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Pharmaceuticals and illicit drugs – A new threat to the application of sewage sludge in agriculture

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HIGHLIGHTS

- The first screen of pharmaceuticals and illicit drugs in sewage sludge in Slovakia.
- 52 target compounds quantified in the investigated samples.
- The highest findings for fexofenadine - 5600 ng/g DM and telmisartan - 3400 ng/g DM
- Aerobic sludge differed from anaerobic sludge in the pattern of pharmaceuticals
- Estimated pharmaceutical loads to soils reach hundreds of kilograms yearly.

GRAPHICAL ABSTRACT

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ABSTRACT

The occurrence of 93 pharmaceuticals, illicit drugs and their metabolites has been investigated in stabilized sewage sludge from five municipal wastewater treatment plants (WWTPs) in the Slovak Republic. The total population connected to the tested WWTPs was approximately 600,000 p.e. which represents >20% of the Slovak population connected to public sewer systems. The sludge production from the five tested plants was >8100 tons in 2016, which is approximately 15% of the total Slovak sewage sludge production in 2016.

The highest total concentration of all pharmaceuticals was found in WWTP Bratislava Devínska Nová Ves (DNV) and Senec - 11,800 and 11,300 ng/g dry matter (DM), respectively. Among individual pharmaceuticals, the highest concentrations were recorded for fexofenadine (mean 2340 ng/g DM, maximum 5600 ng/g DM in Bratislava DNV) and telmisartan (mean 1170 ng/g DM, with a maximum of 3370 ng/g DM in Senec). A principal component analysis revealed differences between pharmaceutical patterns in aerobically and anaerobically stabilized sludge.

The worst-case scenario based on no further degradation of pharmaceuticals between sludge production and field application was used to predict pharmaceutical mass loads in agriculture. For the result, we estimated an annual load to soil in the Slovak Republic of up to several hundred kilograms of pharmaceuticals and drugs, with the maximum for fexofenadine (120 kg/year) and verapamil (29 kg/year).

1. Introduction

There are many types of waste products (according to the newest point of view, waste is often referred to as biosolids or biowaste)

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produced at a wastewater treatment plant (WWTP). The technological line includes screenings, grit, scum, primary sludge, excess sludge, secondary sludge, raw sludge, thickened sludge, dewatered sludge, stabilized sludge, etc. These waste products are usually in the form of a liquid or semisolid liquid, which typically contains 0.25 to 12% solids by weight, depending on the operations and processes used (Metcalf and Eddy, 1991). The stabilized sewage sludge is regarded as the most important (from the perspective of volume or weight) output from WWTPs. Its treatment, reuse, and disposal are the most complex problem that engineers in the field of wastewater treatment are facing at present.

1.1. Sewage sludge production in the European Union (EU)

The specific sewage sludge production is the result of many factors, e.g., primary, secondary and even tertiary treatment efficiency, the treatment technology used (MBR, nutrient removal, P-precipitation, etc.), the method of sludge stabilization (aerobic or anaerobic digestion), operation conditions (sludge age and organic load) and many others. Dry matter (DM) weight per capita production of sewage sludge before processing is in range between 66 and 90 g per person per day (Davis, 1996; Mininni et al., 2015; Metcalf and Eddy, 1991). DM production decreases due to the removal of volatile solids in biological stabilization processes and some inefficiencies of solid-liquid operation (gravity and dynamic thickening and mechanical dewatering) after sludge processing. Therefore, sludge output from WWTPs was theoretically evaluated at 45–56 g DM/capita/day. The actual data from the EU show that the average value for the EU28 is 58.9 g DM/cap.d, which represents a yearly production of approximately 21.5 kg DM of sewage sludge for each EU28 citizen. There is a great variation in waste production in EU countries; the minimum values of specific sewage sludge production refer to Croatia (20.5 g DM/cap.d), Bulgaria (22.6 g DM/cap.d), Romania (23.8 g DM/cap.d), with the highest values for Spain (160.7 g DM/cap.d), Austria (85.8 g DM/cap.d) and Portugal (87.6 g DM/cap.d) (Eurostat, 2017). It is evident that the new EU members have significantly lower sludge production due to a lower number of households connected to sanitary systems. It is expected that the specific production of sewage sludge will increase in these countries along with increasing of sewer and WWTP connections in the coming years. In fact, approximately 11 million tons of sewage sludge DM is estimated to be produced in the EU28 every year (Eurostat, 2017), and in the future, we expect approximately 15 million tons of DM, which represents approximately 60 million tons of fresh matter with assumed treatment and disposal.

1.2. Sewage sludge management in the EU

Sludge contains nutrients, such as nitrogen and phosphorous, and valuable organic carbon matter that is useful when the soil is depleted or tends to erode. The organic matter and nutrients are the two main reasons that make this method of utilization of sewage sludge suitable. Nearly 40% of sludge produced in the EU is estimated to be spread on land for agricultural use and approximately 12% is used in compost and probably will also be applied to soil (in forests, on gardens, etc.). The application of sewage sludge in agriculture varies greatly among EU28 countries. In some countries (Denmark, Ireland, France, Spain and the United Kingdom), more than half of all the sludge production is used in agriculture; on the other hand, in four countries (Malta, Netherlands, Slovenia and Slovakia) no sludge is directly recycled to agriculture, and in four others (Estonia, Finland, Hungary and Romania) the amount is <10% of total sludge production (Eurostat, 2017).

Although sewage sludge contains components beneficial to soil, it also contains contaminants, such as heavy metals, extraneous organic compounds and pathogens. There is clear evidence that, since the mid-1980s, concentrations of heavy metals in sewage sludge have steadily declined due to regulatory controls on the use and discharge of dangerous substances, voluntary agreements and improved industrial practices. These measures have led to the termination or reduction of discharges, emissions and losses of these heavy metals to the environment (Gendebien, 2010; Bloem et al., 2017).

1.3. Pharmaceuticals in sewage sludge

Sewage sludge tends to sorb many potential contaminants from wastewater, such as phthalates, pesticides, phenols, pharmaceuticals, illicit drugs and other organic chemical residues (Chen et al., 2015; Schnaak et al., 1997; Martín et al., 2015; Hörsing et al., 2011). Recent technological advances have increased the sensitivity and selectivity of chromatographic and mass-spectrometric analyses, allowing the determination of these micropollutants in wastewater, surface water, and potable water, as well as in sludge. Among them, illicit drugs and pharmaceutically active compounds are emerging as pollutants that have triggered a major interest in recent years (Verlicchi et al., 2012; Martín et al., 2015; Ivanová et al., 2017), not only due to their potential for ecotoxicological risks but also because of their continuous immission to the environment. This fact has prompted the EU to include some of these pollutants (azithromycin, clarithromycin, erythromycin, diclofenac, estrone, 17α-ethinylestradiol and 17β-estradiol) on the first watch list in order to gather monitoring data in rivers for the purpose of facilitating the determination of appropriate measures to address the risk posed by those substances (European Council Directive, 2013; European Union Decision, 2015). However, no regulation of their content in sewage sludge is present in the current European Union legislation, but we can expect implementation of these measures in a few years into sludge quality requirements.

Many of the studies about the occurrence of pharmaceuticals are focused on raw sewage and contaminant removal in WWTPs (Li, 2014; Golovko et al., 2014; Verlicchi et al., 2012; Tran and Gin, 2017; Park et al., 2017). However, there are a fewer data about the occurrence of these compounds in sewage sludge as the final products that are applied onto soils (Verlicchi and Zambello, 2015; Martínez-Alcalá et al., 2017; Martín et al., 2012; Martín et al., 2015). With regards to stabilized sludge as the output of solid waste from WWTPs, the most studied classes of pharmaceuticals are analgesics/anti-inflammatories, psychiatric drugs and antibiotics. Compounds that occurred at higher concentrations in sludge are ibuprofen (1274 ng/g DM), citalopram (3300 ng/g DM), ciprofloxacin (6300 ng/g DM), norfloxacin (8300 ng/g DM) (Verlicchi and Zambello, 2015).

1.4. Treatment sludge management in the Slovak Republic

There are two types of sludge stabilization in municipal Slovak WWTPs. Aerobic sludge stabilization technology is operated in the Slovak Republic predominantly in small WWTPs with load capacities below 10,000 p.e. There are approximately 640 small and only two large WWTPs (Ružomberok – 325,000 p.e. and Spišská Nová Ves – 48,000 p.e., respectively) with this system of low loaded aerobic sludge stabilization. The annual production of aerobic stabilized sludge is approximately 20–25% of the total produced sludge from Slovak municipal sludge. Due to the relatively good quality of sludge (WWTPs for small villages, towns without an industrial pollution portion), it could be applied to agricultural soil (indirectly as compost). Anaerobic sludge digestion with biogas production and its utilization is realized in 51 municipal WWTPs, with approximately 2.6 mil. connected p.e. Approximately 75–80% of Slovak treatment sludge is produced at these WWTPs (Bodík and Kubaská, 2013).

The amount of sludge produced in municipal WWTPs is permanently monitored and recorded. Annual municipal sludge production varied between 53,000–59,000 t (DM) in recent years, which represents average specific sludge production of approximately 10.4 kg/capita/year. The official data about sludge production and sludge utilization

Table 1
Sludge production in the Slovak Republic and its utilization (Kozáková et al., 2017).

Year	Total	Sludge amount (in tons of DM)				
		Direct soil application	Composted	Landfilled	Energy valorization	Others[a]
2004	53,085	12,067	30,437	4723	0	5858
2006	58,050	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

[a] Temporary stored at WWTP during winter time before utilization previously mentioned ways.

in the Slovak Republic are summarized in Table 1 (Kozáková et al., 2017).

According to published data, it is obvious that the indirect soil application of sludge (as compost) is the most frequent method (approximately 65%) of sludge disposal. Direct sludge application to soil, a method often used in the past, was extremely reduced due to strict requirements on sludge quality (Parliament of the Slovak Republic, 2003). There were some attempts to utilize unpolluted sludge at soils in period 2010 to 2012, however it was not accepted by farmers. A decrease in landfilled sludge is also evident during the last few years, and this tendency will probably continue in the future.

We aimed to study the presence and potential risk of the transfer residual pharmaceuticals from sludge in five Slovak municipal WWTPs with a connection of approximately 600,000 inhabitants. Furthermore, on the basis of different sludge stabilization systems in the investigated WWTPs, we aimed to compare the content of pharmaceuticals in anaerobically vs. aerobically stabilized sludge.

2. Materials and methods

2.1. Characterization of the investigated locations

Five Slovak WWTPs were selected for this study – see on map in Supplementary file No. 1. Two of them are situated in the capital of Slovakia (Bratislava Central and Bratislava – Devínska Nová Ves (DNV)), and three of the WWTPs are from other various places in Slovakia. Three of the tested WWTPs utilize the anaerobic digestion of sludge, and two of them utilize an aerobic sludge stabilization process. Basic relevant information on studied WWTPS are given in Table 2.

2.1.1. WWTP Bratislava Central

The central WWTP in Bratislava is one of the largest wastewater treatment plants in Slovakia, with a capacity of a 620,000 p.e., while currently it operates only to the level of 440,000 p.e. WWTP Bratislava Central consists of a mechanical stage and a biological stage (with nitrification and denitrification), the produced sludge is anaerobically digested (mesophilic fermentation), and the resulting biogas energy is recovered into heat and electric energy. The average wastewater flow is approximately 115,000 m^3/day. The annual sludge production (2016y) is approximately 6650 tons of DM, which represents approximately 41 g DM/(p.e.).day. Final dewatering of the stabilized sludge is realized by centrifuge. The produced sludge is mixed with green biowaste, and after the composting process, is applied in agriculture.

2.1.2. WWTP Bratislava DNV

This treatment plant treats wastewater from a small suburb of Bratislava. It is a treatment plant with the capacity for 76,000 p.e., currently operating only to the level of 45,000 p.e., which also corresponds to the population connected to the plant. WWTP Bratislava DNV consists of a mechanical stage and a biological (nitrification, denitrification and bio-P elimination) stage, the sludge is also anaerobically stabilized, and the generated biogas is energetically recovered. Water flow during last year varied by approximately 7000 m^3/day. Sludge production after anaerobic stabilization is approximately 400 tons of DM/year (24 g DM/(p.e.).day). The sludge is dewatered by centrifuge. After composting with green biosolids, it is applied to soil in line with Slovak sludge legislation.

2.1.3. WWTP Komárno

WWTP Komárno is located in the southern part of the Slovak Republic and has a capacity of 35,000 p.e., which also corresponds to the population connected to the WWTP of the town of Komárno and the small surrounding villages. The WWTP consists of a mechanical pretreatment and a biological stage with anoxic and aerobic sections. The water flow varies by approximately 15,000 m^3/day. Primary and biological sludge is anaerobically digested with biogas production and dewatered by a belt filter press and applied as a compost on soil. Sludge production after anaerobic digestion is approximately 450 tons of DM/year (35 g DM/(p.e.).day).

2.1.4. WWTP Senec

The small town of Senec, with popular recreational lakes, is located near Bratislava and has a WWTP with a load of approximately 22,500 p.e. The technological line consists of a biological stage with predenitrification, nitrification and phosphorus precipitation. Aerobic sludge stabilization is ensured in a low loaded activation system with a yearly sludge production of approximately 200 tons of DM (24 g DM/(p.e.).day). Sludge is dewatered by centrifuge and applied to soil after the composting process.

2.1.5. WWTP Spišská Nová Ves

WWTP Spišská Nová Ves is located in eastern Slovakia. The plant serves the town Spišská Nová Ves and the surrounding small villages, with a total capacity of approximately 50,000 p.e. The mean inflow of wastewater during the last year was 16,500 m^3 per day. This WWTP consists of mechanical pretreatment and biological (RAnDN – Regeneration – Anaerobic zone – Denitrification -Nitrification) stages. High sludge age in the activated sludge processes provides aerobic sludge stabilization, with a yearly sludge production of approximately 400 tons of DM (22 g DM/(p.e.).day). The sludge is dewatered by centrifuge.

Table 2
Basic characteristics of the investigated sludge.

	Population connection	Sludge stabilization method	Sludge production	Total solids content	Pharmaceuticals with concentrations above the LOQ	Total sum of pharmaceutical concentrations
	(p.e.)		tons DM/year	(%)	(No.)	ng/g DM
Bratislava Central	440,000	anaerobic	6650	29.6	43	7460
Bratislava DNV	45,000	anaerobic	400	22.0	40	11,800
Komárno	35,000	anaerobic	450	15.3	36	4870
Senec	22,500	aerobic	200	18.9	45	11,300
Spišská Nová Ves	50,000	aerobic	400	23.0	33	2540

2.2. Sampling and analysis

Samples were taken from the sludge after the stabilization (aerobic and anaerobic) and after dewatering at each of the studied WWTPs during February 2017. We sampled the containers prepared for transport from the WWTP for further processing (compost production). A sample of sludge (approximately 500 ml each day) was taken over three days to obtain approximately 1500 ml of pooled sludge for analysis. Each pooled sample was homogenized and analyzed in triplicate. Sample aliquots were immediately analyzed for basic sludge parameters (water content, suspended solids) – see Table 2, and the rest of the sample was frozen (−20 °C) and transported to an analytical laboratory at the Faculty of Fisheries and Water Protection of USB in Vodňany (Czech Republic).

Pharmaceuticals, illicit drugs and their metabolites from collected sludge samples were extracted by a 2-step extraction procedure described in detail by Golovko et al. (2016). Briefly, 2 g of sludge was extracted with 4 ml of acetonitrile/water (1/1 v/v with 0.1% formic acid), ultrasonicated for 15 min, and the supernatant was filtered through a syringe filter (0.45 μm, regenerated cellulose) into 10-ml vials. In the second step, the same procedure was repeated with 4 ml of acetonitrile/2-propanol/water (3/3/4 v/v/v with 0.1% formic acid). The sludge extracts were mixed and stored in a freezer at −20 °C until the LC-MS/MS analysis.

All LC-MS/MS analyses were performed on a TSQ Quantiva triple-stage quadrupole mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) coupled to an Accela 1250 LC pump (Thermo Fisher Scientific) and an HTS XT-CTC autosampler (CTC Analytics AG, Zwingen, Switzerland). A Hypersil gold aQ column (50 mm × 2.1 mm ID × 5 μm particles; Thermo Fisher Scientific) was used for the chromatographic separation. The internal standard method combined with a matrix of matching standards that was used for the quantification of the target analytes. A detailed description of MS/MS transitions and LC-MS/MS methods has been provided elsewhere (Lindberg et al., 2014, Golovko et al., 2016).

2.3. Concentration and load calculations

The concentration of the pharmaceuticals for the given WWTP was calculated as the arithmetic average of the triplicate results. If at least one of the three analyzed sludge samples was above the LOQ for a given WWTP, the substance was included in the overall results.

The yearly amount of pharmaceuticals in each investigated sludge was calculated from the aforementioned results and the total sludge production at the corresponding WWTP. Then, we summed the data from all studied WWTPs and estimated total yearly amount of pharmaceuticals in sludge by extrapolating this sum to the quantity of sludge produced in Slovakia according to inventory data from 2016 (see Table 1). The fraction applied to soil was estimated with the use of the same data from the national inventory of sludge utilization (multiplication with factor 0.654 as percentage of the sludge applied to soils to total produced sludge – see Table 1 year 2006).

2.4. Principal component analysis

The resulting dataset was censored with the exclusion of all analytes that were below limit of quantification in all samples. We normalized the dataset for each WWTP to sum the compounds to avoid distortion of the data by scattered concentrations among the sludge samples. The analysis was performed using the STATISTICA v.12 software for Windows (StatSoft, Czech Republic).

3. Results and discussion

The LC-MS/MS method for the quantification of 93 pharmaceuticals, illicit drugs and some of their metabolites belonging to 18 therapeutic classes was used for the sludge analysis. The most numerous were psychoactive drugs and their metabolites (27 chemical compounds), antibiotics (15 compounds), antihypertensive drugs (14 compounds), antihistamines (6 compounds), lipid reduction drugs (4 compounds), and other drugs, such as analgesics/nonsteroidal anti-inflammatory drugs (NSAIDs), anti-Alzheimer drugs, anti-Parkinsonian drugs, stimulants, etc. A special group of analytes was 11 illicit drugs and their metabolites. Thirty-three pharmaceuticals were quantified above the LOQ in the sludge from the WWTP Spišská Nová Ves, while up to 45 compounds were determined in the sludge from WWTP Senec. Some pharmaceuticals, such as azithromycin, cetirizine, citalopram, diclofenac, sertraline, telmisartan, valsartan, etc., were quantified in all sludge samples. The highest concentrations of pharmaceuticals were found in sludge from WWTP Bratislava DNV and WWTP Senec (sum of concentrations of all analyzed substances above LOQ - 11,790 and 11,280 ng/g DM, respectively), the lowest sum of concentrations was found in sludge from WWTP Spišská Nová Ves - 2540 ng/g DM - see Table 2. Among the 93 pharmaceuticals, at least one of the samples of sludge contained 52 pharmaceuticals above the LOQ, which are further detailed in the following text.

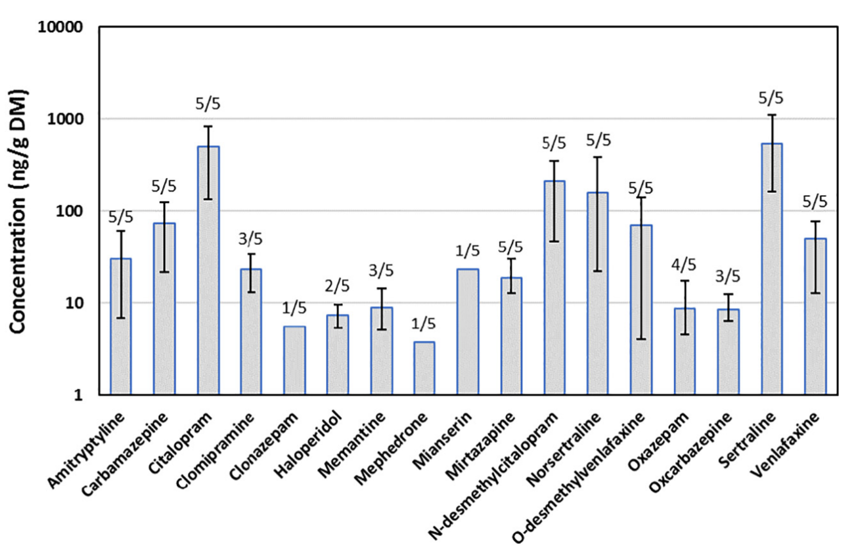

Fig. 1. Concentrations of psychoactive drugs and their metabolites in sewage sludge from Slovak WWTPs (mean, minimum, maximum, and occurrence from five WWTPs).

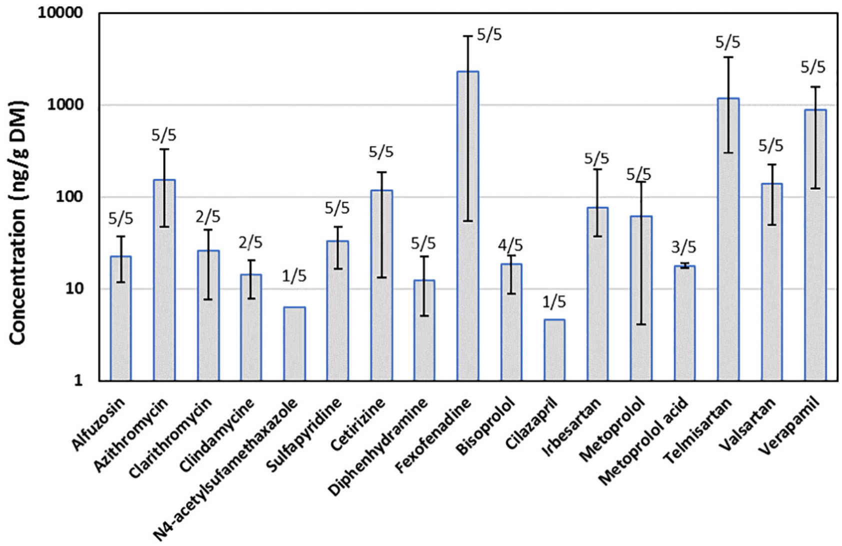

Fig. 2. Concentration of antibiotics, antihistamines, cardiovascular drugs, and their metabolites in sewage sludge from Slovak WWTPs (mean, minimum, maximum, and occurrence from five WWTPs).

3.1. Psychoactive compounds

Citalopram (mean 499 ng/g DM and a maximum of 826 ng/g DM in Bratislava DNV) and sertraline (mean 546 ng/g DM and maximum in Senec 1109 ng/g DM) were present in all the sludge and at high concentration levels - see Fig. 1. Eight compounds were below the LOQ in all analyzed samples (10,11-dihydrocarbamazepine, alprazolam, carbamazepine 10,11-epoxide, cathinone, ketamine, maprotiline, norketamine, oxycodone), and some compounds were found only in one sludge sample at very low concentrations e.g., clonazepam (5.5 ng/g DM in Senec), mephedrone (3.7 ng/g DM in Bratislava Central) and mianserin (23.1 ng/g DM in Senec).

3.2. Antibiotics

Azithromycin showed the highest concentrations among all samples (mean 153 ng/g DM, the most 336 ng/g DM in Senec) – Fig. 2. Nine of 15 antibiotics and their metabolites were below the LOQ in all sludge samples (clindamycin sulfoxide, erythromycin, sulfadiazine, sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxazole, N_1-acetylsulfamethoxazole, trimethoprim). Very low concentrations or values below the LOQ have been reported for these compounds in digested sludge in several studies (Verlicchi and Zambello, 2015; Martín et al., 2015; Nieto et al., 2010), which confirms their low sorption ability or high degradability in sludge.

3.3. Antihistamines

Six antihistamines were found in samples of sludge. Whereas fexofenadine exhibited the highest concentrations among all 93 drugs analyzed - a mean of 2341 ng/g DM, a maximum of 5597 ng/g DM in Bratislava DNV - other pharmaceuticals from this class were present at much lower concentrations. Diphenhydramine and cetirizine were identified in sludge with average concentrations of 12 ng/g DM and 118 g/g DM, respectively – Fig. 2. In contrast,

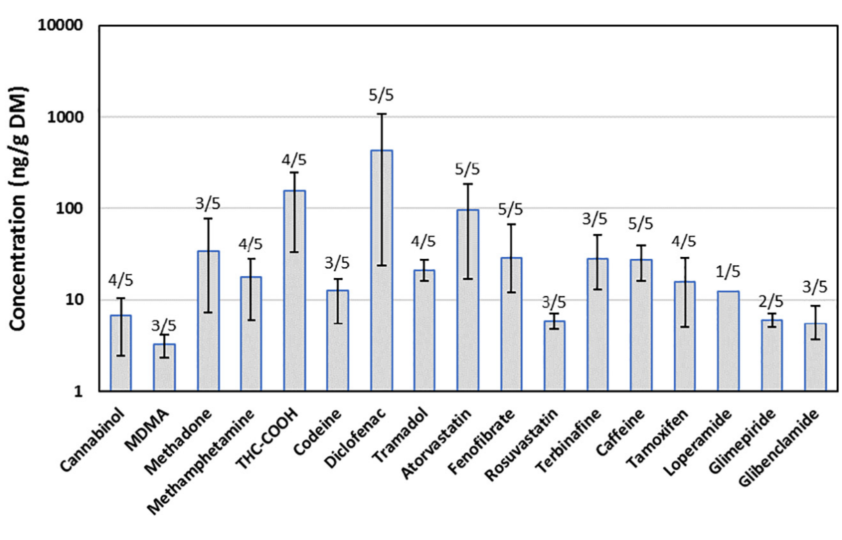

Fig. 3. Concentration of illicit drugs, their metabolites and other therapeutic groups of pharmaceuticals in sewage sludge from Slovak WWTPs (mean, minimum, maximum, and occurrence from five WWTPs).

clemastine, meclozine and orphenadrine were below the LOQ limit in all analyzed samples.

3.4. Cardiovascular pharmaceuticals

Cardiovascular drugs are among the most used in Slovakia and globally, where valsartan, metoprolol, telmisartan and others dominate (Fáberová et al., 2017; Verlicchi et al., 2012). It is expected that some of these will be absorbed into the sludge and remain bound to it even after the aerobic or anaerobic stabilization processes. Our sludge samples were analyzed for 14 cardiovascular drugs, where six of them were below the LOQ (atenolol, diltiazem, disopyramide, pizotifen, propanolol, sotalol). The highest concentrations were observed for telmisartan (mean 1170 ng/g DM, maximum 3370 ng/g DM in Senec), verapamil (mean of 887 ng/g DM, maximum 1560 ng/g DM in Bratislava DNV) and valsartan (mean 138 ng/g DM, maximum 221 ng/g DM in Bratislava DNV) – Fig. 2. Concentrations of other drugs were in the range of tens of ng/g DM (bisoprolol, cilazapril, ibersartan, metoprolol acid and metoprolol).

3.5. Illicit drugs

While data on illicit drugs in wastewater is widely accessible (Thomas et al., 2012; Ort et al., 2014; Macku'ak et al., 2016a), information on their concentrations in sludge is scarce (Gago-Ferrero et al., 2015). We analyzed 11 illicit drugs and their metabolites in sewage sludge samples. In the results, seven of them were found below the LOQ (amphetamine, benzoylecgonine, cocaine, 6-acetylmorphine, 2-

Table 3
Estimated load of pharmaceuticals, illicit drugs and their metabolites to soils in the Slovak Republic.

Compounds	Median concentration of sludge of 5 WWTPs	Total mass in sludge of 5 WWTPs	Estimated mass in Slovak sludge production	Estimated load to soils
	ng/g DM	g/year	kg/year	kg/year
Fexofenadine	1300	25,900	170	110
Verapamil	800	6510	43	28
Citalopram	580	4690	31	21
Telmisartan	530	3630	24	16
Sertraline	490	1910	13	8.1
Diclofenac	330	2750	18	12
N-desmethylcitalopram	220	2260	15	10
THC-COOH	170	1580	10	6.8
Cetirizine	160	1200	7.9	5.1
Valsartan	150	888	5.8	3.8
Norsertraline	110	791	5.2	3.4
Atorvastatin	94	758	5.0	3.2
Carbamazepine	86	643	4.2	2.8
Azithromycin	78	542	3.5	2.3
Metoprolol	53	1010	6.6	4.3
O-desmethylvenlafaxine	52	916	6.0	3.9
Irbesartan	48	452	3.0	1.9
Venlafaxine	44	573	3.8	2.5
Sulfapyridine	33	262	1.7	1.1
Amitryptyline	29	230	1.5	1.0
Clarithromycin	26	11.8	0.077	0.051
Caffeine	26	172	1.1	0.74
Mianserin	23	4.60	0.030	0.020
Clomipramine	23	21.7	0.14	0.09
Bisoprolol	21	168	1.1	0.72
Alfuzosin	21	111	0.72	0.47
Tramadol	20	128	0.84	0.55
Methamphetamine	19	200	1.3	0.86
Terbinafine	18	23.4	0.15	0.10
Metoprolol acid	17	125	0.82	0.54
Codeine	16	46.7	0.31	0.20
10,11-dihydro-10,11-dihydroxy carbamazepine	16	130	0.85	0.56
Mirtazapine	15	110	0.72	0.47
Methadone	15	133	0.87	0.57
Tamoxifen	15	115	0.75	0.49
Clindamycine	14	56.0	0.37	0.24
Fenofibrate	13	300	2.0	1.3
Loperamide	13	2.50	0.016	0.011
Diphenhydramine	7.8	51.2	0.34	0.22
Haloperidol	7.4	6.40	0.042	0.027
Memantine	7.3	41.1	0.27	0.18
Cannabinol	7.1	75.6	0.50	0.32
Oxcarbazepine	6.8	50.1	0.33	0.22
Oxazepam	6.6	47.9	0.31	0.21
N_4-acetylsufamethoxazole	6.3	42.1	0.28	0.18
Glimepiride	6.1	36.4	0.24	0.16
Rosuvastatin	5.9	5.70	0.037	0.024
Clonazepam	5.5	1.10	0.007	0.005
Cilazapril	4.5	1.80	0.012	0.008
Glibenclamide	4.2	29.8	0.19	0.13
Mephedrone	3.7	24.4	0.16	0.11
MDMA	3.3	29.0	0.19	0.12
Total		59,800	390	256

oxy-3-hydroxy-LSD, MDA, MDEA). The dominant drug in Slovak wastewater is methamphetamine (Mackuľak et al., 2014; Ort et al., 2014; EMCDDA, 2017), which ranges from 600 to 1000 ng/L in wastewater in Bratislava. However, the methamphetamine sorption affinity to sewage sludge is relatively low, since its average concentration in sludge was only 18 ng/g DM, with the highest concentration recorded in sludge from Bratislava Central - 28 ng/g DM. THC-COOH exhibits a significantly higher sorption ability, resulting in a concentration of 245 ng/g DM and 213 ng/g DM in Bratislava DNV and in Bratislava Central, respectively. Concentrations of THC-COOH in wastewater at the entrance to both WWTPs averaged approximately 200 ng/L, and in effluents were very often under the LOQ in the years 2014–2016 (Mackuľak et al., 2015; Mackuľak et al., 2016b). This very high efficiency of removal shows either light biodegradability of THC-COOH or high sorption on the sludge, which confirms our data. The other two compounds present in influent to WWTPs, cannabinol and MDMA, were also found in sludge, but at very low levels (mean 7 ng/g DM and 3 ng/g DM for cannabinol and MDMA, respectively) – Fig. 3.

3.6. Other therapeutic groups of pharmaceuticals

Representatives of other therapeutic classes, such as analgesics/NSAIDs, lipid regulators, antifungals, stimulants, antidiabetic, cancer and diarrhea drugs, were also found (Fig. 3). It is worth mentioning that diclofenac from the family of analgesics/NSAIDs had a mean concentration in the sludge of 434 ng/g DM, with the highest concentration observed in Senec – 1080 ng/g DM. We also recorded the presence of caffeine (mean 27 ng/g DM, maximum Senec 39 ng/g DM) and atorvastatin as a lipid reduction compound (mean of 97 ng/g DM, maximum 183 ng/g DM in Bratislava DNV) (Fig. 3). Tramadol, which belongs to prevailing compounds in Slovak wastewaters with concentrations ranging between 288 and 2070 ng/L (Mackuľak et al., 2016a; Mackuľak et al., 2016b), showed relatively low concentrations in sludge (21 ng/g DM, maximum 27 ng/g DM). This observation indicates its minimal sludge sorption, which also corresponds to data in the available literature (Verlicchi and Zambello, 2015; Hörsing et al., 2011).

3.7. Pharmaceutical loads for land application

The investigated WWTPs belong to large and medium scale treatment and together contribute 15% of the total sludge production in Slovakia. We performed basic mass flux calculations taking into account some assumptions. We assumed that this selection was an enough representative of the national scenario to estimate the amount of pharmaceuticals that can be applied with the sludge to the soil. We did not include further degradation during other steps prior to application (composting, etc.), so this estimation could be used as the worst case scenario. The obtained results are summarized in Table 3.

The data show the values of active pharmaceutical ingredients (APIs) and some metabolites applied to agricultural land within the range of hundred grams to low hundreds of kilograms, with the highest values being estimated for fexofenadine (110 kg/year), verapamil (28 kg/year) and citalopram (20 kg/year). The data in Table 3 are ordered according to their median concentrations, and it is obvious that the order of applied amounts of APIs is slightly different. That is caused by the methodology of estimation, where the input of each WWTPs is calculated separately. The contribution of different therapeutic classes to the total load of pharmaceutically active compounds to soils in the Slovak Republic is illustrated in Fig. 4. Antihistamines dominate with 45%, followed by the groups of cardiovascular and psychoactive compounds. Metabolites and diclofenac representing NSAIDs are two other groups that contribute >2%. The compound profile of this load differs significantly from the pollution pattern in effluent from WWTPs as a consequence of the distribution between water and the solid phase in combination with sludge stabilization. In general, relatively stable compounds with high affinity to sludge sorption can be transported to the environment via this pathway (Narumiya et al., 2013; Dong et al., 2016).

3.8. Pharmaceuticals in aerobic vs. anaerobic sludge

Sludge from WWTPs is stabilized in a low loaded activated sludge system (strictly oxic conditions – ORP > 100 mV) or under an anaerobic digestion system with biogas production (strictly anaerobic conditions – ORP < −200 mV). Two of our tested sludges were from aerobic systems

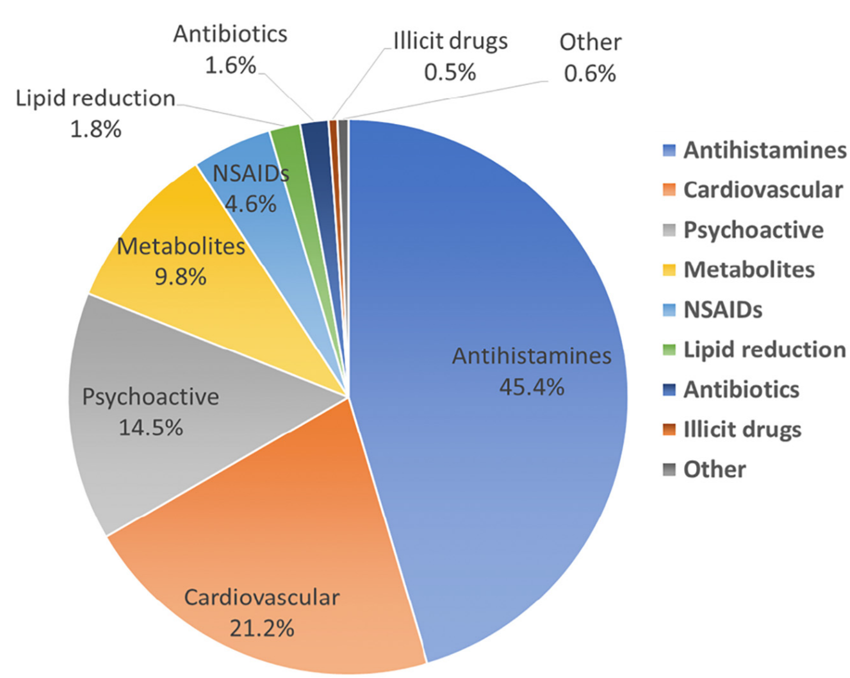

Fig. 4. Estimated profile of the pharmaceuticals and related compounds in total soil loads in Slovakia.

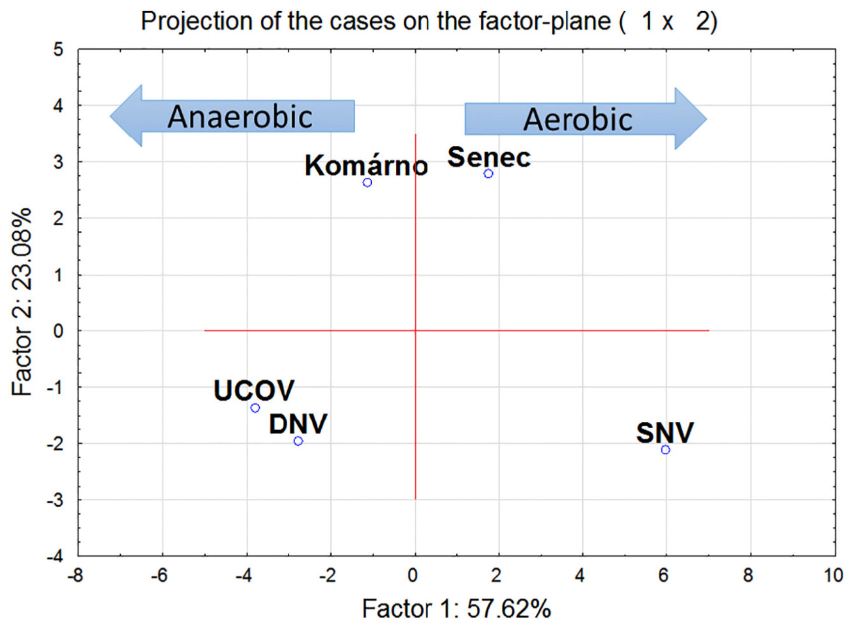

Fig. 5. PCA plot of the tested WWTP sludge samples in the factor planes 1 vs. 2. Factor 1 seems to correlate with type of the sludge condition (aerobic vs anaerobic), factor 2 were not able to match with corresponding variable.

(Senec, Spišská Nová Ves) and three sludges were stabilized under anaerobic conditions (Bratislava Central, Bratislava DNV and Komárno).

We can assume that the stability of target analytes under the aforementioned conditions could be influenced differently depending on the processes in the sludge matrix. The selected stabilization treatment could consequently lead to a different pattern of pharmaceuticals and their transformation products released to the environment. We used a PCA analysis of normalized datasets to reveal qualitative differences or similarities in the patterns of pharmaceuticals among the tested sludge samples.

There are clearly separate types of sludge from aerobic and anaerobic treatments along the axis of factor 1 in Fig. 5. Another well distinguished group is the WWTPs in Komárno and Senec along the axis of factor 2; however it was not possible to find any technological explanation for this observation. Looking at the correlation of factor 1 with the presence of target compounds in aerobic sludge, we can state that these sludge samples may be characterized by a significantly higher contribution of terbinafine, azithromycin and irbesartan to the sum of all compounds. The plot of APIs contribution to the sum of all compounds is shown in Fig. 6. Telmisartan (1950 ng/g DM vs. 651 ng/g DM), sertraline (800 ng/g DM vs. 377 ng/g DM), azithromycin (292 ng/g DM vs. 60 ng/g DM), irbesartan (117 ng/g DM vs. 50 ng/g DM) prevail in aerobic sludge.

In contrast, fexofenadine, THC-COOH, citalopram and *N*-desmethylcitalopram had significantly greater contributions to the total concentration of pharmaceuticals in anaerobic sludge. For

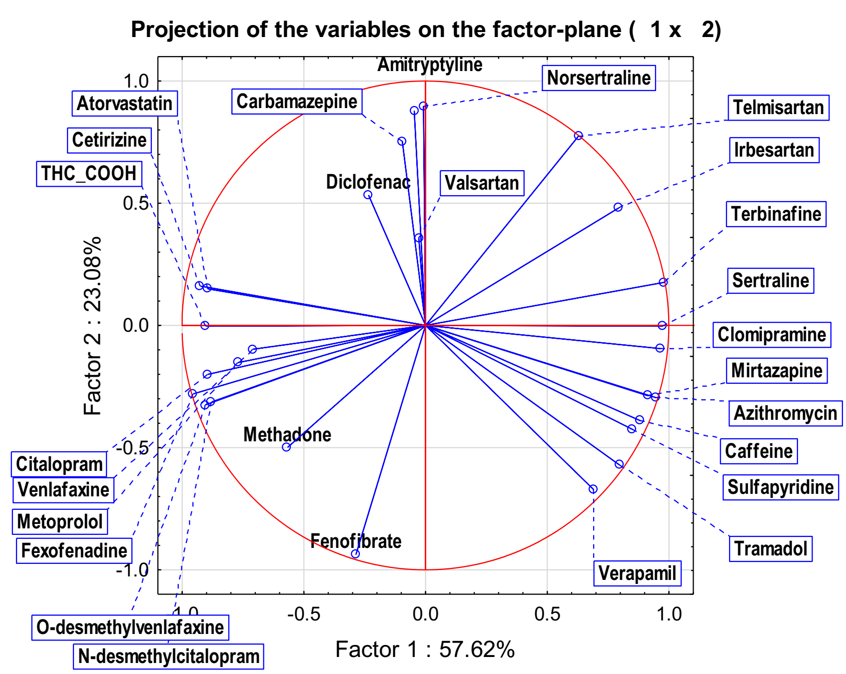

Fig. 6. PCA plot of variables (contribution of given compound to the sum of all compounds in the sludge sample) in the planes of factors 1 and 2. Vectors to right indicate compounds.

example, the average concentration of fexofenadine in aerobic sludge was 686 ng/g DM, while in anaerobic sludge, it was up to 3440 ng/g DM. Similarly, the anaerobic sludge was dominated by citalopram (593 ng/g DM vs. 358 ng/g DM), THC-COOH (196 ng/g DM vs. 33 ng/g DM), metoprolol (85 ng/g DM vs. 29 ng/g DM), and others. The metabolite metoprolol acid was observed in all samples of anaerobic sludge but was not present in any aerobic sludge sample.

In Slovakia, similarly to other developed countries, the majority of sludge is stabilized in anaerobic systems, and therefore, the comparison of the content of pharmaceuticals in aerobic and anaerobic sludge is relatively rare. Some authors (Verlicchi and Zambello, 2015; Martín et al., 2015) report significantly higher concentrations of azithromycin in aerobically stabilized sludge (680 ng/g DM) compared to anaerobic sludge (35 ng/g DM), which also corresponds to our results (292 ng/g DM versus 60 ng/g DM). In aerobic sludge, tramadol had a concentration of 43 ng/g DM, which also corresponds to our values of 23 ng/g DM in aerobically stabilized sludge. Taking into account the produced mass of aerobic sludge, we can state that their effect on the total load will be minor; however, it can introduce to the environment some specific compounds, such as azithromycin and terbinafine.

4. Conclusions

The presented paper summarizes the results of the first screen of pharmaceuticals, illicit drugs, and their metabolites in stabilized sewage sludge of the Slovak Republic. Aerobically and anaerobically treated sludge from five Slovak WWTPs representing approximately 15% of the total sludge production in Slovakia were analyzed. The sludge samples contained 52 different compounds out of a total of 93 screened.

Based on these data, we were able to estimate individual quantities of sludge-bound contaminants applied to the soil, ranging from hundreds of grams to hundreds of kilograms yearly. Because 70% of Slovak sludge production is utilized directly or indirectly in soil, these results show this common practice in a different light and justify moving recent EU legislation forward. There is an attempt to reuse macronutrients (organic carbon, nitrogen, phosphorus) to improve the soil's production properties, but there is also concern about the presence of emerging pollutants and their (almost unknown) impact on plants, living organisms and humans. It is very likely that to prevent potential exposure risk, most of the sludge with a high pharmaceutical content will be subjected to heat treatment (drying, incineration, pyrolysis, gasification, etc.).

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.04.001.

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