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Original Article

Trends of Amphetamine Type Stimulants DTR Mass Load in Poznan Based on Wastewater Analysis

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Abstract

Background: The aim of this study was to determine the monthly DTR mass load of amphetamine-type compounds in Poland as well as an investigation of cyclical behaviour by using time series analysis and especially trends analysis.

Methods: Amphetamine, methamphetamine and MDMA (ecstasy) were detected in wastewater samples collected from the main Wastewater Treatment Plant in the city of Poznan using liquid chromatography / tandem mass spectrometry (LC-MS-MS). Back-calculations used in the sewage epidemiology approach were applied to estimate the DTR mass load level of the drugs analyzed. Trends analysis was performed by fitting the data to a simple linear regression and then by using smoothing by means of a moving average (Mat lab 2013a). Trend analysis displays a steady tendency of increase or decrease throughout time series. When we plot the observation against time, we may notice that a straight line can describe the increase or decrease in the series as time goes on. Simple linear regression and method of last squares to estimate parameters of a straight-line model were used. Additionally, a lagged plot (autocorrelation plot) was used to investigate an appearance of correlation between amphetamines throughout time.

Results: Trends analysis showed the slight increase in consumption of amphetamine and decreasing trend in case of ecstasy and methamphetamine within the investigated period. There is also visible, strong correlation between ecstasy and methamphetamine consumption which cannot be stated in case of amphetamine.

Conclusion: Trends analysis is a very useful tool to analyse the increasing or decreasing tendency in consumption of illicit drugs based on the DTR mass load data.

Keywords: Illicit drugs, Amphetamines, Time series, Wastewater, HPLC-MS/MS

Introduction

In the last few years, a new approach, termed 'sewage epidemiology', has been applied in order to estimate the DTR mass load level of illicit drugs recommended by EMCDDA. This method proposed by Daughton and Thernes (1) was first implemented by the Zucatto research group (2-4) to estimate the DTR mass load level of cocaine in some Italian cities, based on the analysis of surface and wastewater samples. Such investigations have been conducted in the last few years in other European countries, such as Belgium (5-7), United Kingdom (8), Italy - Florence (9), Spain (10-14), Croatia (15), Switzerland (16) and also in Canada (17) and the United States of America (18).

The application of wastewater analysis to the investigation of illicit drug use represents an innovative approach to the monitoring of the illicit drug problem. This method is most useful for drug surveillance at the community level. It could be used as a drug surveillance tool to assist public health and law enforcement officials in identifying patterns of drug use across municipalities of all sizes. Furthermore, because wastewater sampling and analysis can be conducted on a daily, weekly

or monthly basis, the data can be used to give a real-time measure that provides communities with more opportunities (19) for monitoring the impact and effectiveness of prevention and intervention activities.

The analysis of wastewater is based upon samples drawn from the total liquid waste produced by a population. The mass flow of analytes contains information about the overall DTR mass load. The results do not provide specific information about who has consumed which drugs, or what specific doses of drugs may have been taken. It is not possible to determine directly whether an observed variation in wastewater samples reflects changes in the number of active users (prevalence), or whether it relates to changes in levels of use (patterns of use, dosage) among users (20).

Undoubtedly, there are difficulties in using wastewater measurements of drugs to make inferences about the prevalence of users. The approach may, for example, be subject to limitations in the accuracy of estimates regarding collective DTR mass load parameters, and these levels of uncertainty might be further increased by potential sources of error and variability related to the assumptions that are required for the calculations. Uncertainties surrounding the analysis of wastewater samples may include, for example, issues concerning epidemiological questions, such as the phenotypes of users or behavioural variations in patterns of drug taking. Uncertainties may be related to individual differences in drug metabolism, such as the levels of drug metabolites in the blood. Information on human excretion rates for different substances is important for calculating the original amount of drugs consumed, but the data that is available on this topic is very limited because these values have generally been obtained from rather small samples of healthy volunteers, and they may not be representative of the metabolic responses of chronic drug users (21). Additionally, closed water systems may serve transient human populations, and as a result, it may be difficult to characterize the population served by a given wastewater treatment system because of various sorts of changes that may occur in the resident populations of a given area (for instance, due to people congregating at inner-city venues during weekends). This may lead to problems in determining whether an apparent rise in observed drug use measurements is due to an increase in DTR mass load by the resident population, or to an increase in the number of consumers because of changes in the resident population (19). Moreover, the samples obtained from a wastewater system will be affected by leakage or heavy rainfalls. The greatest loss of drug residues is likely to occur during storms. The analysis of wastewater samples may also be confounded by the sudden or unexpected introduction of high concentrations of chemical agents.

Finally, information is required about substance transport or degradation in wastewater systems. The physical, chemical and biological transformation processes of solutes may have an important effect upon the ability to make meaningful estimates, and knowledge about such processes is incomplete, even for conventional pollutants (3, 4, 19). A number of complex chemical and biological interactions occur within sewer systems. Very little is known about how drugs and their metabolites in wastewater systems may be affected by biotransformation or sorption in sewer biofilms and sediments. Drug loads may also be affected by natural processes such as changes in wastewater temperature (3, 22).

There are many advantages of the sewage methodology but it also requires further optimisation and standardization various important param-eters like sample collection and back-calculation (23, 24).

Because there are still many areas of uncertainty, the aim of this research was to identify trends that allowed the monitoring of changes in patterns than calculating levels of DTR mass load or the numbers of illicit drug consumers. This approach makes estimation easier because the aforementioned factors do not have such significance as conducting repeated measures and sampling under the same conditions over time (with a constant time interval) (25). For this purpose, different tools from the "box" of Time Series were used to show trends that occurred during a two-year study of amphetamine in wastewater in Poznan.

In the present study a near-two sampling campaign was conducted at a single Wastewater Treatment Plant (WWTP) in Poznan, which served almost the whole city of Poznan at the time of our study. Sewage epidemiology was applied for amphetamine-like stimulants. Back-calculations from concentrations in influent wastewater to the amount of DTR mass load of illicit drug were conducted based on correction factors, flow rates, and the number of inhabitants.

Materials and Methods

Wastewater samples

The study was performed in Poznan, the fifth biggest city in Poland in 2009/2010. The samples were collected from the central wastewater treatment plant, which at that time served almost the whole city and its suburbs, a total of approximately 687 000 people. 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. Analysis of the samples was performed on the same day, just after collection.

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 (1mg/mL) – were diluted to 10ng/µL with methanol and stored in the dark at -20^{0}C. All other reagents were acquired from J.T. Beker (USA).

Sample treatment and analysis

Samples were filtered on a glass microfiber filter GF/A 1.6 µm (Whatman, Kent, U.K.) prior to extraction, were spiked with 15 ng of each internal standard and the pH was adjusted to 7.0±0.4 with a phosphate buffer (pH=7.0). Solid-phase extraction of the substances to be analyzed was performed using Bakerbond Narc-2 mixed mode cartridges, which were conditioned with methanol (2 mL), followed by deionized water (2 mL) and then a phosphate buffer (2 mL, 0.1M, pH 7.0). Next, the sample was passed through the cartridges under a vacuum at a flow rate of 10mL/min. When the sample was eluted under gravity, the column was washed with deionized water (2 mL), followed by hydrochloric acid (0.1M, 0.5mL), followed by methanol (0.5 mL). A 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 10L) were pooled and dried under a nitrogen stream.

Liquid Chromatography – Tandem Mass Spectrometry

The pooled and dried samples were redissolved 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 a capillary column (Agilent Zorbax XDBC18, 4.6x50mmx1.8µm) at a flow rate of 0.45 mL/min. The mobile phase consisted of solutions: water with formic buffer (pH~3.2) and acetonitrile with a gradient from 10 to 70% of acetonitrile (6.5 min.). The capillary voltage was 4000 V and the temperature was 300^{0}C, while the auxiliary and collision gas was N_2. 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. For amphetamine (m/z 136) and amphetamine D-6 (m/z 142.1) the analyzed product ions were: 119.1

(m/z), 91.1 (m/z) and 125.1 (m/z), 93.1 (m/z) respectively. Two product ions of methamphetamine (m/z 150.1) were: 163 (m/z) and 105.1 (m/z), for the deutered methamphetamine-D9 (m/z 159.2) were: 125.1 (m/z) and 93.05 (m/z). In case of MDA (m/z 180.1) and MDA-D5 (m/z 185.1) the analyzed product ions were: 163 (m/z), 105.1 (m/z) and 168.1 (m/z), 110.1 (m/z) respectively. But in case of MDMA (m/z 194.1) and MDMA-D5 (m/z 165) the product ions were: 163.1 (m/z), 105.1 (m/z) and 165 (m/z), 107.1 (m/z) respectively (26).

An 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 carried out according to Funk methodology (27), including testing homogeneity, linearity, homogeneity of variances (precision), outliers and securing the lower range limit. 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.

Back-calculation of community drug use

Estimation of community drug use was done according to the method described by (2). Because surveys conducted in Poland reveal that amphetamine is a commonly used illicit drug, the group of amphetamine-like stimulants was chosen for analysis (amphetamine, methamphetamine, MDA, MDMA, MDEA). In the case of amphetamines, the substances which are used as drug target residues (DTR) are the parent drugs, because all are excreted mainly as unchanged compounds. The concentrations of these substances were very low and therefore the dried residues of two untreated wastewater samples (each 10L) after filtration and SPE extraction were pooled and combined by redissolving them 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 mean flow monthly rate in the WWTP (Wastewater Treatment Plant) to give the amount of DTR (grams) discharged per month. This value was then divided by the number of people served by 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. Cocaine DTR mass load was originally estimated by Zuccato from the data for its major metabolite, benzylececgonine (BE), so the molar ratio of 1.05 was applied to compensate for the higher molecular weight of BE comparing with cocaine. In the case of amphetamines, the parent drug is determined and therefore the molar ratio is 1, so the correction factor for the estimation takes into consideration only the percentage of the drug dose excreted as DTR (for amphetamine this is 30, for methamphetamine 43 and for MDMA 65). Correction factors (the fraction of the consumed parent drug extracted as DTR in urine and the parent drug-to-DTR molar mass ratio) were 3.3 for amphetamine, 2.3 for methamphetamine and 1.5 for ecstasy. Finally, the amount of illicit drugs consumed monthly by 1000 people was estimated.

Statistical evaluation

Statistical analysis of the results was performed with MATLAB V. 7.12.0.635 software. Time series were analyzed in order to understand the underlying structure and function that produced the observations. Understanding the mechanisms of a time series allows a mathematical model to be developed that explains the data in such a way that prediction, monitoring, or control can occur. It is assumed that a time series data set has at least one systematic pattern. The most common patterns are trends and seasonality. Trends are generally linear or quadratic. To find trends moving averages or regression analysis are often used. Seasonality is a trend that repeats itself systematically over time. A second assumption is that the data exhibits enough of a random process so that it is hard to identify the systematic patterns within the data. Time series analysis techniques often employ some type of filter to the data in order to dampen the error. Other potential patterns got to do with lingering effects of earlier observations or earlier random errors A Time Series was constructed and trend analysis was carried out to indicate any in-

crease or decrease tendencies. Trend analysis was performed by fitting the data to a simple linear regression and then by using smoothing in meaning of a moving average. Analysis of the correlation was performed using plots of cross-correlation. Autocorrelation refers to the correlation of a time series with its own past and future values. Time series are very complex because each observation is somewhat depending on a previous observation and often is influenced by more than one previous observation. Random errors are also influential from one observation to another. These influences are called autocorrelation—dependent relationships between successive observations of the same variable. The challenge of time series analysis is to extract the autocorrelation elements of the data, either to understand the trend itself or to model the underlying mechanisms.

Time series reflect the stochastic nature of most measurements over time. Thus, data may be skewed, with meaning and variation not constant, abnormally distributed, and not randomly sampled or independent. Another abnormal aspect of time series observations is that they are often not evenly spaced in time due to instrument failure, or simply due to variation in the number of days in a month. Autocorrelation is also sometimes called "lagged correlation" or "serial correlation", which refers to the correlation between members of a series of numbers arranged in time. Positive autocorrelation might be considered a specific form of "persistence", a tendency for a system to remain in the same state from one observation to the next. Autocorrelation can be exploited for predictions: an auto correlated time series is predictable, probabilistically, because future values depend on current and past values. Finally, simple indices were calculated to demonstrate how the DTR mass load data may be presented in a comparative form.

Results

Based on the determination of DTR in wastewater samples population-standardized mean monthly loads of DTR were calculated (Table 1).

In order to check trends that occurred during the near-two-year monitoring of amphetamine-like substances in wastewater in Poznan. Trends analysis was performed. A time series is a set of measurements of a variable that are ordered over time. In our case it is DTR mass load (mg/month/1000 inhabitants) ordered through the months from July 2009 to December 2010. One of the aims of time series analysis is to look for a steady tendency of increase or decrease over time. Such a tendency is called a trend. The tendency may take the form of seasonality or cyclical behaviour. It is important that such kinds of variables cannot be forecast at all because the changes or movement of DTR mass load over several months are a mathematically "random walk". In Fig. 1 non-regular increase and decrease values of the DTR mass load for amphetamine, methamphetamine and ecstasy can be seen. It can be seen that two cyclical peaks occurred together for amphetamine, methamphetamine and ecstasy (December 2009 and March to July 2010). This is proof of the seasonality of the illicit drug DTR mass load in Poznan. It is impossible to state cyclical behaviour in a two-year study and therefore the authors omitted this subject.

Figure 1 presents a fitted line with parameters of regression to each time series. We cannot give much credence to regression results but the position and regression parameters clearly indicate that the ecstasy and methamphetamine DTR mass load decreased during the period of investigation (slope value -0.32 and -0.37 respectively). In the case of amphetamine, a slight increase (slope 0.0034) can be observed. Notice that the plots showed a cyclical increase and in the case of methamphetamine and ecstasy there was an increase in DTR mass load from October 2009 to May 2010. There are as many as five peaks (a sharp increase in consumption) (December 2009, March 2010, May 2010, September and November 2010) in the case of ecstasy, three for methamphetamine (December 2009, March 2010, September 2010) and only two for amphetamine (December 2009, May 2010). It is important to bear in mind that the analysis did not concern values but only trends.

Table 1: Values of DTR mass load (mg/month/1000 inh.) and index for amphetamine, methamphetamine and ecstasy

		Amphetamine			Methampetamine			Ecstasy		
Month	Flow rate~ (*10^3 m^3 /month)	DTR conc. (ng/L)	DTR* mass load (mg/month/ 1000inh)	Index of DTR mass load with base period- June'09	DTR conc. (ng/L)	DTR massload (mg/month/ 1000inh)	Index of DTR massload with base period- June'09	DTR conc. (ng/L)	DTR massload (mg/month/ 1000inh)	Index of DTR massload with base period- June'09
Jun' 09	5323	0.25	1.99	100	1.22	9.42	100	1.56	12.12	100
Jul' 09	5658	0.32	2.64	132.4297	1.35	10.78	114.4271	1.54	12.64	104.3053
Aug'09	4898	0.40	2.84	142.5201	1.46	10.42	110.7072	1.29	9.21	76.02929
Sep '09	4619	0.34	2.27	113.8554	1.38	9.30	98.78942	1.38	9.27	76.5099
Oct' 09	4908	0.30	2.18	109.7139	1.37	9.83	104.4197	1.39	10.04	82.84571
Nov' 09	4898	0.30	2.18	106.1747	1.26	8.84	93.8834	1.82	12.61	104.0594
Dec' 09	5270	0.48	3.64	182.8564	1.41	10.76	114.2954	1.71	13.00	107.2723
Jan' 10	3616	0.34	1.78	89.48293	1.41	7.42	78.7427	2.03	10.69	88.24257
Feb' 10	3660	0.25	1.24	62.26908	1.37	6.70	71.12244	1.92	9.39	77.46205
Mar' 10	5039	0.27	1.97	99.13153	1.03	7.56	80.25698	1.83	13.44	110.8787
Apr' 10	3871	0.29	1.63	82.07831	0.96	5.38	57.15196	1.83	10.21	84.20792
May'10	4291	0.59	3.68	184.5683	0.81	5.08	53.92163	1.82	11.39	93.99752
Jun '10	3223	0.69	3.22	161.5964	1.22	3.90	41.38261	1.75	8.20	67.69802
Jul '10	3586	0.25	1.32	66.4508	1.35	3.60	38.21918	1.14	5.97	49.26238
Aug '10	4127	0.24	1.43	71.89759	1.46	3.99	42.43283	1.12	6.72	55.47772
Sep '10	3867	0.23	1.32	66.26506	1.38	4.30	45.65148	1.27	7.14	58.91089
Oct '10	3452	0.48	2.43	122.008	1.37	6.24	66.26633	1.17	5.90	48.64851
Nov '10	4257	0.64	3.97	199.3976	1.26	5.41	57.40682	1.29	8.02	66.13861
Dec '10	4216	0.71	4.34	217.7159	1.41	5.58	59.22162	1.02	6.27	51.51815

All three amphetamine-type stimulants showed only one common peak, in December 2009. Furthermore, there were two common peaks for ecstasy and methamphetamine, in December 2009 and March 2010, and two for amphetamine and ecstasy (December 2009 and May 2010). At this point it is important to realize that linear or polynomial regression fitting is generally used for prognosis or forecasting time series. In our case it was only used for detecting a trend. There is no sense in presenting a coefficient of determination or errors for these regression lines because errors of regression models for time series may not be independent of one another and the coefficient of determination is not a reliable measure of fitting. Trend analysis does not enjoy the theoretical strengths that regression analysis does in a non-time series context. The main advantage of trend analysis is that when the model is appropriate and the data exhibit a clear trend, simple analysis may be carried out.

Fig. 2 shows the autocorrelation of a time series or cross-correlation between two time series. The correlation function is an important diagnostic tool for analyzing time series in the time domain. We use an autocorrelation plot, or correlogram, to better understand the evolution of a process through time by the probability of a relationship between data values separated by a specific number of time steps. A correlogram plots correlation coefficients on the vertical axis, and lag values on the horizontal. Cross-correlation is a measure of the degree of the linear relationship between two time series. A high correlation between time series at a specific lag might indicate a time delay. The plots on the diagonal are a collerogram and off-diagonal elements are cross-correlation. We focused on cross correlation because a correlogram is not useful when the data contains a

trend; index data at all lags will appear to be correlated because a data value on one side of the mean tends to be followed by a large number of values on the same side of the mean. A strong correlation was indicated between ecstasy and methamphetamine, especially in a lag interval from -5 to 5, which corresponds to the December 2009 -May 2010 time period. This is proof that DTR mass load ecstasy and methamphetamine is more correlated than amphetamine with ecstasy or amphetamine with methamphetamine.

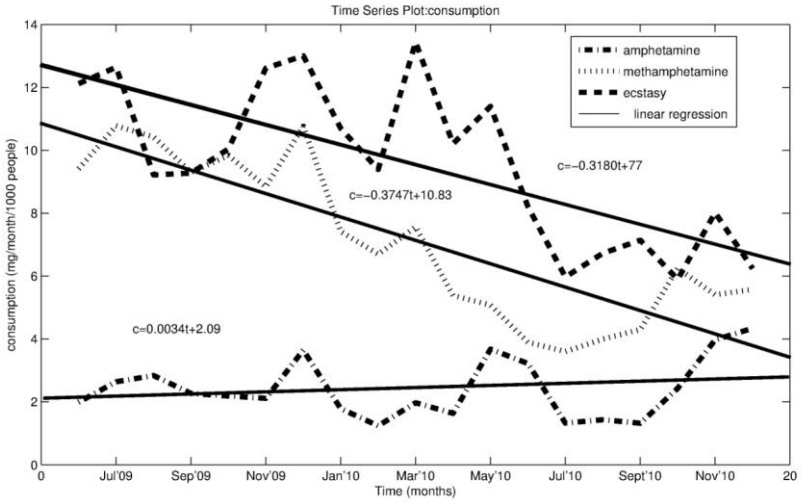

Fig. 1: Amphetamine, methamphetamine and ecstasy DTR mass load (mg/month/1000inh.) from June 2009 to December 2010 with fitted regression line(c-DTR mass load, t-time)

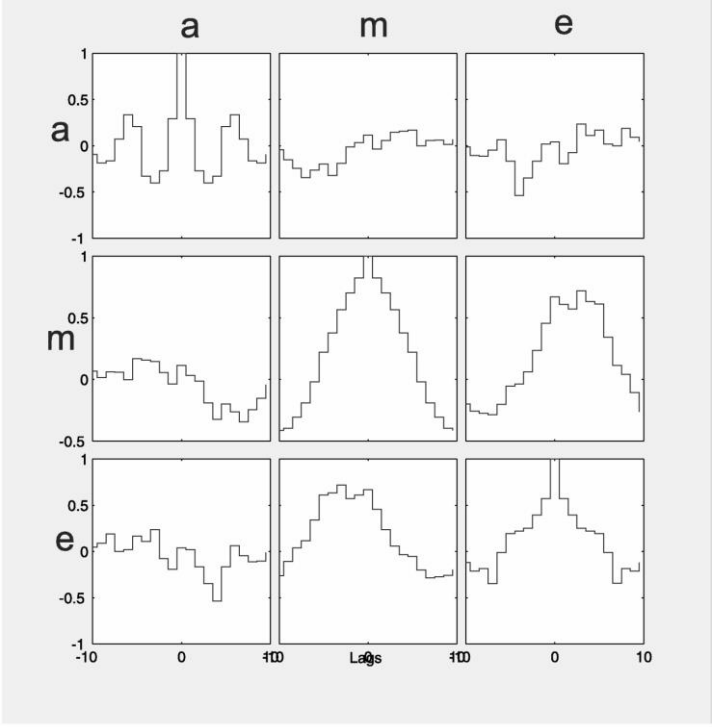

Fig. 2: Correlation plot. (a-amphetamine, m-methamphetamine, e-ecstasy)

Discussion

Estimation of DTR mass load or the number of doses consumed per day per 1000 by wastewater analysis is quite a promising method for gathering data about the problem of illicit drugs. Analysis of illicit drugs in wastewater becomes a great tool to assist public health and law enforcement officials in identifying patterns of drug use and to reduce the harmful consequences associated with drug use. The methods used for this kind of estimation although improving, are still inaccurate.

There is still a lack of in-depth knowledge about the following: the rate of DTR's reaching to STP, the stability of DTR's in various conditions, the velocity of DTR's conveyed and, generally speaking, recovery, that is, the differences between the real amount of DTR's which are discharged into the sewage system and the quantity measured and calculated. There are no proved models which could be used for comparison and calculation purposes. If such a model exists, it would be useful in the system where it was created.

According to those facts, the authors put emphasis on trends that occurred during the near-two-year monitoring of amphetamine-like substances in wastewater in Poznan. In case of amphetamine a slight increase can be observed but ecstasy and methamphetamine have a decreasing tendency during the investigated period. The autocorrelation of a time series indicates that there is a correlation between ecstasy and methamphetamine consumption. We are not able to compare these results with the results of other authors cited in this paper, because trends analysis has not been done by them (1,5-18). We can compare only the DTR mass loads of analyzed illicit drugs what is presented in Table 2 (26). It is very difficult to compare the results because of the differences within the whole methodology itself (sampling for instance) or back- calculation (the data of the population).

Table 2: Amphetamines-group substances consumption. Comparison with previously all published data for Europe

City/Area (Country)	Consumption units	Amphetamine	Ecstasy	Methamphetamine	References
Zagreb (Croatia)	mg/day/1000 ihb.	9.7	3.6		15
Milan (Italy)	mg/day/1000 ihb.	8.9	8.9	10.35	3
London (UK)	mg/day/1000 ihb.	79	5.1	5.52	2
Poznan (Poland)	mg/day/1000 ihb.	0.26	0.47	0.53	This study
Lugano (Switzerland)	mg/day/1000 ihb.	-	10.9		3
Catalonia (Spain)	mg/day/1000 ihb.	-	200 (young aduts)		10
Catalonia Spain	mg/day/1000 ihb.	76	400	24	14
North-East Spain	mg/day/1000 ihb.	193	60 (aged 15-34)	1.5 (aged 15-34)	13

- Not determined

Based on the trends analysis we can also observe the sharp increase in some months, like December or May which could be caused by end- of- term exams at Poznan universities.

If we want to describe the relative change of DTR mass load, we can construct a simple index of DTR mass load, that is, a number that measures the changes in a set of measurements over time.

Suppose we are interested in comparing the DTR mass load of amphetamine of any time period to DTR mass load in July 2009. The index for any month during monitoring period is defined as follows:

Index number for period i = 100 (Value in period i / Value in base period).

It is very important to understand that a change in the index from period to period may not be interpreted as a percentage except when one of the two periods is the base period.

Table 1 shows the months, the values of DTR mass load and DTR mass load index for amphetamine, methamphetamine and ecstasy from June 2009 to December 2010. Using indexes makes it possible to compare the DTR mass load of amphetamines with various correction coefficients resulting from a different molar ratio and pharmacokinetics. It can be seen that the indexes of methamphetamine and ecstasy smoothly decrease; the index of amphetamine shows more variation than the others but seems not to change or increase smoothly. Looking at Table1, it is clear that in December 2009 and May 2010 the value of the DTR mass load index for amphetamine stood at over 180 (compared with June 2009). This fact is hard to interpret but it may be connected with end-of-term examinations at Poznan's universities (there are five universities and several colleges of higher education in Poznan). The indexes were made on the basis of the first June 2009 period. As time passes, the relevance of any base period in the past decreases in terms of comparison with values in the present. Therefore, it is sometimes useful to change the base period and move it closer to the present. There is a simple way of changing the base period of an index. We need to change the index number of the new base period so that it will equal 100 and change all other numbers using the same operation. Thus we divide all numbers in the index by the index value of the proposed new base period and multiply by 100:

New index value = 100 (Old index value / Index value of new base).

Using indices of DTR mass load instead of values of DTR mass load makes data more comparable and much clearer to interpret. The form of index seems to be a more comfortable tool to assist public health and law enforcement officials in identifying patterns of drug use across municipalities of all size.

Conclusion

The application of wastewater analysis to the investigation of illicit drug use represents an innovative approach to the monitoring of this problem. Many scientific works about wastewater analysis have been published in journals over last ten years. Many of them focused on estimating the DTR mass load or number of illicit drug users. Maximum effort is put into accuracy and effective estimation, and each year, this estimation becomes more and more reliable. In this work, the authors placed greater emphasis on trends occurring during the near-two-year monitoring of wastewater. Looking for trends in DTR mass load figures is probably devoid of uncertainties that occur during estimation data, such as DTR mass load per day month, number of consumers etc. Carrying out a trend analysis requires these to be estimated, but in fact the most important task is to maintain the invariable and repeatable condition of sampling and analysis. Monitoring amphetamine-like substances in wastewater at the Poznan Wastewater Treatment Plant indicated a decrease in the DTR mass load of ecstasy and methamphetamine and a slight increase in amphetamine DTR mass load. Cross-correlation analysis showed a correlation between methamphetamine and ecstasy and a lack of correlation between amphetamine and others. Data filtered by a moving average revealed that from June to September 2009 the DTR mass load had increased to a great extent in terms of ecstasy and methamphetamine and less in the case of amphetamine. It followed a decrease after the plateau phase from September 2009 to March 2010 in the case of methamphetamine and July in the case of ecstasy. Although it would be very valuable, it is not possible to compare these results with previous research (1-5,13) because they carried out a daily quantification in the short term, while our research was focused on monthly DTR mass load

in a long time period, which was required by the authorities given in the acknowledgements.

Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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References

1. Daughton CG, Jones-Lepp TL (2011). *Pharmaceuticals and personal; care products in the environment" Scientific and Regulatory Issues.* American Chemical Society. Washington. pp. 348-64.
2. Zuccato E, Chiabrando C, Castiglioni S, Calamari D, Bagnati R, Schiarea S, Fanelli R (2005). Cocaine in surface waters: a new evidence tool to monitor community drug abuse. *Environ Health Glob,* 4(14):1-7.
3. Zuccato E, Chiabrando C, Castiglioni S, Bagnati R, Fanelli R (2008). Estimating community drug by wastewater analysis. *Environ Health Perspect,* 116(8): 1027-1032.
4. Zuccato E, Castiglioni S, Bagnati R, Chiabrando C, Grassi P, Fanelli R (2007). Illicit drugs, a novel group of environmental contaminants. *Water Res*, 116(42): 961-968.
5. Nuijs ALN, Pecceu B, Theunis L, Dubois N, Charlier C, Jorens PG, Bervoets L, Blust R, Neels H, Covaci A (2009). Cocaine and metabolites in waste and surface water across Belgium. *Environ Pollut,* 157(1): 123-129.
6. Nuijs ALN, Mougel JF, Tarcomnicu I, Bervoets L, Blust R, Jorens PG, Neels H, Covaci A (2011). Sewage epidemiology – A real time approach to estimate the DTR mass load of illicit drugs in Brussels, Belgium. *Environ Int,* 37(3): 612-621.
7. Tarcomnicu I, Nuijs ALN, Simons W, Bervoets L, Blust R, Jorens PG, Neels H, Covaci A (2011). Simultaneous determination of 15 top-prescribed pharmaceuticals and their metabolites in influent wastewater by reversed-phase liquid chromatography coupled to tandem mass spectrometry. *Talanta,* 83(3): 795-803.
8. Kasprzyk-Hordern B, Dinsdale RM, Guwy AJ (2009). Illicit drugs and pharmaceuticals on the environment – Forensic applications of environmental data. Part 1: Estimation of the usage of drugs in local communities. *Environ Pollut,* 157(6): 1773-1777.
9. Mari F, Politi L, Biggeri A, Accetta G, Trignano C, Di Padua M, Bertol E (2009). Cocaine and heroin in waste water plants: A 1-year study in the city of Florence, Italy. *Forensic Sci Int*, 189(1-3): 88-92.
10. Boleda RM, Galceran MT, Ventura F (2009). Monitoring opiates, cannabinoids and their metabolites in wastewater, surface water and finished water in Catalonia, Spain. *Water Res*, 43(4): 1126-1136.
11. 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. *Journal of Chromatogr A*, 1216(15): 3078-3089.
12. Bueno MMJ, Ucles S, Hernando MD, Fernandez-Alba AR (2011). Development of a solvent-free method for the simultaneous identification/quantification of abuse and their metabolites in environmental water by LC-MS/MS. *Talanta*, 85(1): 157-166.
13. Postigo C, Lopez de Alda MJ, Barcelo D (2010). Drugs of abuse and their metabolites in Ebro river basin: occurrence in sewage and surface water, sewage treatment plants removal efficiency, and collective drug usage estimation. *Environ Int,* 36:75-84.
14. Huerta-Fontela M, Galceran MT, Martin-Alonso J, Ventura F (2007). Ultra-performance liquid chromatography-tandem mass spectrometry analysis of stimulatory drug abuse in wastewater and surface waters. *Anal Chem,* 79: 3821-9.
15. 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(8): 2686-2693.
16. 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(7): 859-866.
17. 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(10): 3179-3185.
18. Burlet-Hunt SL, Snow DD, Damon T, Shockley J, Hoagland K (2009). The occurrence of illicit and therapeutic pharmaceuticals in wastewater effluent and surface water in Nebrasca. *Environ Pollut,* 157(3): 786-791.
19. European Monitoring Centre for Drugs and Drugs Addiction (EMCDDA), Assessing illicit drugs in wastewater. Potential limitations of a new monitoring approach 2008.
20. European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), Annual Report 2010. The state of the drug problem in Europe. Lisbon: EMCDDA, 2010. http: // www. emcdda.europa.eu/publications/annual-report/2010.
21. Baselt RC, Casarett LJ (1972). Biliary and urinary elimination of methadone and its metabolites in the rat. *Biochem Pharmacol*, 21: 2704-2712.
22. Thai PK, Jiang G, Gernjak W, Yuan Z, Yin Lai F, Mueller JF (2014). Effects of sewer conditions on the degradation of selected illicit drug residues in wastewater. *Water Res,* 48: 538-547.
23. Van Nuijs ALN, Castiglioni S, Tarcomnicu I, Postigo C, Lopez de Alda M, Neels H, Zuccato E, Barcelo D, Covaci A (2011). Illicit drug consumption estimation derived from wastewater analysis: A critical *Sci Total Environ,* 409: 3654-3577.
24. Vaquez-Roig P, Blasco C, Pico Y (2013). Advances in the analysis of legal and illegal drugs in the aquatic environment. *Trends Anal. Chem,* 50: 65-77.
25. Reid MJ, Langford KH, Mørland J, Thomas KV (2011). Quantitative assessment of time dependent drug-use trends by the analysis of drugs and related metabolites in raw sewage. *Drug and Alcohol Dependen,* 119(3): 179-186.
26. Klos J, Nowicki P, Kokot Z (2013) Pilot study of the estimation of amphetamines consumption in the Polish city of Poznan, *J Forensic Res,* 4:5, http://www.omicsonline.org/pilot-study-of-the-estimation-of-amphetamines-consumption-in-the-polish-city-of-poznan-2157-7145.1000203.php?aid=20398
27. Funk W, Dammann V, Donnevert G (2007). *Quality Assurance in Analytical Chemistry.* Wiley-VCH Varlag GmbH & Co. KGaA, Weinheim. Germany.