Clean water – Healthy city. Using of WBE for monitoring of the consumption of illicit drugs in the city Prague

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Abstract. The results of the three years long project named *Municipal wastewater as a diagnostic medium of the capital city of Prague* are presented. The post informs about regular, almost two-year long monitoring of municipal wastewater at selected sampling points of the Prague sewerage network. In addition to selected illicit drugs and some pharmaceuticals (e.g. methamphetamine, MDMA, THC, cocaine, fentanyl), nicotine and its metabolites and the ethanol metabolite ethyl sulphate were monitored in municipal wastewater. 24-hour composite samples each fourth day (the first year) or eighth day (the second year) were collected. There were obvious differences in consumption of individual drugs in different parts of the city.
For the purposes of the project, analytical methods standardly used in the laboratory for the determination of monitored substances were extended.
The project included a Socio-economic study of spatial distribution of the population and identification of risk areas in terms of population lifestyle.
Keywords: wastewater-based epidemiology, illicit drugs, nicotine, alcohol, monitoring

1. Introduction

Wastewater analysis is a "relatively" simple matter. With the development of instrumental technology in this area, we are able to detect the presence of individual substances in subnanogram quantities using high-end, highly sensitive analytical instruments. The project **Clean Water – Healthy City. Municipal wastewater as a diagnostic medium of the Capital City of Prague.** loosely followed the project **Determination of the amount of illegal drugs and their metabolites in municipal wastewater – a new tool for supplementing data on drug consumption in the Czech Republic,** which also monitored contamination of municipal wastewater in selected sewers of the sewer network of the capital city of Prague. One of the main outputs of this project was the development of a methodology (Očenášková, V. et al. 2015), which was also used for measurement in this concept, in which up-to-date data on the consumption of illicit substances and data on newly monitored markers were obtained.

The purpose of the project was to obtain objective data, i.e. also data from the part of the population that was not included in the questionnaire actions on the monitored topic, did not have health problems related to the use of illegal substances, etc.

The basis of wastewater-based epidemiology (WBE) was laid at the turn of 1999 to 2000 by expressing the hypothesis that municipal wastewater can be considered as a diluted urine sample. (Daughton, C. G. Ternes, T. A., 1999, 2001) and was first applied in the Catchment Area of the River Po (Zuccato et al., 2005). Initially, the epidemiological approach to wastewater was used to monitor the consumption of illicit substances in the monitored site and to refine the estimate of prevalence and drug use in the population. Since its inception, this field has been developing very rapidly and thanks to the ever-improving analytical technique, it is possible to monitor a wide range of substances in very low concentrations.

Municipal wastewater contains a complex mixture of chemicals including human metabolites – biomarkers. Quantitative measurement of these specific substances will provide information such as diet, population health, disease incidence, alcohol and pharmaceuticals consumption, and population exposure to environmental contaminants, e.g. pesticides.

Since the beginning of the year 2018, the project **Clean Water – Healthy City. Municipal wastewater as a diagnostic medium of the Capital City of Prague.** has been solved at TGM WRI, p. r. i. In addition to illicit substances and certain drugs, metabolites of alcohol (ethyl sulfate), nicotine (cotinine and trans-3-hydroxycotinine) and pesticides were also monitored. Monitoring took place for two years at selected locations of the sewer network of the capital city of Prague. The planned project output was a map layer of the Prague Geoportal, where, in the case of regular monitoring, the consumption of drugs in individual parts of Prague will be recorded.

The project also included **The socioeconomic study of the spatial distribution of the population and identification of risk areas from the point of view of the lifestyle of the**

inhabitants. This study was subcontracted by ACCENDO – Centre for Science and Research.

The aim of the project was to obtain up-to-date data on drug consumption in individual parts of Prague (according to the possibilities of collection on individual sewers), as well as on other substances that were monitored in the project. Their list is available in Table 1.

Table 1: List of monitored substances

Group of substances	***Name***
"Classic" drugs	11-nor-9-carboxy-delta-9-THC
	3,4-methylene dioxy-methamphetamine
	Methamphetamine
	Amphetamine
	Cocaine
	Cocaethylene
	Benzoylekgonine
	Lysergic acid diethylamide
	Heroin
	Morphine
Substitution treatment	Methadone
	2-ethyliden-1,5-dimethyl-3,3-diphenylpyrolidine (EDDP)
	Buprenorphine
New synthetic drugs (NSD)	Ethylcatinone
	Entedron
	Methylon
	4-methylethkatinone
	Mephedrone (4-methylmethkatinone)
	Normefedron
	Alpha-pyrolidinovalerophenone
Abused medicines	*cis*-tramadol
	Nordiazepam
	Fentanyl
	Norfentanyl
Nicotine and its metabolites	Nicotine
	Cotinine
	trans-3-hydroxycotinin
Ethanol metabolite	Ethyl sulphate
Precursor for drug production	Ephedrine

These substances were not yet regularly monitored; they are not subject to the relevant legislation. The detection of their occurrence and concentrations in wastewater is significant not only for the above reasons, but since some of these substances are not completely removed in the wastewater treatment process, they also enter surface waters in which they can have an impact on the environment.

The obtained and analytically processed data were interpreted through the map portal. A potential option was the use of the Geoportal of Prague.

Based on analysis and measurement in wastewater, methods can be developed to evaluate the effectiveness and cost-effectiveness of measures to prevent and control the use of those substances (illicit drugs). The methods can be set up to be usable for different types of evaluations.

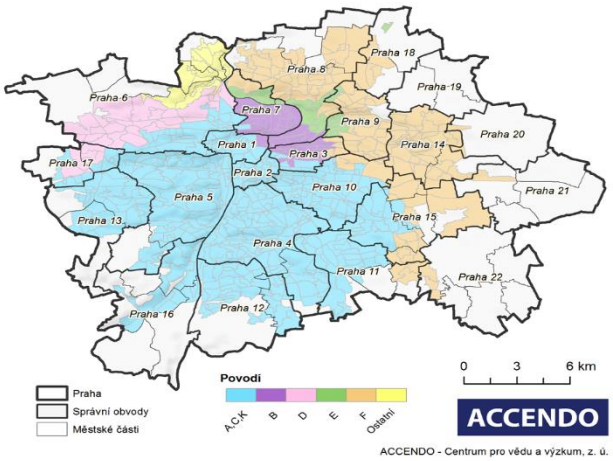
2. Project solution

2.1. *Sampling and basic pre-preparation of samples*

In cooperation with the employees of the WWTP, six sampling profiles were selected on the sewer network of the capital city of Prague, a sampling plan was prepared and the conditions of sampling and transport of samples to the laboratory were prepared. Sampling profiles were on the total tributary to the Prague WWTP (ÚČOV) and also on individual sewers – ACK, B, D, E and F. Sampling took place from the beginning of April 2018, in 2018 at approximately four-day intervals, in 2019 at eight-day intervals. In addition to these samplings, a weekly monitoring campaign for the international SCORE project was carried out in 2019. 24-hour composite samples of municipal wastewater were taken.

Samples were taken in clean sample containers of suitable material (glass, polypropylene (PP)). After transport to the laboratory, these samples were further processed by established operating procedures. The aliquot of the taken sample was used for analysis; preservation was not be carried out. After sampling, the samples were kept cool and dark at a temperature of up to 8 °C. If the samples could not be analyzed within 72 hours of sampling, they were frozen and stored at -20 ± 4°C Before analysis, samples were centrifuged (4,500 rpm, 15 min) and solid particles were removed from the sample by filtration through disposable membrane filters from regenerated cellulose of 0.45 µm.

Figure 1 shows a map of the Prague sewer network showing the areas under the individual sewers.



Source: Prague WWTP, ACCENDO

Figure 1. Prague sewer network

2.2. *Development of analytical methods – introduction of procedures for the determination of nicotine and its metabolites, ethyl sulfate*

(ethanol metabolite) and new psychotropic substances in wastewater

The analytical method (LC-MS/MS) for determination of illicit drugs, which was described in the methodology of application of wastewater based epidemiology for determination of illegal substances (drugs) (Očenášková, V. et al. 2015), was extended to the determination of new psychotropic substances (NSD, nordiazepam, fentanyl, norfentanyl) for the purposes of the Clean Water – Healthy City project.

Two completely new methods were introduced for the determination of nicotine and its metabolites and for the determination of ethyl sulphate. All these methods described in detail in Pospíchalová 2020 were validated and accredited.

Used technique: Agilent 1200 RR liquid chromatograph with binary pump, Applied Biosystem 4000 Q Trap triple quadrupole mass detector, Aspec GX-271 autosampler (Gilson). The determination of all substances was performed with Synergi Hydro-RP80A chromatography column (150 × 2.0 mm, 4)m); Hypersil Gold 20 × 2,1 mm, 12 µm was used for SPE-online pre-concentration of compounds.

According to the chemical properties of the substances, the following procedures were used:

Determination of selected drugs by LC-MS/MS with online preconcentration in ESI + mod (most of compounds from table 1).

Determination of selected drugs by LC-MS/MS with online preconcentration in ESI – mod (11-nor-9-carboxy-delta-9-THC).

Determination of nicotine and its selected metabolites by LC-MS/MS with online preconcentration in ESI + mod.

Determination of ethyl sulphate by LC-MS/MS in ESI - mod

2.3. Reverse calculation of consumption of monitored substances

Additional data can be obtained from the concentrations (ng/l) of the monitored substances in the analyzed municipal waste water samples, such as daily mass loads of monitored substances or their consumption expressed in g/day/1000 inhabitants (unit of mass may be different if necessary). For these calculations, it was necessary to know the daily flow of wastewater on the day of sampling of the 24-hour composite sample and the number of inhabitants connected to the sewer network in the area. In order to determine the consumption of a particular substance, it was necessary to determine the so-called correction (recalculation) factor. Correction factors were used from literary sources.

3. Examples of results

Sampling started in April 2018, initially mainly "classic" drugs were monitored, and later (July 2018) substances for which new methods were developed were added. Sampling ended in December 2019.

About 500 wastewater samples were collected and analyzed. The findings of the monitored drugs from the group identified in Table 1 as "classic" drugs (with the exception of heroin and LSD that were not found in any sample) and other substances (cis-tramadol, nor-diazepam, nicotine and its metabolites, ethyl sulphate and ephedrine) were positive in all samples. Methadone and its metabolite 22-ethylidene-1.5-dimethyl-3.3-diphenylpyrrolidine (EDDP) were found in almost all samples. Fentanyl was found in concentrations close to the limit of determination. Buprenorphine and most substances from the new synthetic drug group (NSD) were not found in any sample. Findings of some NSDs (mephedrone) were quite unique. Heroin is metabolized to 6-acetylmorphine, which rapidly degrades; 6-acetylmorphine was not be determined in any sample. However, another metabolite of this drug, morphine, is also a metabolite of other substances, including prescribed drugs. Determining what proportion of morphine findings were caused by heroin degradation is virtually unrealistic. A typical weekend drug is ecstasy. Differences in drug consumption between working and weekend days are presented in Figure 2.

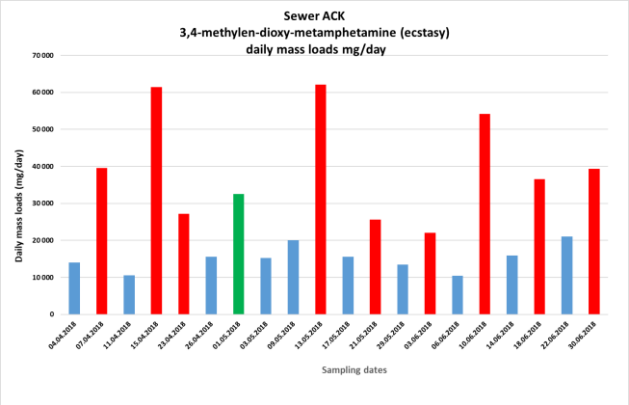


Figure 2. Daily mass loads of ecstasy (ACK sewer). Working days are marked in blue, weekend days in red and in green is marked national holiday.

Other figures show some results of wastewater monitoring for 2018.

Figure 3 shows very well the differences between the consumption of various drugs in different parts of Prague, the map showing the catchment areas of the individual sewers is shown in Figure 1.

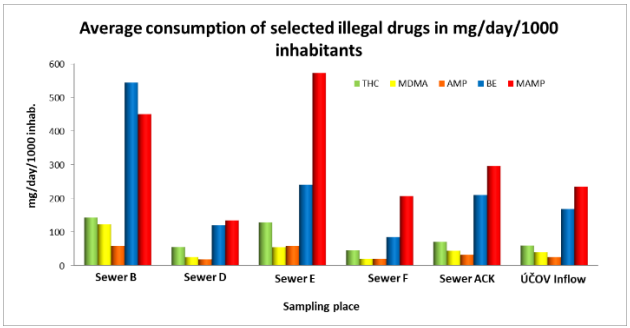


Figure 3. Average consumption of selected illegal drugs in mg/day/1000 inhabitants. THC - tetrahydrocannabinol (marijuana), MDMA – ecstasy, AMP – amphetamine, BE – benzoylecgonine, main metabolite of cocaine, MAMP – methamphetamine (meth, Crystal)

Tobacco consumption is presented in Figure 4. Important metabolites are especially cotinine and trans-3-hydroxycotinine, stable nicotine metabolites. Nicotine enters wastewater not only as unmetabolised from smoked cigarettes and other tobacco products, but also, for example, from products used in weaning (Castiglioni S. et al. 2014, Ort Ch. et al. 2014).

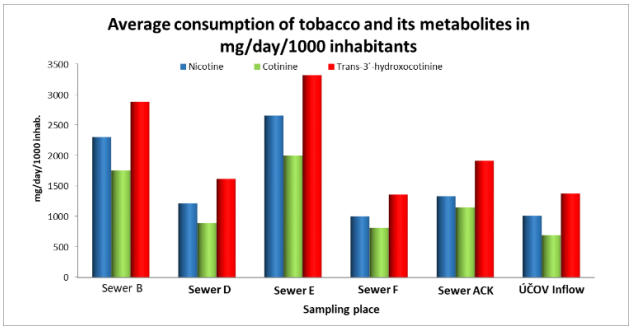


Figure 4. Average consumption of tobacco and its metabolites in mg/day/1000 inhabitants

The legal drug alcohol was also monitored in the project. Its consumption was monitored through the metabolite ethyl sulphate. Although only <0.1 % of the alcohol consumed is excreted in this way (Mastroianni 2016), wastewater findings were clearly the highest of all monitored substances (Figure 5).

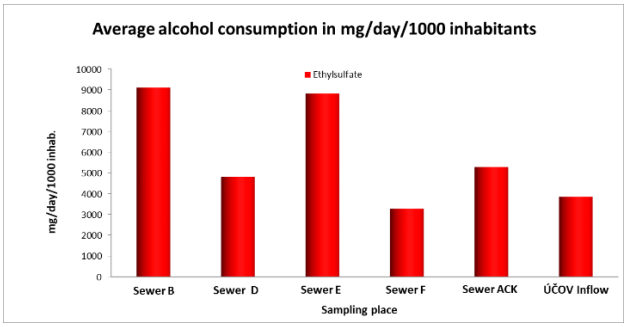


Figure 5. Average alcohol consumption in mg/day/1000 inhabitants (ethyl sulfate - monitored stable metabolite of ethanol)

4. Conclusion

In total, almost 500 wastewater samples were collected and processed as part of the project and about 2000 analyses were carried out.

The results were gradually processed; recalculations of the carry-outs of individual monitored substances were carried out both in the partial monitored areas (individual sewers of the Prague sewer network) and on the tributary to the WWTP of the capital city of Prague.

These results showed significant differences in drug consumption in different parts of Prague. For example, cocaine consumption was highest in areas close to the center of Prague, which are also popular neighborhoods to live in.

Socioeconomic study of spatial distribution of the population and identification of risk areas from the point of view of the lifestyle of the population was carried out; the subcontractor of this study is ACCENDO Institute – Center for Science and Research.

The laboratory participated in the international SCORE project, which also includes, in addition to weekly monitoring of municipal wastewater in the monitored site, in this case Prague, participation in an interlaboratory comparison of tests. The results of this monitoring were presented on the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) website.

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