWTP Prague OWL	More than 100,000	Vltava	491,633	44,989
CWWTP Prague NWL	More than 100,000	Vltava	706,012	64,601
WWTP Dolní Chabry	2 to 10,000	Drahanský brook	4,632	264
WWTP Velké Přílepy	2 to 10,000	Podmoráňský brook	2,935	190
WWTP Horoměřice	2 to 10,000	Únětický brook	3,450	274
WWTP Tuchoměřice	2 to 10,000	Únětický brook	1,816	149

The method for determining trace substances in water used for the analyses in this project was developed according to the procedure published by Postigo et al. [12]. This method has been used in the hydrochemical laboratory of TGM WRI for more than ten years, and new substances are gradually being included among the determined compounds depending on the current situation on the drug scene. Fully automated on-line SPE and LC-MS/MS methods of determination in ESI+ or ESI- mode are accredited for surface and wastewater. The laboratory annually participates in the international comparison of tests, which takes place within the framework of the global drug situation monitoring under the auspices of the SCORE-network (<https://score-network.eu/>).

The concentrations of a selected group of substances listed in Tab. 3 were monitored in the samples.

Tab. 3. List of monitored substances

Group of substances	Name, abbreviation and limit of determination
"Classic" drugs	11-nor-9-carboxy-delta-9-THC (nor-THC); 0.2 ng/l
	3,4-methylene-dioxy-methamphetamine (MDMA); 0.1 ng/l
	Methamphetamine (MAMP); 0.1 ng/l
	Amphetamine (AMP); 0.3 ng/l
	Cocaine (CO); 0.04 ng/l
Substitution treatment	Benzoylcegonine (BE); 0.06 ng/l
	Methadone (MET); 0.2 ng/l
Nicotine and its metabolites	EDDP (2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine); 0.3 ng/l
	Cotinine (COT); 10 ng/l
	<i>trans</i> -3-hydroxycotinine (T3H-COT); 10 ng/l

## Collection and pretreatment of surface water samples

Sampling was carried out by employees from the TGM WRI Department of Hydrobiology. In the same profiles, they monitored the effect of wastewater on microbial contamination of the Vltava [7]. For hydrochemical analyses, samples taken during 2022 and 2023 at approximately two-month intervals were used.

Samples were collected in polypropylene containers. After transport to the laboratory, these samples were further processed according to the relevant standard operating procedures. After collection, the samples were kept cool and dark at a temperature of up to 8 °C. If samples could not be analysed within 72 hours of collection, they were frozen and stored at  $-20 \pm 4$  °C. Before analysis, samples were centrifuged (4,500 rpm, 15 minutes) and solid particles were removed from the sample by filtration through disposable regenerated cellulose membrane filters with a porosity of 0.45 µm.

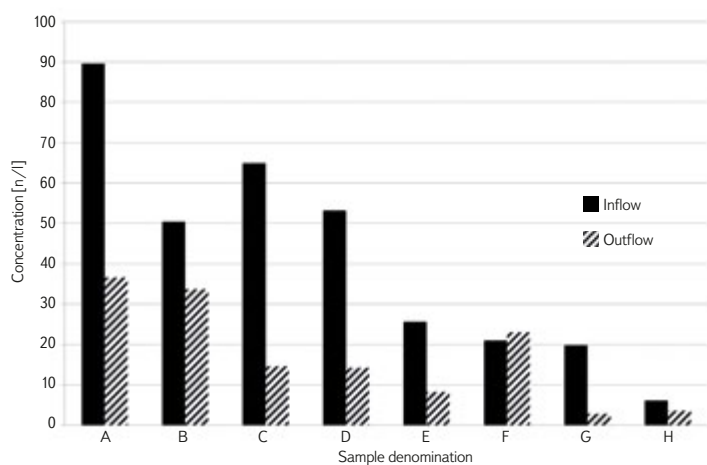
Based on the chemical properties of the substances, the following procedures were used for analysis:

- Determination of selected drugs by liquid chromatography with on-line preconcentration and mass detection in ESI+ mode (MDMA, MAMP, AMP, CO, BE, MET, EDDC).
- Determination of selected drugs by liquid chromatography with on-line preconcentration and mass detection in ESI- mode (nor-THC).
- Determination of nicotine and its selected metabolites by liquid chromatography with on-line preconcentration and mass detection in ESI+ mode (NIC, COT, T3H-COT).

Analytical procedures are described in detail in Pospíchalová et al. [13].

## RESULTS AND DISCUSSION

When we analyse untreated wastewater, the findings of the drugs listed in *Tab. 2* are positive in all samples. In the case of surface water analysis, the situation is different. Ecstasy, benzoylecgonine, cotinine, and *trans*-3-hydroxycotinine were found in all samples analysed in this pilot study. Methadone and its metabolite EDDP were determined only in some sampling profiles. Amphetamine was found sporadically, mostly at the limit of detection. This is also consistent with our findings within the DRAGON project (No. VG20122015101), in which we had the opportunity to compare the concentration of selected drugs in the influent and effluent of some WWTPs [14]. Amphetamine was removed most successfully (85–100 %), methamphetamine, ecstasy and benzoylecgonine only 40–50 % (*Tab. 4, Fig. 9*). Other compounds were not monitored in the DRAGON project.



*Fig. 9. Removal of benzoylecgonine, the main metabolite of cocaine, at different treatment plants [14]*

*Tab. 4. Examples of removal of illicit compounds at wastewater treatment plants [14]*

Analyte	Methamphetamine			Amphetamine			Ecstasy		
	Inflow [ng/l]	Outflow [ng/l]	Residual content [%]	Inflow [ng/l]	Outflow [ng/l]	Residual content [%]	Inflow [ng/l]	Outflow [ng/l]	Residual content [%]
<b>A</b>	4,070	392	10	173	11.3	7	17	3.9	23
<b>B</b>	1,410	319	23	24.4	0	0	4.04	2.33	58
<b>C</b>	1,030	449	44	62.2	3.23	5	2.99	1.08	36
<b>D</b>	1,120	193	17	102	15.9	16	61.9	16.8	27
<b>E</b>	484	148	31	44.8	3.12	7	7.98	8.72	109
<b>F</b>	232	202	87	36	3.72	10	10.7	18.1	169
<b>G</b>	250	139	56	28.4	2.12	7	4.36	3.62	83
<b>H</b>	276	151	55	36.6	0	0	7.86	4.74	60

## Vltava – Trojská lávka control profile

In the Vltava – Trojská lávka control profile, all monitored substances occurred in very low concentrations, mostly close to the limit of detection. Concentrations of nor-THC, a metabolite of marijuana, ranged from the limit of detection of 0.2 ng/l to 2.8 ng/l, with 45 % of findings below the limit of detection. From the group of amphetamines, methamphetamine was determined in all samples; the concentrations ranged from 0.3 ng/l to 2.9 ng/l. The limit of detection for MAMP is 0.1 ng/l. AMP was below the limit of detection (0.3 ng/l) in all samples. From the perspective of the Czech drug scene, amphetamine is primarily a metabolite of MAMP, not a drug used on its own. At the same time, it is successfully removed in wastewater treatment plants (*Tab. 3*). The party drug ecstasy (MDMA) was detected at concentrations between the detection limit of 0.1 ng/l and 8.3 ng/l, with 50 % of the findings below 1.0 ng/l. The samples were taken on weekdays and MDMA is a typical weekend drug. The concentrations of this drug may have influenced by this. Cocaine and its main metabolite benzoylecgonine were detected in all analysed samples (*Fig. 10*), the determined amounts ranged between 0.8–2.03 ng/l (BE) and 0.22–0.59 ng/l (CO). Apart from the findings on 5 June 2023 and 24 October 2023, the ratio of concentrations of these compounds corresponds, as only 1–9 % of cocaine is excreted unchanged, while 35–53 % leaves the body as benzoylecgonine. The reasons for the unusual findings on the above-mentioned days cannot be explained. On 5 June 2023, the concentration of cocaine was 4.48 ng/l and benzoylecgonine was 4.69 ng/l, while on 24 October 2023 it was 1.82 ng/l and 1.71 ng/l, respectively. Methadone used for substitution treatment and its metabolite EDDP were also found in the control profile; methadone in only three samples at values close to the limit of detection (0.2 ng/l), its metabolite in all samples. Its concentration in water was very stable, between 0.4 and 0.6 ng/l. The concentration of the licit drug nicotine and its metabolites is always higher than that of illicit drugs, with both metabolites occurring in surface waters in particular.



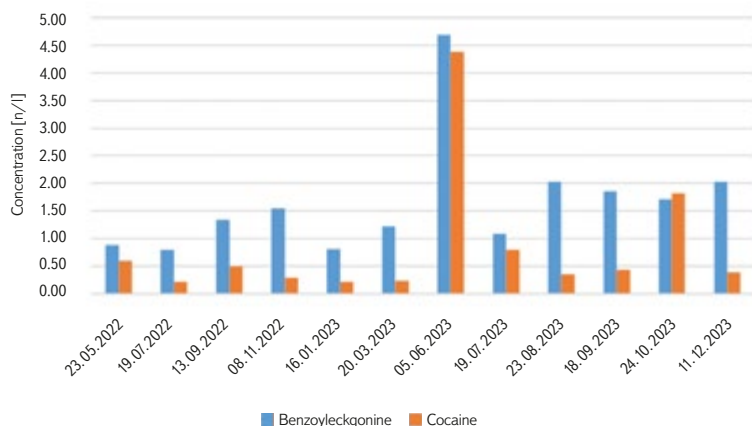


Fig. 10. Concentrations of cocaine and benzoyllecgonine in the Vltava control profile Vltava – Trojská lávka

## Vltava – Podbaba profile

The Vltava – Podbaba sampling profile, situated below both outputs of treated wastewater from the Prague WWTP into the recipient, shows significantly higher findings of individual monitored substances. Concentrations of the marijuana metabolite nor-THC are in the range of 0.7–4.4 ng/l, i.e. approximately 2× higher. Again, amphetamine was not present in detectable amounts, with concentrations slightly above the limit of detection in only two cases. Methamphetamine was detected in all samples, at concentrations ranging from 7.9 to 36.0 ng/l, i.e. at concentrations up to 10 times higher than in the control profile. Methamphetamine is removed significantly less than amphetamine in the treatment process (Tab. 3). Another of the monitored amphetamines, ecstasy, was also found in significantly higher concentrations in the Vltava – Podbaba profile; from 10.5 ng/l to 65.4 ng/l, i.e. up to 8 times higher concentrations. MDMA is removed approximately as successfully as pervitin (MAMP), i.e. 40–50 %. Cocaine and benzoyllecgonine concentrations were also higher in this profile than in the control profile, with the exception of the sample from 5 June 2023, which ranged between 0.4 ng/l and 2.2 ng/l (CO) and 1.1 to 3.4 ng/l (BE). The concentration of cocaine on 5 June 2023 was 21.6 ng/l and benzoyllecgonine 19.0 ng/l, again in an unusual ratio, approximately 4 times higher than in the control profile. A higher concentration of cocaine metabolite (BE) was also detected on 23 August 2023, at 19.3 ng/l, but the concentration of cocaine in this case was low (0.4 ng/l). Methadone and EDDP were

determined in all samples; methadone between 1.4–5.4 ng/l and EDDP between 2.8–8.9 ng/l, again in concentrations several times higher. The concentration of these two substances is always relatively stable, which results mainly from the regular use of methadone as an opioid for substitution treatment. Nicotine metabolites were determined in all samples; cotinine at concentrations of 13–74 ng/l, and *trans*-3-hydroxycotinine at concentrations of 18–46 ng/l. In this profile, relatively high nicotine findings were also found in more than half of the samples. The highest concentration was determined in the sample taken on 25 March 2022, at 1,040 ng/l, which also corresponds to the highest values for COT and T3H-COT.

Fig. 11 compares the concentrations of cocaine metabolites in the Vltava – Trojská lávka control profile and in the profile below the wastewater outlet from Prague CWWTP.

## Drahanský brook profile

WWTPs discharging treated wastewater (WW) into monitored streams are in the same category according to population equivalent (PE); see Tab. 2. The characteristics of individual streams are presented in the previous chapter. The average flow rate of Drahanský brook is the lowest of the monitored streams, but the number of people connected to Dolní Chabry WWTP is the highest of the monitored WWTPs (with the exception of Prague WWTP) and the annual volume of treated water discharged is also large. Therefore, the stream experiences the least dilution of this treated WW.

Positive THC metabolite findings were found in 75 % of the samples taken, with the concentrations ranging between the values at the detection limit, i.e. 0.2 ng/l and 4.2 ng/l. Illicit substances from the amphetamine group MDMA and MAMP were found in all analysed samples; again, amphetamine did not occur in values above the limit of detection. Ecstasy (MDMA) concentrations ranged from 0.3 to 17.4 ng/l, with 92 % of samples containing up to 9.4 ng/l. Pervitin was present in the tested samples at concentrations of 12.5 to 90.7 ng/l; these values are higher than in the Vltava – Podbaba profile. Cocaine and benzoyllecgonine were present in detectable amounts in all analysed samples, and their ratio was consistent. Cocaine concentrations ranged from 0.85 to 5.89 ng/l, while benzoyllecgonine values ranged from 2.4 ng/l to 51.7 ng/l. These values are also higher than in the Vltava – Podbaba profile. Substitution treatment contributes to the Drahanský brook contamination in the case of methadone, with concentrations of 1.5–15.6 ng/l, and its metabolite EDDP, with 5.0–26.2 ng/l. The situation is the same as for the previous analytes; the values are higher than in the Vltava profile below Prague CWWTP. Nicotine metabolites were also present in all tested samples, with concentrations ranging between

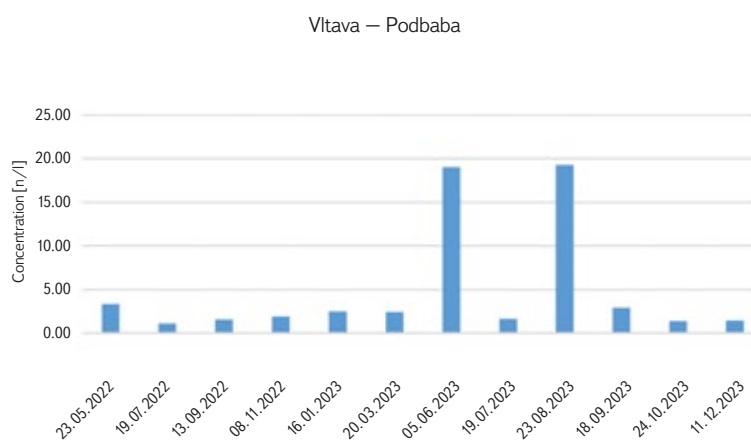


Fig. 11. Comparison of the concentration of the main metabolite of cocaine, benzoyllecgonine, in the Vltava control profile above the Prague CWWTP Vltava – Trojská lávka and in the Vltava profile below the outlet of the treated wastewater from the Prague CWWTP into the Vltava river

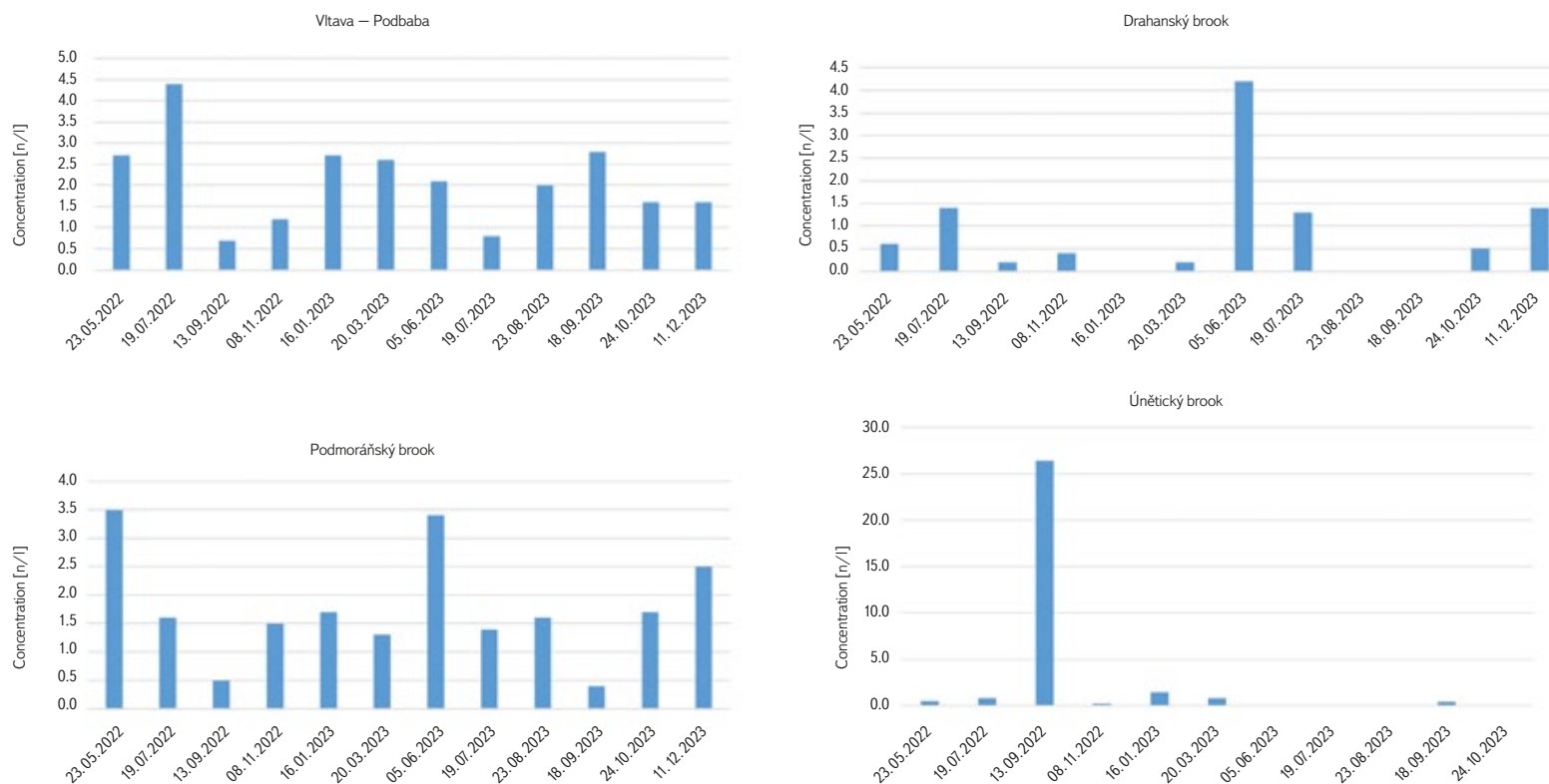


Fig. 12. Comparison of THC metabolite concentrations in monitored streams

15 and 53 ng/l for cotinine and 21–107 ng/l for *trans*-3-hydroxycotinine. Nicotine was detectable in 42 % of samples, with concentrations of 120 to 525 ng/l. In this case, the values were lower than in the recipient below Prague CWWTP.

### Podmoráňský brook profile

The average flow of Podmoráňský brook is 24 l/s; the average amount of discharged wastewater is 11.8 l/s; the number of people connected to the treatment plant in Velké Přílepy is almost half lower than in the Drahanický brook. Therefore, the treated water in Podmoráňský brook is more diluted than in Drahanický brook. The metabolite nor-THC, which represents marijuana, was found in surface water samples at concentrations of 0.4–3.5 ng/l in all tested samples. The concentrations are similar to those in samples of Vltava water below Prague CWWTP. Ecstasy was also found in all samples, at values between 2.2 and 34.2 ng/l. Methamphetamine at concentrations of 18.5–168 ng/l was also detected in all samples, and its metabolite amphetamine was present in detectable amounts in two-thirds of the tested samples, at concentrations between 0.3 and 2.5 ng/l. Cocaine and benzoylecgonine were also present in 100 % of samples, with cocaine concentrations ranging from 0.06 to 11.1 ng/l, and the respective metabolite in concentrations of 0.54 to 17.0 ng/l. Methadone was detectable in only three samples, EDDP in all but one sample, ranging from the limit of detection to 1.0 ng/l. The findings of these substances representing substitution treatment are related to the numbers of people using this treatment in the monitored area. Substances representing the legal drug nicotine were present in all samples in the case of both metabolites, nicotine was determined in two thirds of the samples. Their concentrations ranged from 122 to 685 ng/l (NIC), 21 to 74 ng/l (COT) and 31 to 206 ng/l (T3H-COT). The Podmoráňský brook therefore burdens the Vltava less than the Drahanický brook.

### Únětický brook profile

The Únětický brook has the largest water content of all the monitored tributaries of the Vltava; the average flow rate is 100 l/s. At 19 km<sup>2</sup>, the catchment area is also the largest, and the length of the stream is similar or the same as that of the other tributaries – 4.1 km. The Únětický stream is fed by the outlets of two WWTP, Horoměřice and Tuchoměřice, which serve a total of 5,266 connected residents. The annual volume of discharged water is 423,000 m<sup>3</sup>/year. Due to the high flow rate, the greatest dilution of treated WW occurs in the recipient, 64 % of the samples contained the THC metabolite, nor-THC, above the limit of detection; the concentrations were below 1.0 ng/l, with the exception of the sample taken on 13 September 2022 with the concentration of 26.4 ng/l. Ecstasy was determined in all samples, at concentrations between 0.9–5.1 ng/l. In one analysed sample, from 18 September 2023, the MDMA concentration was higher – 15.4 ng/l. During this period, social events were taking place in the monitored location, which, given that ecstasy is a typical party drug, could have influenced the concentration found. Pervitin was in detectable amounts in all collected and analysed samples. Amphetamine was always below the limit of detection, while pervitin concentrations were in the range of 2.9 to 14.1 ng/l. Cocaine (CO) and benzoylecgonine (BE) were detected in all samples of surface water collected; measured values for CO ranged between 0.27–17.5 ng/l and 1.25–59.3 ng/l for BE. The findings of this drug are relatively high, which may again be related to the sociodemographic and socioeconomic characteristics of the monitored locations; for example, in Horoměřice, it can be assumed that the residents belong to an affluent population in which cocaine is popular. The opioid methadone, used for substitution treatment, and its metabolite EDDP were present in 100 % of the samples; their concentrations were relatively stable throughout the project, which is related to its method of application. For methadone, concentrations were between 0.6 and 1.3 ng/l, for EDDP between 2.2 and 5.1 ng/l. Nicotine was determined in 55 %

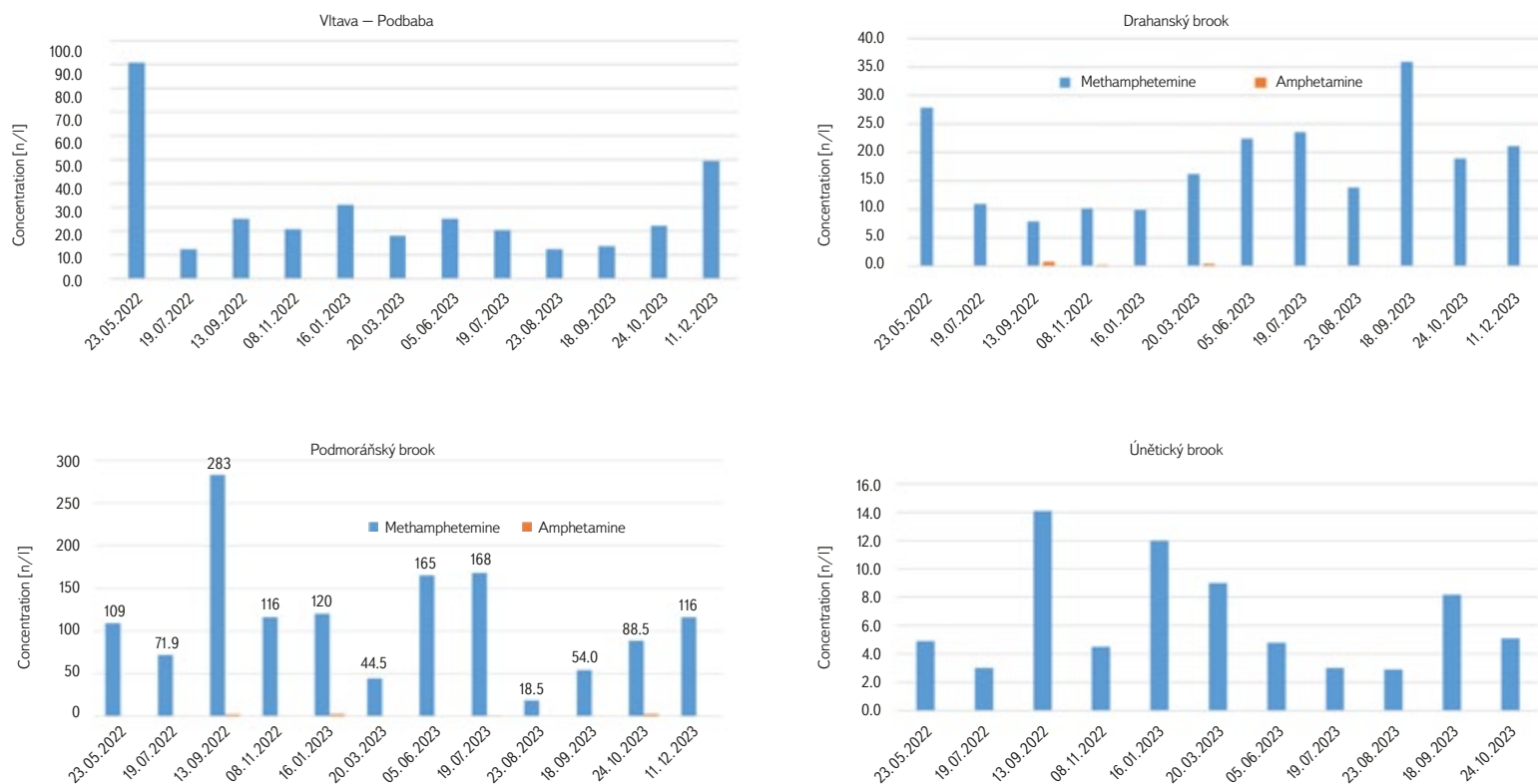


Fig. 13. Comparison of methamphetamine and amphetamine concentrations in monitored streams

of the analysed samples, with values ranging between 138–415 ng/l. All samples were positive for both cotinine and *trans*-3-hydroxycotinine, at concentrations of 13–142 ng/l and 15–277 ng/l, respectively.

At the end of this chapter, Figs. 12 and 13 compare the concentrations of the most commonly used drugs in the Czech Republic, marijuana and methamphetamine, in the monitored streams.

## CONCLUSION

The study is small in scope, however, it does confirm that even treated wastewater contains drug residues and their metabolites and is thus a source of both licit and illicit drugs entering into surface waters. Their quantity is influenced by the character and quality of the specific WWTP, the concentration of monitored substances in untreated urban wastewater and, last but not least, the ratio of the amount of discharged water to the size of the recipient. It also depends on the sociodemographic and socioeconomic characteristics of the monitored locations, which have an impact on the type of drugs used. Studies focusing, for example, on the influence of these substances on the behaviour of aquatic animals, have shown that these compounds have an undesirable effect on the environment as well.

We expect that this will change in the future thanks to the fundamental revision of Council Directive 91/271/EEC of 21 May 1991, on urban wastewater treatment, which introduces new principles for the treatment of urban wastewater, including quaternary treatment, which should remove micropollutants present in urban wastewater. These micropollutants undoubtedly include both illegal and legal drugs.

## Acknowledgements

The article was written with the support of Institutional Funds for the Development of the Research Organization TGM WRI under internal grant No. 3600.52.24/2022 and project No. SS02030008 “Centre for Environmental Research: Waste and Circular Economy and Environmental Safety” (CEVOOH) and other institutional funds.

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The Czech version of this article was peer-reviewed, the English version was translated from the Czech original by Environmental Translation Ltd.

DOI: 10.46555/VTEI.2024.11.003

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