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Pilot Study of the Estimation of Amphetamines Consumption in the Polish City of Poznan

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Abstract

According to the European Monitoring Centre for Drugs and Drug Addiction Annual Report 2011 in many European countries amphetamines or ecstasy is the second most commonly used illicit substance after cannabis. More reliable and objective method based on the 'sewage epidemiology' was applied to estimate the level of amphetamines consumption in Poland. Amphetamine, methamphetamine and MDMA (ecstasy) were determined in wastewater samples collected from the main Wastewater Treatment Plant in the Polish city of Poznan (about 687,000 people) using liquid chromatography/tandem mass spectrometry (LC-MS-MS). Back-calculations used in the 'sewage epidemiology' approach were applied to estimate the level of consumption of analyzed drugs. Analysis of variance (ANOVA) was applied to check the monthly differences between determinations. Two-year profiles of consumption for amphetamine methamphetamine and ecstasy were calculated. Results from our city indicate that consumption of amphetamines is one order of magnitude lower than in Western Europe. Therefore, there is a different profile of amphetamines consumption. Consumption of amphetamine is several times lower, but the consumption of ecstasy is relatively high. ANOVA analysis confirmed significant differences between monthly loads of determined illicit drugs.

Keywords: Drug abuse; Amphetamines; Wastewater treatment plants; Amphetamines consumption

Introduction

Amphetamines are a term that includes both amphetamine and methamphetamine, both of which stimulate the central nervous system. Ecstasy is a group of synthetic substances chemically related to amphetamines but with some differences in their effects. The most common substance belonging to the ecstasy group is 3,4-methylenedioxy-methamphetamine (MDMA), but other analogues are also sometimes found in ecstasy tablets (MDA, MDEA). Illicit amphetamine use is associated with a broader set of negative consequences, such as short-term negative effects (restlessness, tremor, anxiety, dizziness), a 'crash' or coming-down after-effect (depression, sleeping difficulties, suicidal behavior), and also psychological and psychiatric effects of long-term use (psychosis, suicidal behavior, anxiety and violent behavior) [1].

The United Nations Office on Drugs and Crime (UNODC 2011) reported that in 2009-2010 4.5% of the world's population aged 15-64 used cannabis, 1.3% used amphetamines, 0.6% Ecstasy-group stimulants and cocaine 0.5% [2]. According to the European Monitoring Centre for Drugs and Drug Addiction Annual Report 2011, in many European countries amphetamines or ecstasy are the second most commonly used illicit substances after cannabis. Significant methamphetamine use is restricted to the Czech Republic and Slovakia (our neighbors) but recent reports indicate an increase in amphetamine use in some countries in northern Europe [3]. Based on surveys conducted in Poland in 2008, cannabis and amphetamines are the most commonly used illicit drugs [4].

The official figures on the prevalence of drug abuse are currently derived from population surveys integrated with crime statistics, medical records, drug production and seizure rates. Such estimation is subjective and very expensive. In 2005, the Zuccato group estimated the level of consumption of cocaine based on the determination of cocaine and their metabolite in surface water and wastewater [5]. This methodology appeared to be much more relevant and objective. Nevertheless, the procedure needs to be standardized to make comparison of results in many countries more reliable, but the methodology gives an objective insight into the level and profile of illicit drugs consumed by local community in real time. Recently, many reports concerning the estimation of illicit drug abuse using 'sewage epidemiology' have been published in many European countries, such as Belgium [6-8], United Kingdom [9], Italy - Florence [10], Spain [11-13], Croatia [14], Switzerland [15] and also in Canada [16] and the United States of America [17-19].

According to surveys conducted in Poland amphetamine is a commonly used illicit drug, and this is why the group of amphetamine-like stimulants was chosen for analysis (amphetamine, methamphetamine, MDA, MDMA, MDEA) [4]. The aims of this study were to implement 'sewage epidemiology' to estimate the level of amphetamines consumed in Poland's fifth largest city, Poznan, describe the profile of amphetamines used and monitor the long-term trends in amphetamines use.

Materials and Methods

Wastewater treatment plant in poznan

The Wastewater Treatment Plant is located in north-east of Poznan, in the township of Kozieglowy. The facilities are located on the right bank of the Warta river and occupies an area of approximately 60 hectares. The central wastewater treatment plant has undergone several

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modifications to meet present demands from the year 1995 to 2009. The new and highly efficient biological section was constructed, which incorporates a system enabling the integrated biological removal of carbon, nitrogen and phosphorus. The capacity of the plant is one of the largest of such facilities of Poland. During the time when samples were collected the central plant served almost the whole city with the suburbs, about 687,000 people.

Standards and reagents

All pure standards: amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA or ecstasy), 3,4-methylenedioxyethylamphetamine (MDEA) and their deutered molecules used as internal standards: amphetamine–d_6, methamphetamine-d_9, MDA-d_5, MDEA-d_5 and MDMA-d_5 were purchased from Certilliant, a Sigma-Aldrich' Company. The standards-solutions in methanol (1 mg/mL)-were diluted to 10 ng/µL with methanol and stored at -20°C in the dark. All other reagents were acquired from J.T. Beker (USA).

Sample collection and processing

Two wastewater samples (10 L each) were collected twice a week, on Monday and on Wednesday, from June 2009 to December 2010. All samples were collected at the same point before any chemical and physical treatment with the exception of sedimentation and the mean flow rate was 130,000 m^3/day. The analyses of the samples were performed the same day just after collection.

Sample treatment and analysis

Samples were filtered on a glass microfiber filter GF/A 1.6 µm (Whatman, Kent, U.K.) prior to the extraction and were spiked with 15 ng of each internal standards and the pH was adjusted to 7.0 ± 0.4 with phosphate buffer (pH=7.0). Solid-phase extraction of analyzed substances was performed using a Bakerbond Narc-2 mixed mode cartridges, which were conditioned with methanol (2 mL) followed by deionized water (2 mL) followed by phosphate buffer (2 mL, 0.1 M, pH 7.0). Next the sample was passed through the cartridges under vacuum at a flow rate of 10 mL/min. When the sample eluted under the gravity, the column was washed with deionized water (2 mL) followed by hydrochloric acid (0.1 M, 0.5 mL) followed by methanol (0.5 mL). Vacuum was applied and the cartridges were dried for 20-30 min. The analytes were eluted into a vial with a mixture of chloroform: isopropanol: ammonium hydroxide (80:30:3, 2 mL). The eluates of two samples (each 10 L) were pooled and dried under a nitrogen stream.

Liquid chromatography-tandem mass spectrometry

Pooled and dried samples were redisolved in 200 µL of mobile phase, centrifuged and transferred into glass vials for instrumental analysis. 20 µL of the solution were injected in the LC-MS spectrometer (Agilent HPLC 1200 series, 6410B Triple Quad LC/MS System). Chromatographic separation was performed using capillary column (Agilent Zorbax XDBC18, 4.6×50 mm×1.8 µm) at a flow rate of 0.45 mL/min. The mobile phases were solutions: water with formic buffer (pH~3.2) and acetonitrile with the gradient from 10 to 70% of acetonitrile (6.5 min.). The capillary voltage was 4000 V and the temperature was 300°C, the auxiliary and collision gas was N2. The collision energy and tube lens were optimized for each analyte and standards separately. All selected analytes were analyzed in positive ionization mode (ESI+). Identification and quantification were performed using two characteristic transitions in multiple reaction monitoring (MRM) mode for the fragmentation products of the protonated or deprotonated pseudomolecular ions of each substance and each deutered analogue (Table 1).

8-point calibration curve was built at 4, 8, 12, 16, 20, 24, 28, 32 ng for amphetamine, methamphetamine, MDA and MDMA and the solutions were spiked with 30 ng of all internal standards. Validation was done according to Funk methodology, including testing homogeneity, linearity, homogeneity of variances (precision), outliers and securing the lower range limit [20,21]. The matrix effect was determined by analyzing 50 mL of wastewater samples spiked with internal standards. The recoveries for the whole process of sample preparation, filtration and extraction were set within the range 0.80-0.93. The detection limits (LOD) and quantification limits (LOQ) for the whole method were calculated by spiking wastewater samples with different amounts of the substances analogously like at the calibration curve. The results all of validation activities are shown in Table 2.

Back-calculation of community drug use

The first time back-calculation of illicit drug consumption was carried out was by Zuccato et al. [5,19,20]. Based on the determination of the major metabolite of cocaine (benzoylecgonine, BE) in wastewater and surface water, cocaine consumption in a few Italian cities was estimated. This approach has been developed for other illicit drugs. For each drug the substance, called drug target residues (DTRs), is specified. An ideal DTR is a major and exclusive excretion product, metabolite or unchanged parent drug, which is stable in wastewater. Amphetamines are excreted mainly as unchanged compounds, so the DTRs for them were parent drugs. The concentrations of these substances in wastewater samples were very low and therefore the dried residues of two untreated wastewater samples (each 10 L) after filtration and SPE extraction were pooled and joined together by redissolving in the mobile phase to perform HPLC-MS-MS analysis. The mean concentrations of DTR in ng/L of all samples collected in one month were multiplied by the monthly mean flow rate in the wastewater treatment plant (WWTP) to give the amount of DTR (grams) discharged per month. This value was then divided by the number of people served by the WWTP to estimate the grams of DTR excreted in wastewater per person per month and finally normalized to a value of grams per month per 1000 people. To estimate the consumption of the illicit drug a correction factor is applied, which takes into account the percentage of the parent drug excreted as the chosen DTR and the parent drug-to-DTR molar mass ratio. The correction factors for amphetamines were 3.3, 2.3 and 1.5 for amphetamine, methamphetamine and ecstasy respectively. As a result, the amount of illicit drugs consumed monthly by 1000 people was estimated.

It is also possible to estimate the number of doses consumed by local community (1000 people) per day or month by dividing the

Substance	Retention time (min)	Fragmentor Voltage	Precursor ion m/z	Product ion I m/z and collision energy (eV)	Product ion II m/z and collision energy (eV)
Amphetamine-D6	3.4	60	142.1	125.1 (8)	93.1 (17)
Amphetamine	3.4	70	136	119.1 (5)	91.1 (17)
MDA-D5	3.6	70	185.1	168.1 (5)	110.1 (21)
MDA	3.6	60	180.1	163 (5)	105.1 (21)
Methamphetamine-D9	3.8	80	159.2	125.1 (5)	93.05 (17)
Methamfetamina	3.8	80	150.1	119.1 (8)	91 (17)
MDMA-D5	3.9	80	199.1	165 (9)	107.1 (25)
MDMA	3.9	80	194.1	163.1 (5)	105.1 (25)

Table 1: Conditions for MRM determination of illicit drugs.

Substance	y=bx+a		Residual standard deviation	Process standard deviation	Processvariationcoefficient (%)	Decision limit DL (ng/L)	Detection Limit LOD (ng/L)	Quantification limit LOQ (ng/L)
	a	b				(DIN 32645)		
Amphetamine	531	1270	2.57	0.20	1.12	0.36	0.71	1.07
						Range 1-2.4		
Methamphetamine	15555	126.7	6.96	0.55	3.06	0.81	1.65	2.32
						Range 3.5-7		
MDMA	40.28	1143	6.09	1.11	6.09	0.89	1.77	2.66
						Range 3-4.8		

Table 2: Validation according to Funk (German Standard DIN 32645).

Month	Concentration (ng L^{-1})			DTR massloads mg/ month/1000ppl			Drug consumption mg/ month/1000ppl			Number of doses dose/ month/1000ppl		
	AMF (± 0.03)	MET (± 0.07)	MDMA (± 0.07)	AMF (± 0.03)	MET (± 0.07)	MDMA (± 0.07)	AMF (± 0.03)	MET (± 0.07)	MDMA (± 0.07)	AMF (± 0.03)	MET (± 0.07)	MDMA (± 0.07)
Jun'09	0.25	1.22	1.56	1.99	9.42	12.12	6.573	21.66	18.18	0.22	0.72	0.14
Jul'09	0.32	1.35	1.54	2.64	10.78	12.64	8.70	24.78	18.96	0.29	0.82	0.14
Aug'09	0.40	1.46	1.29	2.84	10.42	9.21	9.37	23.98	13.82	0.31	0.80	0.10
Sep'09	0.34	1.38	1.38	2.27	9.30	9.27	7.49	21.40	13.91	0.25	0.71	0.10
Oct'09	0.30	1.37	1.39	2.18	9.83	10.04	7.22	22.61	15.06	0.24	0.75	0.11
Nov'09	0.30	1.26	1.82	2.18	8.84	12.61	7.22	20.34	18.92	0.24	0.68	0.14
Dec'09	0.48	1.41	1.71	3.64	10.76	13.00	12.02	24.76	19.50	0.40	0.82	0.15
Jan'10	0.34	1.41	2.03	1.78	7.42	10.69	5.89	17.06	16.04	0.19	0.57	0.12
Feb'10	0.25	1.37	1.92	1.24	6.70	9.39	4.09	15.40	14.08	0.14	0.51	0.11
Mar'10	0.27	1.03	1.83	1.97	7.56	13.44	6.52	17.38	20.16	0.22	0.58	0.15
Apr'10	0.29	0.96	1.83	1.63	5.38	10.21	5.40	12.38	15.31	0.19	0.41	0.12
May'10	0.59	0.81	1.82	3.68	5.08	11.39	12.13	11.68	17.01	0.40	0.39	0.13
Jun'10	0.69	0.83	1.75	3.22	3.90	8.20	10.63	8.97	12.31	0.35	0.30	0.09
Jul'10	0.25	0.69	1.14	1.32	3.60	5.97	4.38	8.28	8.96	0.15	0.27	0.07
Aug'10	0.24	0.66	1.12	1.43	3.99	6.72	4.73	9.19	10.08	0.16	0.31	0.08
Sept'10	0.23	0.76	1.27	1.32	4.30	7.14	4.36	9.89	10.71	0.14	0.33	0.08
Oct'10	0.48	1.24	1.17	2.43	6.24	5.90	8.02	14.36	8.85	0.27	0.48	0.06
Nov'10	0.64	0.87	1.29	3.97	5.41	8.02	13.11	12.44	12.02	0.44	0.41	0.09
Dec'10	0.71	0.91	1.02	4.34	5.58	6.27	14.32	12.83	9.41	0.47	0.43	0.07

Table 3: Results of determination of amphetamine, methamphetamine and MDMA (ecstasy) from June 2009 to December 2010.

	Sums of Squares (SS)	Degree of Freedom (dF)	Mean Squares (SS/df) (MS)	F-statistic	Prob>F (p)
	Amphetamine				
Columns	65.9156	18	3.66198	13902.48	5.24361e-097
Error	0.015	57	0.00026		
Total	65.9307	75			
	Methamphetamine				
Columns	449.216	18	24.9564	106918.78	2.97059e-122
Error	0.013	57	0.0002		
Total	449.229	75			
	MDMA				
Columns	452.88	18	25.16	187006.79	3.57393e-129
Error	0.008	57	0.0001		
Total	452.887	75			

Table 4: Results of ANOVA analysis.

consumption value by a single typical dose for each illicit drug. A typical oral dose for amphetamine and methamphetamine is 30 mg, but for MDMA (ecstasy) it is 100 mg. But it is recommended to focus on the loads of illicit drugs estimated for 1000 people per day or loads of DTR excreted per day per 1000 people and these results should be monitored for a period of time to give an objective insight into the level and profile of illicit drugs consumed by local community.

Statistical analysis

Graphical method and Shapiro Wilk test were used for testing the normal distribution of samples. To check whether the mean value of determinations in one month differs from mean in another the analysis of variance-one-way ANOVA- was performed (Matlab v. 7120635). The null hypothesis assumed the all monthly means of determinations are equal. It was interested not only if there are any differences among means but even more important which pairs of means differ significantly, that is why series of t-tests were performed. Because this procedure has a pitfall when you compare more than five variables (means) multiple comparison procedure was applied to compensate for multiple tests. It is easier to visualize the difference between monthly means using a graph which is presented in Figure 1. The procedure applied for the collection of samples and determination of illicit substances did not allow daily variations in the drugs consumed to be compared.

Results and Discussion

The results of determination of amphetamine, methamphetamine and ecstasy in wastewater samples are collected in Table 3. The normal distribution of variables was checked by graphical method and Shapiro-Wilks test ($p>0.05$). Based on the determination of DTR in wastewater samples population-standardized mean monthly loads of DTR were calculated. To check an increasing or decreasing tendency between months one way ANOVA and Multiple Comparison Test were applied. The results of ANOVA are presented in Table 4. Based on the F and p-values the null hypothesis was rejected. P-values are very below the cut-off level of 0.05% therefore it can be stated that

the differences between monthly means are very high significant. The Multiple Comparison Test visualized the results and showed significant differences between means of monthly DTR loads (mg/month/1000 people) except: February-July-September 2010 and June 2009-March 2010 in case of amphetamine; April-September 2010 and August-December 2009 in case of methamphetamine (Figure 1).

Processing of DTR extraction data allowed us to calculate the consumption rate (mg/month/1000 inhabitants) for the amphetamines group substances (in our case amphetamine, methamphetamine and ecstasy) as described in the section named "Materials and methods" (Figure 2). Because of the low limit of DTRs in wastewater, significantly more samples were subjected to analysis. Comparison of drug consumption with other reports [19,11,14] by other Western European scientists using a similar methodology shows significant differences in consumption amounts and the profiles of amphetamine-group substances (Table 5). Generally speaking, consumption of amphetamines is one order of magnitude lower than in the countries compared. The mean drug consumption of amphetamine amounts to 0.33% (incase of London) to 2.9% (in case of Milan) of overall drug consumption. In case of ecstasy and methamphetamine, the percentage range is 5.30-13.25% (in case of Catalonia even 0.24%) and 5.1-9.67% respectively. Because drug consumption is standardized per day and per thousand people, these results seem to be comparable. An interesting fact is that cocaine was detected only once during the two-year study period. It

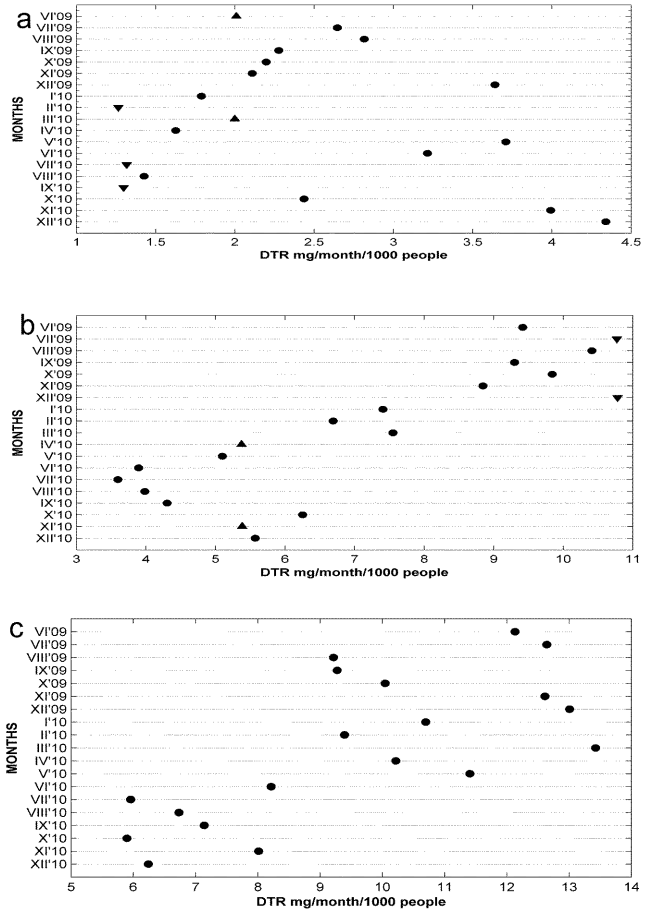

Figure 1: (A) Amphetamine. (B) Methamphetamine. (C) MDMA (ecstasy). Multiple Comparisons. Visualization of the difference between group means (DTR loads/mg/month/1000 people). Triangle in the vertical line-no significant difference, circle-significant difference.

City/Area (Country)	Consumptionunits	Amphetamine	Ecstasy	Metham-phetamine	References
Zagreb (Croatia)	mg/day/1000 ihb.	9.7	3.6		Terzic et al. [14]
Milan (Italy)	mg/day/1000 ihb.	8.9	8.9	10.35	Zuccato et al. [19]
London (UK)	mg/day/1000 ihb.	79	5.1	5.52	Zuccato et al. [20]
Poznan (Poland)	mg/day/1000 ihb.	0.26	0.47	0.53	This study
Lugano (Switzerland)	mg/day/1000 ihb.	-	10.9		Zuccato et al. [20]
Catalonia (Spain)	mg/day/1000 ihb.	-	200		Boleda et al. [11]

Table 5: Amphetamines-group substances consumption. Comparison with previously published date for Western Europe region.

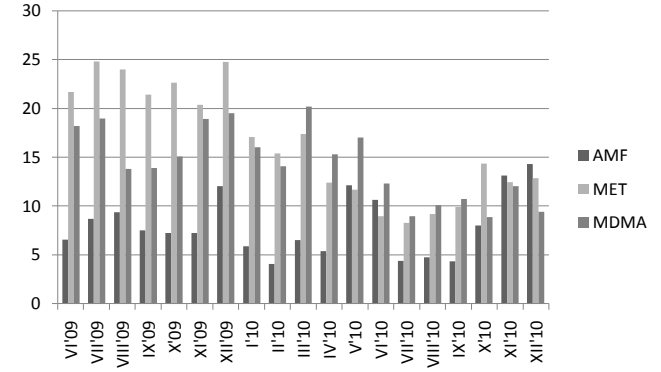

Figure 2: Mean monthly consumption (mg/month/1000 people) of amphetamine (AMF), methamphetamine (MET) and MDMA (ecstasy) from June 2009 to December 2010.

is important to remember that these data were collected in different time periods (a few weeks monitoring was compared with a continuous two-year study) but in the authors' opinion, for general comparison purposes this approach is justified. The choice of city where our studies were carried out resulted from the author's place of residence and work, and also poll based data not indicating that Poznan is a city with special addiction problem. Therefore, it is possible that monitoring in other cities might give different results. The official Polish statistics do not offer any integrated data on the consumption of different types of illicit drugs in Poznan and other Polish cities. Official data are based on the number of people treated and hospitalized. Our calculations indicate that amphetamine consumption in Poznan (mean 0.26 mg/day/1000 inh.) is several times lower than in Western Europe (Table 5) and slight lower compared to London. Consumption of ecstasy is relatively high, but still makes up 13.25% at the most. An interesting fact is that the amphetamine to ecstasy consumption rate in case of Poznan is 0.55, while it is 2.7, 1.4, and 7.6 for Zagreb, Milan and Catalonia respectively. This indicates a different profile of consumption and a different 'liking for illicit drug' among Poznan's inhabitants. In London and Milan, the data show a higher level of methamphetamine consumption compared to Poznan. At this point it is important to take into consideration the sample collection and processing, which can be distinguished from other authors with regard to the amount of samples prepared and analyzed. Using a 20 L sample for analysis made this more complicated (because of the matrix effect) and more laborious, but it did make it possible to detect traces in wastewater. When interpreting the comparison of consumption in Poznan and other cites, it is important to note that this comparison concerns only those cities where the concentration of

amphetamine-type drugs was especially high. In other cites which were monitored, where the authors did not give figures for amphetamine consumption, it was under the detection or quantification limit using a 0.5 L sample. It is possible that in these cities the level of consumption is comparable with Poznan. It is therefore worth noting that the low level of consumption in Poznan compared with other cites may result from the selection of cities.

In our study an attempt was made not only to estimate the number of mg of consumed illicit drugs by local population but to indicate trends in consumption. To achieve this aim, the authors changed the way of collecting samples compared with others research (see Sample collection and processing). The data obtained allowed us to generate two-year profiles of consumption for amphetamine methamphetamine and ecstasy (Figure 2). The profile indicates a gentle decrease in the monthly consumption of amphetamine in Poznan from May 2010 to the end of the monitoring period. It is difficult to make a comparison with other works because there are no such long-term monitoring data and these data would need to be compared over the same period of time.

Conclusion

Wastewater analysis and monitoring of illicit drug content and calculation of consumption is a promising complementary tool for the assessment of trends in illicit drug abuse. Our research was the first of its kind carried out in Poland. The monitoring focused particularly on the amphetamine group. Consumption of amphetamine is lower than in other compared European countries, but consumption of ecstasy is relatively high. The profile of consumption of drug abuse substances is different in city of Poznan.

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References

1. Barceloux DG (2012) Medical Toxicology of Drug Abuse. Synthesized Chemicals and Psychoactive Plants, Wiley, New Jersey, USA.
2. World drug report (2011) United Nations Office Drugs and Crime (UNODC), New York, USA.
3. Ferri M, Bo A (2013) EMCDDA Best Practice Promotion in Europe: an internet based dissemination tool. Adicciones 25: 3-6.
4. Malczewski A, Kidawa M, Struzik M, Strzelecka A (2010) EMCDDA. National Report (2009 data) to the EMCDDA by the Retinox National Focal Point. Poland. New Development, Trends and in-depth information on selected issues.
5. Zuccato E, Chiabrando C, Castiglioni S, Calamari D, Bagnati R, et al. (2005) Cocaine in surface waters: a new evidence-based tool to monitor community drug abuse. Environ Health 4: 14.
6. van Nuijs ALN, Pecceu B, Theunis L, Dubois N, Charlier C, et al. (2009) Cocaine and metabolites in waste and surface water across Belgium. Environ Pollut 157: 123-129.
7. van Nuijs AL, Mougel JF, Tarcomnicu I, Bervoets L, Blust R, et al. (2011) Sewage epidemiology--a real-time approach to estimate the consumption of illicit drugs in Brussels, Belgium. Environ Int 37: 612-621.
8. Tarcomnicu I, van Nuijs ALN, Simons W, Bervoets L, Blust R, et al. (2011) Simultaneous determination of 15 top-prescribed pharmaceuticals and their metabiltes in influent wastewater by reversed-phase liquid chromatography coupled to tandem mass spectrometry. Talanta 83: 795-803.
9. Kasprzyk-Hordern B, Dinsdale RM, Guwy AJ (2009) Illicit drugs and pharmaceuticals in the environment--forensic applications of environmental data. Part 1: Estimation of the usage of drugs in local communities. Environ Pollut 157: 1773-1777.
10. Mari F, Politi L, Biggeri A, Accetta G, Trignano C, et al. (2009) Cocaine and heroin in waste water plants: a 1-year study in the city of Florence, Italy. Forensic Sci Int 189: 88-92.
11. Boleda MA, Galceran MA, Ventura F (2009) Monitoring of opiates, cannabinoids and their metabolites in wastewater, surface water and finished water in Catalonia, Spain. Water Res 43: 1126-1136.
12. Bijlsma L, Sancho JV, Pitarch E, Ibanez M, Hernandez F (2009) Simultaneous ultra-high-pressure liquid chromatography-tandem mass spectrometry determination of amphetamine and amphetamine-like stimulants, cocaine and its metabolites, and cannabis metabolite in surface water and urban wastewater. J Chromatogr A 1216: 3078-3089.
13. Martínez Bueno MJ, Uclés S, Hernando MD, Fernández-Alba AR (2011) Development of a solvent-free method for the simultaneous identification/quantification of drugs of abuse and their metabolites in environmental water by LC-MS/MS. Talanta 85: 157-166.
14. Terzic S, Senta I, Ahel M (2010) Illicit drugs in wastewater of the city of Zagreb (Croatia)--estimation of drug abuse in a transition country. Environ Pollut 158: 2686-2693.
15. Berset JD, Brenneisen R, Mathieu C (2010) Analysis of illicit and illicit drugs in waste, surface and lake water samples using large volume direct injection high performance liquid chromatography-Electrospray tandem mass spectrometry (HPLC-MS/MS). Chemosphere 81: 859-866.
16. Metcalfe C, Tindale K, Li H, Rodayan A, Yargeau V (2010) Illicit drugs in Canadian municipal wastewater and estimates of community drug use. Environ Pollut 158: 3179-3185.
17. Bartelt-Hunt SL, Snow DD, Damon T, Shockley J, Hoagland K (2009) The occurrence of illicit and therapeutic pharmaceuticals in wastewater effluent and surface waters in Nebraska. Environ Pollut 157: 786-791.
18. Chiaia-Hernandez AC, Banta-Green CJ, Field JA (2011) Interpreting methamphetamine levels in a high-use community. Environ Sci Pollut Res Int 18: 1471-1477.
19. Zuccato E, Chiabrando C, Castiglioni S, Bagnati R, Fanelli R (2008) Estimating community drug abuse by wastewater analysis. Environ Health Perspect 116: 1027-1032.
20. Zuccato E, Castiglioni S, Bagnati R, Chiabrando C, Grassi P, et al. (2008) Illicit drugs, a novel group of environmental contaminants. Water Res 42: 961-968.
21. Funk W, DammannV, Donnevert G (2007) Quality Assurance in Analytical Chemistry: Applications in Environmental, Food, and Materials Analysis, Biotechnology, and Medical Engineering. (2nd edn) WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany.