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TREMEPOL

Transformation Products of Emerging Pollutants in the Aquatic Environment

Work Package 5 – Results dissemination

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Summary

Emerging pollutants are thought to be responsible for the most significant share of environmental, human health and economic risk. Although the universe of chemical pollutants is enormous, we only monitor and have studied a minor fraction.

The study of the fate of the emerging pollutants and their transformation products in wastewater-treatment plants (WWTP) is of paramount importance, since it can provide valuable information on the human consumption of various drugs. Sewage epidiomiology is the new field that was develloped for the estimation of illicit drug use based on measurements of urinary excreted illicit drugs and their metabolites in untreated wastewater. Within this approach, human metabolic excretion products resulting from drug consumption are rapidly collected and pooled by the sewage systems, providing valuable evidence of the amount and type of drug consumed by a population.

The main objective of this research is the contribution to the current knowledge on the actual burden of micropollutants on the environment and on the effect they have on the human health.

Introduction

Urban wastewaters contain a large number of substances, including several human metabolites. Besides serving to control the types and the amounts of pollutants discharged into the environment, the analysis of micropollutants present in wastewater can also provide valuable information about patterns of human use of these substances. Human metabolic excretion products, both parent compounds and TPs, resulting from drug use are rapidly collected and pooled by the sewage systems. Analyzing these substances in the influent wastewaters could provide evidence of the amount and type of drug used by a population (Daughton, 2001).

The official ways to obtain figures about the use of drugs are surveys integrated with medical records and drug production data. These tools give a useful general picture of drugs, but estimates of use rates and drug use prevalence may be inaccurate and have also several limitations. One of the main disadvantages is the inclusion of substantial time lags that negatively impact the reliability, validity and utility of such data (van Nuijs et al., 2011). In the case of illicit drugs the biased selection of the population that is needed may lead to an underestimation of the use rates and also to incorrect estimates since most of the information is obtained from the users (Castiglioni et al., 2013). There is a recent option to get this information based on data obtained from influent wastewater, which can be used to back-calculate the mass loads of the parent drugs and/or metabolites. These loads allow the estimation of its use (knowledge of the drug metabolism and excretion patterns is required). Including served population by the corresponding wastewater treatment plant (WWTP) in the equation, it is possible to make a comparison with results from different locations (van Nuijs et al., 2011). Several studies have already used these methodologies to back calculate drug use (Bones et al., 2007, Zuccato et al., 2008, Postigo et al., 2008, Ort et al., 2014, Been et al., 2014, United Nations, 2012). One of the main advantages of calculating the loads by analyzing the influent wastewaters is that it allows a rapid detection of changes in the patterns of illicit drugs as well as the facility for comparing data.

Greece is amongst the most affected countries by the severe economic crisis plaguing Europe since 2008. The inability to continue financing its public debt caused significant cuts in the public sector, including healthcare. In 2010, the Greek government requested a loan from the EU, which was the first request for a resave due to the impossibility of keeping limiting the public debt. The effects of this crisis in Greece directly affect its population, with growing unemployment rates (up to 30%), a substantial decline in per capita income and a significant increase in poverty. Another consequence has been a great decrease in public spending for health, which is now less than any of the other pre-2004 European Union members (Kentikelenis et al., 2014, Stuckler & Basu, 2013). Some studies point to a change in the use of drugs, especially since in 2012 the Greek government surpassed the Troika's demands for cuts in drug spending (Kentikelenis et al., 2014, European Commission 2012, European Commission 2013). These studies also suggest an increment in the use of some illicit drugs. However, there are not extensive studies using chemical data to support and quantify these hypotheses so far. The analysis of wastewater offers great possibilities for the obtaining of solid data to confirm and expand the knowledge in this regard.

The objective of this work is to use the tools offered by analytical chemistry and sewage epidemiology and relate the use of various classes of licit and illicit drugs with the effects of the economic crisis. Contradictory factors, like severe cuts in expenditure for drugs and increase of mental illnesses due to the stress associated to the crisis, may affect the licit drug use in the population. Moreover, the use of illicit drugs in the community cannot be predicted. This study aims to provide information on changes in use patterns of licit and illicit drugs during the last 5 years (2010 – 2014) and relate this data to social parameters such as the unemployment rate or the decrease in the public health investments through the evaluation of the obtained use trends of the different studied substances. It should be emphasized that the studied WWTP serves half the population of Greece and it is located where the effects of the crisis are more noticeable.

Study of licit and illicit drugs, quantified through sewage-based epidemiology

In order to achieve the aforementioned objective, antidepressants, anxiolytics, antipsychotics, antibiotics, antiepileptics, analgesics, NSAIDs, diuretics, antihypertensives, antiulcers and steroids (a representative sample of the licit drugs used in a modern society) were considered, as well as the main illicit drugs and their metabolites. The concentrations of these substances in influents were monitored from 2010 to 2014 in Athens. Applying methods of back-calculation to each of the substances, daily use values were estimated and results were discussed and correlated with the severe socioeconomic changes.

Samples

All the influent wastewater samples considered in this study were collected from the WWTP of Athens (Greece) during five sampling campaigns conducted from 2010 to 2014.

The WWTP of Athens is designed with primary sedimentation, activated sludge process with biological nitrogen and phosphorus removal and secondary sedimentation. The average sewage flow for the periods of study was 720,000 m^3 day^{-1} for a typical dry day. For the load calculations the specific day flow has been used. As for the travel distances of wastewater in the sewer, the closest connected household is 0.5 km and the most remote is 30 km away. The residential population connected to the WWTP based on official census excluding the commuters is 3,700,000 and the number of people estimated based on number of house connections is 4,562,500. This WWTP has a design capacity to serve a population equivalent of 5,200,000, being by far the largest of Greece and one of the largest in the world. For the drug use estimations, we used the census value for the residential population.

In each campaign influent wastewater samples (24-hour composite flow proportional samples) were collected during 7 consecutive days from 5/12 to 11/12 in 2010, on 8 days from 3/4 to 10/4 in 2011, on 8 days from 2/4 to 9/4 in 2012, on 11 consecutive days from 6/3 to 16/3 in 2013 and on 8 days from 11/3 to 18/3 in

2014. This allows investigation of drug use trends throughout the week and over the course of the study period. All wastewater samples were collected in pre-cleaned high-density polyethylene (HDPE) bottles. Untreated and treated wastewater samples were immediately filtered with glass fiber filters (pore size 0.7 μm) after arrival at the laboratory. Samples were acidified with a 1 M HCl solution to pH 2.5 ± 0.10 and stored in the dark at 4 °C until analysis.

Analysis and Evaluation of the data

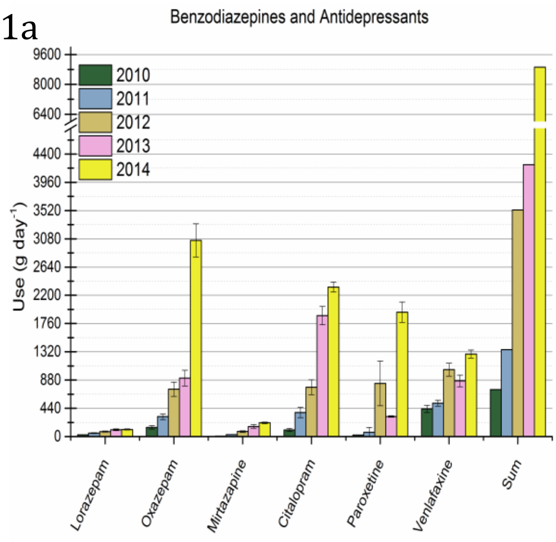
The analytical method for the determination of 148 substances is based on solid phase extraction and further analysis by LC-MS/MS, according to Thomaidis et al. (2015). The approach for the estimation of the drug usage can also be retrieved from the publication (Thomaidis et al. 2015).

Results for licit and illicit drugs

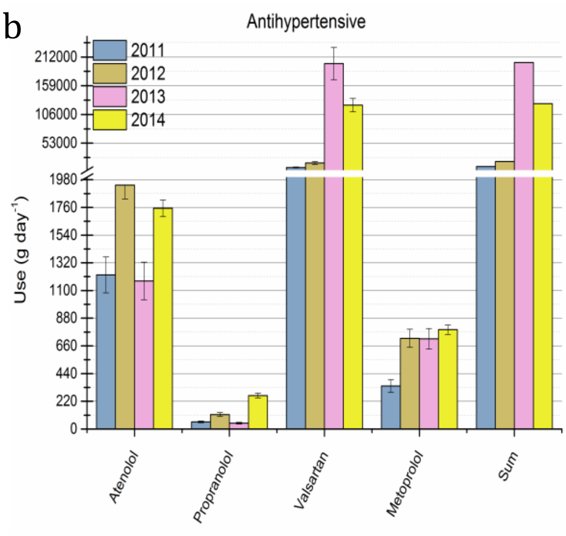
Pharmaceuticals

Results showed high concentrations of pharmaceuticals in the influent wastewater samples in all five sampling campaigns. Up to 128 out of the 148 target drugs and related metabolites were detected, in concentrations in some cases above 200 μg L^{-1}. In figure 1, the variations in use in terms of mean loads (g day^{-1}) in the analyzed influent samples are presented for the most relevant compounds.

1a



1b



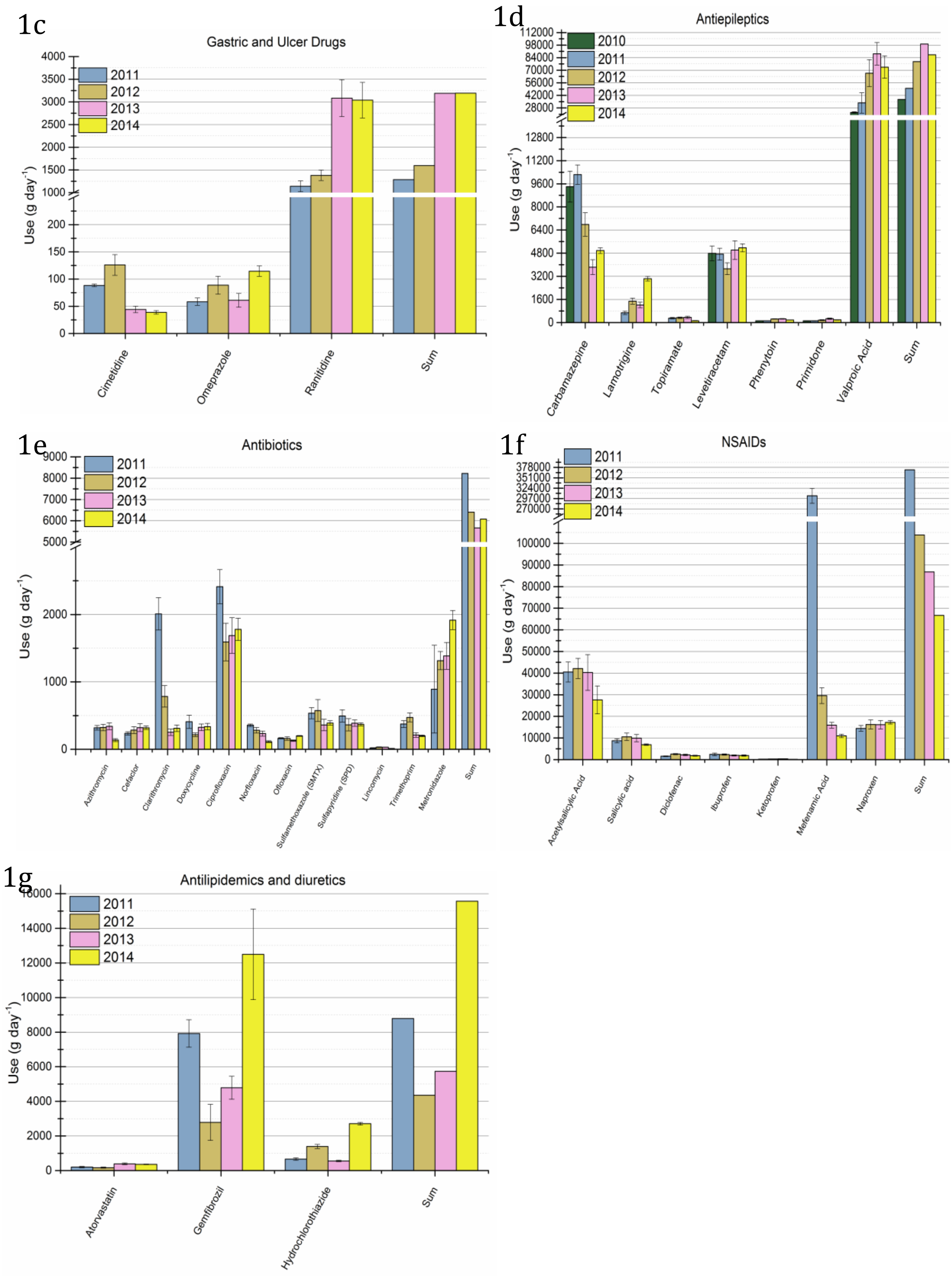


Figure 1. Estimated use (mean values, g day^{-1}) for selected pharmaceuticals.

Different patterns were observed for the various therapeutic families of monitored compounds between the different sampling periods. While the use of some substances increased significantly, such as benzodiazepines and antidepressants, the use of others decreases considerably (e.g. NSAIDs). Significant differences were also observed in the used amounts of some compounds within the same therapeutic family.

Variations in the use of benzodiazepines anxiolytics and various types of antidepressants are shown in Figure 1a. A pronounced increase in the overall use of both antidepressants (TeCAs, SSRIs and SNRIs) and benzodiazepines was observed (from ~36,000 to ~500,000 doses day^{-1} for the substances), with a remarkable increase for all the studied compounds of these therapeutic families. The oxazepam and citalopram cases are remarkable, with high increase during the sampling period reaching use rates of 203,638 and 116,416 doses day^{-1} (3055 and 2328 g day^{-1}), respectively. The observed increase in use of paroxetine, an antidepressant widely used (reaching values up to 96,833 doses day^{-1} (1,937 g day^{-1})), is also noteworthy. For compounds of these therapeutic families for which data on excretion and increased bioavailability were not available (e.g. sertraline/norsetraline, amitriptyline, bromazepam or temazepam), an increase of the same magnitude in the concentrations detected in the influent wastewater samples was also observed. The detected levels for the studied antipsychotics (e.g. clozapine, olanzapine) in the influent wastewaters followed the trend of the other psychiatric drugs and showed a marked increase every year from 2010 to 2014.

Other families of drugs with an increase in use during the period studied were antihypertensives (Figure 1b), highlighting the large increase in the use of valsartan. The average use of this compound increased from 25,988 doses day^{-1} (8,316 g day^{-1}) in 2011 to over 625,000 doses day^{-1} (>200 kg day^{-1}) in 2013, with a slight decrease in 2014. Another antihypertensive drug with a pronounced increase was propranolol. A similar but less pronounced pattern was found for the anti-ulcer drugs. A significant increase in use of ranitidine during the study period was observed, from 7,607 doses day^{-1} (1,141 g day^{-1}) (2011) to values above 20,000 doses day^{-1}

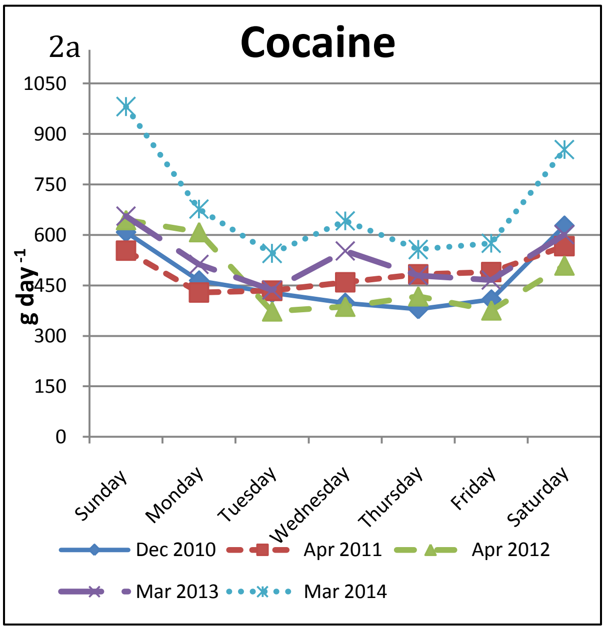
(3,040 g day^{-1}) (2013, 2014) (Figure 1c). The family of antiepileptics showed a constant increase in overall use. However, important differences in the use of drugs within this family were observed. While a significant decrease in use was observed for carbamazepine (which is one of the most widely prescribed and very important compound for the treatment of epilepsy, neuralgia and some psychiatric diseases, such bipolar affective disorder), an increased even more pronounced was determined for valproic acid (from 11,494 doses day^{-1} (22,988 g day^{-1}) in 2010 to 36,761 doses day^{-1} (73,521 g day^{-1}) in 2014, Figure 1d), a compound which has very similar applications. A significant increase was also observed for lamotrigine, reaching 15,180 doses day^{-1} (3036 g day^{-1}) in 2014.

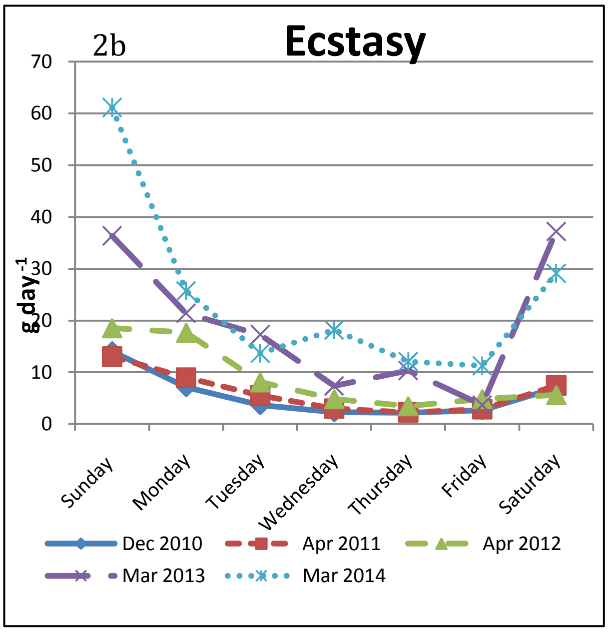
The use of antibiotics (Figure 1e), which are among the most frequently prescribed drugs for humans in modern medicine, showed a slight decrease between the different periods studied as it can be observed in the Sum graphic. Significant decreases were observed in the use of the macrolide clarithromycin (from 4,019 doses day^{-1} (2010 g day^{-1} in 2011 to 624 doses day^{-1} (312 g day^{-1}) in 2014) and for the fluoroquinolone ciprofloxacin (from 4,827 doses day^{-1} (2413 g day^{-1}) in 2011 to 3,558 doses day^{-1} (1,779 g day^{-1}) in 2014), two widely prescribed antimicrobials. These compounds were detected at the highest concentrations in the present study, in comparison with other investigations conducted in influent wastewater in other countries (Guerra, 2014). To a lesser extent, reduction in the use of norfloxacin, sulfamethoxazole or trimethoprim were also observed. An exception can be found in the significantly higher use of metronidazole throughout the sampling period (from 1,784 doses day^{-1} (892 g day^{-1}) in 2011 to 3,834 doses day^{-1} (1,917 g day^{-1}) in 2014).

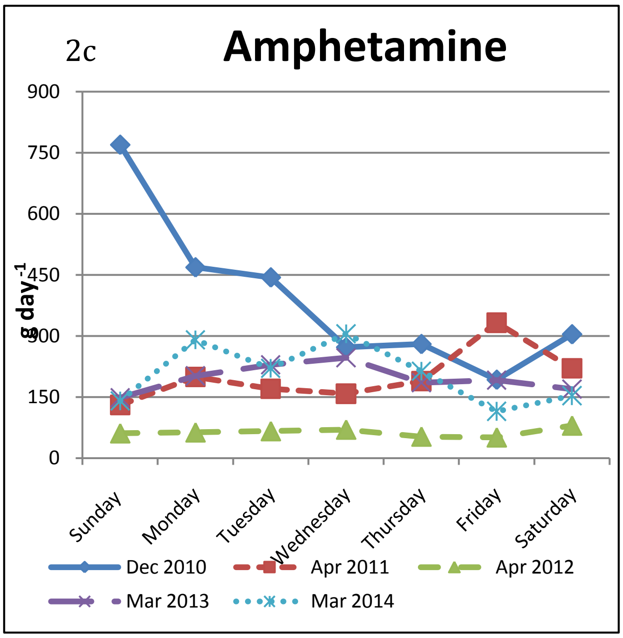
Non-steroidal anti-inflammatory drugs (NSAIDs) were the substances detected at highest concentrations and with the highest use rates (Figure 1f). These drugs, which are used for treatment of acute pain and inflammation, are administered both orally and topically and are available as prescription and over-the-counter (non-prescription) (Jelic et al., 2011). The overall use of these drugs remained stable with no significant differences except for mefenamic acid, for which a high decrease was observed in its use. For antilipidemics and the diuretic hydrochlorthiazide, no significant variations could be derived (Figure 1g). Gemfibrozil showed a high variation of its use, with a significant decrease in 2012, and significant increase in 2014. A significant increase in 2014 was observed for hydrochlorthiazide, as well, but not in 2013. In general, hydrochlorthiazide is associated with antihypertensive substances like valsartan and other sartans and angiotensin-converting enzyme (ACE) inhibitors (like Captopril, Ramipril and others not included in this study). Therefore, sound conclusions for this compound could not be derived.

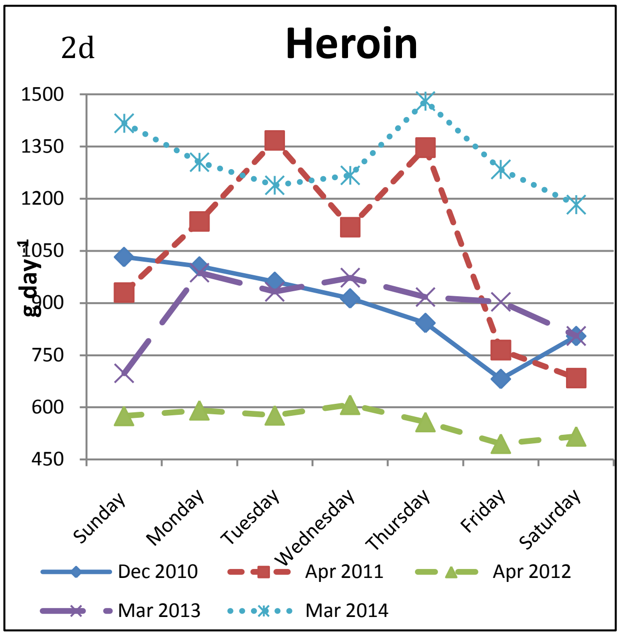
Illicit drugs

Drugs of abuse in general showed a clear pattern of use during the week. For some of these substances a sharp increase in use during the weekend was detected in all the monitored periods. The highest concentrations were detected in samples of Saturdays, Sundays and, in some cases, of Mondays.






2c
Amphetamine
g day -1
900
750
600
450
300
150
0
Sunday
Monday
Tuesday
Wednesday
Thursday
Friday
Saturday
Dec 2010
Apr 2011
Apr 2012
Mar 2013
Mar 2014


2d
Heroin
g day-1
1500
1350
1200
1050
900
750
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Monday
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Wednesday
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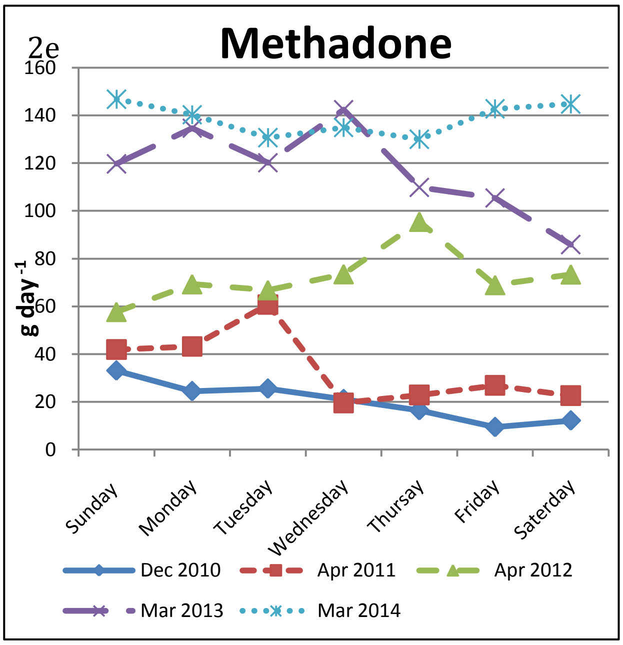
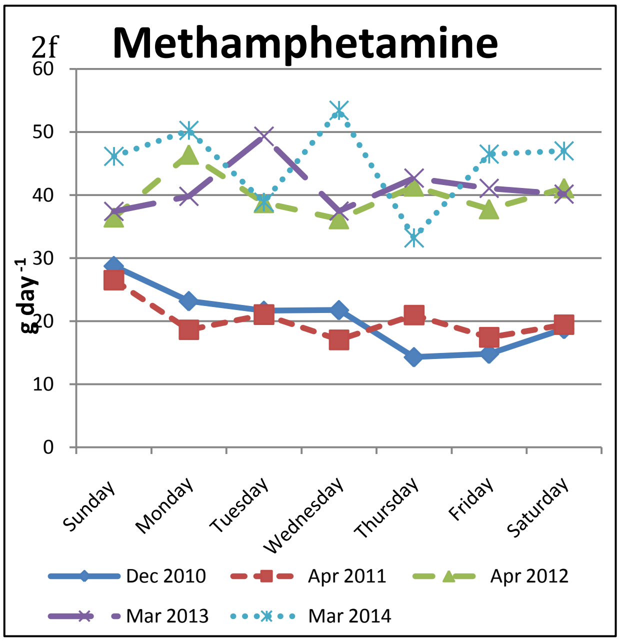
Friday
Saturday
Dec 2010
Apr 2011
Apr 2012
Mar 2013
Mar 2014


2e
Methadone
g day -1
160
140
120
100
80
60
40
20
0
Sunday
Monday
Tuesday
Wednesday
Thursay
Friday
Saterday
Dec 2010
Apr 2011
Apr 2012
Mar 2013
Mar 2014


2f
Methamphetamine
g day -1
60
50
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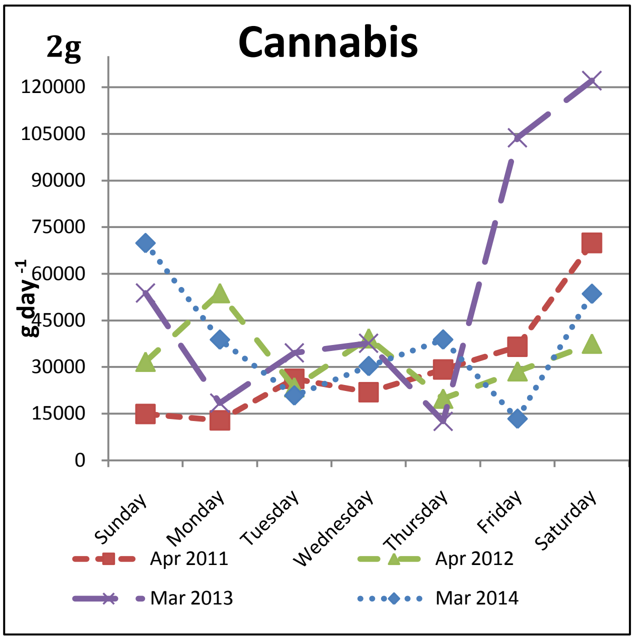

Figure 2. Daily use (g day^{-1}) of drugs of abuse through the five years investigation (2010-2014).

Figures 2a, 2b and 2c show the graphs of daily use (seven days for five different sampling campaigns) for cocaine, ecstasy and amphetamine, respectively. These substances are representative cases of the pattern mentioned above. In the case of ecstasy, changes in daily use were very strong, with use rates up to ten times higher than the ones detected on weekdays, suggesting that the use of this substance is practically restricted to the eve of non-working days. The weekly variation for amphetamine in the sampling period of 2010 is very similar to the ecstasy trends. However, variations throughout the week were not noticeable in the other four sampling campaigns. This is maybe related to the fact that the recreational use of this drug has decreased and the amounts detected correspond to its additional use as therapeutic drug in hospitals, since this substance is widely used in the treatment of some diseases, such as attention deficit hyperactivity disorder (ADHD) or narcolepsy. A strong trend was also clearly observed for cocaine, although the differences between weekends and weekdays are not as high as in the case of ecstasy. Cocaine use seems to be much more widespread, with higher g day^{-1} values. In this case, significant use during weekdays was also detected. Regarding this substance, noteworthy were the high values observed in the samples corresponding to

Mondays, reflecting a very high intake of this substance also on Sundays.

By contrast, other substances exhibited a relatively more constant use throughout the week. An example of this pattern can be found for heroin and methadone (Figure 2d and 2e, respectively), for which the use remained constant and did not increase significantly during the weekends. Another example of this pattern was observed for methamphetamine (Figure 2f). This is a so called “street” drug, and its pattern was clearly different than that of recreational drugs (e.g. cocaine, ecstasy) and whose use strongly increases during weekends. The “street” drugs of abuse, like heroin and methamphetamine (known also as sisa in Greece), are more related to marginal conducts, and their use was relatively constant throughout the week.

The case of cannabis use is noteworthy (Figure 2g), since this drug somehow perceived as a recreational one. Although some studies showed an increase in its use during the weekends (Viana et al., 2011), most of them did not report weekly patterns (Ort et al., 2014, Thomas et al., 2012). In the present study, a correlation between use and the specific day of the week was not clearly observed. Use of THC was not constant but varied significantly without following a specific pattern. An exception was the results for Friday and Saturday in the monitoring campaign of 2013. The high values reported in those days can be distorted by the proximity of the Carnival of Athens, which may be strongly related to a higher use of this substance. This suggests that the pattern of use of illicit drugs could vary considerably from one country (or city) to another (Ort et al., 2014, European Union, 2013).

Table 1. Weekly drug use and percentage of population under use per year.

Compound	December 2010		April 2011		April 2012		March 2013		March 2014	
	Weekly use (Kg)	Population under use* (%)	Weekly use (Kg)	Population under use* (%)	Weekly use (Kg)	Population under use* (%)	Weekly use (Kg)	Population under use* (%)	Weekly use (Kg)	Population under use* (%)
Cocaine	3.31	0.12	3.42	0.12	3.31	0.12	3.70	0.13	4.83	0.17
Heroin	6.24	0.74	7.35	0.87	3.92	0.47	6.22	0.74	9.18	1.09
Amphetamine	2.73	0.33	1.39	0.17	0.44	0.053	1.37	0.16	1.44	0.17
Methamphetamine	0.14	0.017	0.14	0.017	0.28	0.033	0.29	0.034	0.32	0.038
MDMA (Ecstasy)	0.039	0.0014	0.043	0.0015	0.063	0.0023	0.13	0.0048	0.17	0.0061
THC (cannabis)	-	-	211.6	6.0	234.4	6.7	382.9	10.9	265.5	7.59
Methadone	0.14	-	0.24	-	0.51	-	0.82	-	0.97	-
Codeine	1.04	-	1.21	-	1.37	-	2.03	-	2.03	-

**based on the average dose and for a population of 3,700,000 people.*

Regarding the variation with respect to the different sampling periods, different patterns were observed. In addition to Figure 2, the weekly use for each substance is summarized in Table 1, as well as the estimated population under use, based on the average dose and for a population of 3,700,000 people. Cocaine use amongst the five sampling periods showed no significant variations. The use of this substance remained about 3.3 kg week^{-1} (except in 2014, where the use increased to 4.83 kg week^{-1}). However, amphetamine use (2.73 kg week^{-1} in 2010) declined significantly in 2011 (1.39 kg week^{-1}) and 2012 (0.44 kg week^{-1}) with a slight increase in 2013, which remained in 2014. On the contrary, ecstasy experienced a significant increase. The estimated use of this substance in 2014 was 0.17 kg week^{-1}, while the 2010 estimated use being only 0.039 kg week^{-1}. Use of cannabis increased from 211.6 kg week^{-1} in 2011 to 265.5 kg week^{-1} in 2014. The high estimated use in 2013, 382.9 kg week^{-1}, can be related to the proximity of the Carnival of Athens, as explained before.

Significant changes were detected in methamphetamine use associated with the so called "sisa", a newly introduced form of the drug related to marginal conducts. The weekly estimated use of this substance increased from 0.14 kg week^{-1} in 2010 and 2011 to values above 0.28 kg week^{-1} in 2012 and onwards. Methadone use also experienced a significant increase during these years, from 0.14 kg week^{-1} in 2010 to 0.97 kg week^{-1} in 2014. Heroin abuse has changed during the study period (3.9 – 9.2 kg week^{-1}) without a defined trend. The amount of morphine due to codeine use has been taken into account for the calculation of heroin use. However, the therapeutic use of morphine was not taken into account due to the absence of data and the unwillingness of the Greek state to provide this data. The therapeutic morphine may represent a significant portion of the total amount of this substance in wastewater. This may lead to an overestimation of the heroin use. This is in agreement with the fact that the percentage of people under use of this substance (0.74 %) is significantly higher than the one published in the European Drug Report 2013 (0.25 – 0.3%). One solution to this problem would be to perform the back-calculation from a minor metabolite (6-MAM), but concentrations obtained for this compound are below the limit of quantitation in most cases. Therefore, heroin use data may be overestimated and the detected changes in use may also be a consequence of variations in the use of therapeutic morphine. It is noteworthy that codeine use was also increased in 2013 and 2014 (Table 1). However, since use data could not be derived from the relevant ministries, no sound conclusions can be drawn about the use of heroin from these data.

Occurrence of new designer drugs of abuse and their transformation products

The aim of this study is a comprehensive quantitative target screening of emerging pollutants in environmental samples, which involves a generic sample preparation, a UPLC-QTOF-MS/MS method and post-acquisition evaluation of the data. An in-house database was built with information of retention time, MS and MS/MS ions for 2327 compounds, including pesticides, pharmaceuticals, drugs of abuse, industrial chemicals, doping compounds, as well as metabolites and transformation products. Out of this database, 745 new designer drugs, drugs of abuse and psychoactive pharmaceuticals (PP) were examined more thoroughly.

Samples

Influent and effluent wastewater samples were collected from the WWTP of Athens (Greece) during two sampling campaigns conducted on 2014 and 2015.

In each campaign influent and effluent wastewater samples (24-hour composite flow proportional samples) were collected during 7 consecutive days from 11/3 to 18/3 in 2014 and from 4/3 to 11/3 in 2015. Moreover, 12 influent 2-hour composite flow proportional samples were collected for 2 days (Tues. 12/3 & Sat. 15/3/2014).

This allows investigation of drug use trends throughout the week and over a weekday and weekend. All wastewater samples were collected in pre-cleaned high-density polyethylene (HDPE) bottles. Untreated and treated wastewater samples were immediately filtered with glass fiber filters (pore size 0.7 μm) after arrival at the laboratory. Samples were stored in the dark at 4 °C until analysis.

Analysis & Evaluation of the data

Optimization was performed in order to minimize false negative results and a validation protocol is proposed in order to evaluate the performance criteria of the HRMS method. The samples were analyzed and evaluated with sophisticated software (Bletsou et al., 2015). Identification points were attributed to each analyte and quantitation was also carried out.

Results

The results obtained revealed that analytes from different categories, such as NPSs, alkaloids, DoAs, central nervous system (CNS) pharmaceuticals and metabolites of those compounds, were present in the wastewater samples in the range of few ng/L and μg/L levels. Moreover, intra-day and intra-week variation was estimated and time profiles were derived for most of the compounds. 183 compounds were detected at least once in influent wastewaters and 163 in effluent wastewaters. The detection of NPSs and DoAs in wastewater can provide valuable information on the use of these substances in the community.

Some compounds are present in both studies and are presented in more detail in the sewage-based epidemiology study, where more data are available. The advantage of this HRMS method is the possibility for post-acquisition evaluation of the data and the retrospective analysis. In that sense, compounds that are reported for the first time, or newly released compounds will be the focus of that second study.

Stimulants – Amphetamines

Among others, 29 stimulants in total (Table 2) were detected in wastewater samples in concentration levels from 0.0013 μg/L (ethylamphetamine) – 3.62 μg/L (Phenelzine), in influent wastewaters.

Table 2. Stimulants and amphetamines detected in wastewater samples

		CAS Number	Influent wastewater Identification Points	Effluent wastewater Identification Points
1	Cocaine (COC)	50-36-2	5	n.d.
2	Benzoylecgonine (BECG)	519-09-5	≥5	2
3	Ecgonine methyl ester (EME)	7143-09-01	2	n.d.
4	Amphetamine	300-62-9	≥5	n.d.
5	Methamphetamine (MA)	537-46-2	2.5	n.d.
6	Dimethylamphetamine	1009-69-	2.5	2.5

		4		
7	Ethylamphetamine	457-87-4	2	2.5
8	3,4-methylenedioxyamphetamine (MDA)	4764-17-4	≥5	2
9	3,4-methylenedioxy-N-methylamphetamine (MDMA)	42542-10-9	2	2
10	PMMA (para-Methoxy-N-methylamphetamine)	3398-68-3	5	5
11	Metaraminol (3,β-dihydroxyamphetamine)	337376-15-5	2.5	n.d.
12	Pholedrine (p-hydroxy-methylamphetamine)	6114-26-7	n.d.	2.5
13	4-methyl-2-hexanamine	105-41-9	2	2
14	Mephentermine	100-92-5	2	2.5
15	Phenelzine	51-71-8	2	2.5
16	Pyrovalerone	3563-49-3	n.d.	2.5
17	Phendimetrazine	17140-98-6	2	2
18	Midodrine	133163-28-7	2	2.5
19	Heptaminol	372-66-7	2.5	2.5
20	Cathine/ norpseudoephedrine	492-39-7	2.5	2.5
21	Nikethamide	59-26-7	n.d.	2.5
22	Pemoline	2152-34-3	2	n.d.
23	Aminorex	2207-50-3	2	2
24	Dimefline	1165-48-6	n.d.	2.5
25	Ethamivan	304-84-7	n.d.	≥5
26	TMA (trimethoxyamphtamine)	1082-23-1	2	2
27	3,4-DMA (dimethoxyamphtamine)	120-26-3	2	2
28	4-Methyl-pyrrolidinopropiophenone (MPPP)	28117-80-8	2	2
29	2 C-D (2,5-dimethoxy-4-methylphenethylamine)	24333-19-5	2	2.5

Apart from the well-known amphetamine-derivatives, such as AMP, MA, MDA, MDMA and MDEA, some compounds are reported for the first time in the same concentration levels in wastewater samples; such as 2,5-dimethoxy-4-methylphenethylamine (2C-D), dimethoxyamphtamine, trimethoxyamphtamine, ethylamphetamine, dimethylamphetamine, para-Methoxy-N-methylamphetamine (PMMA), 4'-Methyl-α-pyrrolidinopropiophenone (MPPP), cathine, aminorex, phendimetrazine, mephentermine, midodrine, heptaminol, bemegride and pemoline.

Opiates -Opioids

5 compounds were detected in influent wastewaters, morphine, normorphine, methadone, codeine & EDDP, while 2 more metabolites were detected in effluent wastewaters, norcodeine and hydrocodone. This is a proof of the degradation processes that take place during the wastewater treatment procedure.

More drugs

From the sympathomimetics drugs, 9 were detected, among them ephedrine, norephedirne and etafedrine, as well as metanephrine, phenylephrine, apophedrin, isoetharine, methoxamine and nylidrin.

Antidepressant drugs from different categories, such as SSRIs (selective serotonin reuptake inhibitors), SNRIs (serotonin-norepinephrine reuptake inhibitors), TCAs (tricyclic antidepressants) and MAOIs (monoamine oxidase inhibitors) were also present in the samples.

Moreover, benzodiazepines, with oxazepam as a characteristic example were present in the samples, following the same trend during the week with that presented in the first study. Antiepileptics, barbiturates, anesthetics and antipsychotics (clozapine) were also detected in the samples.

Intra-day and intra-week variation of parent compounds and their metabolites

The intra-day concentration profile of parent and metabolites can be used to provide additional evidence for the identification the metabolites. Figure 3 shows this for clarithromycin and two related metabolites, N-desmethyl clarithromycin and hydroxyclarithromycin. As the concentration in influent wastewater was measured every two hours, it can be observed that the profiles are identical for the metabolites and for clarithromycin.

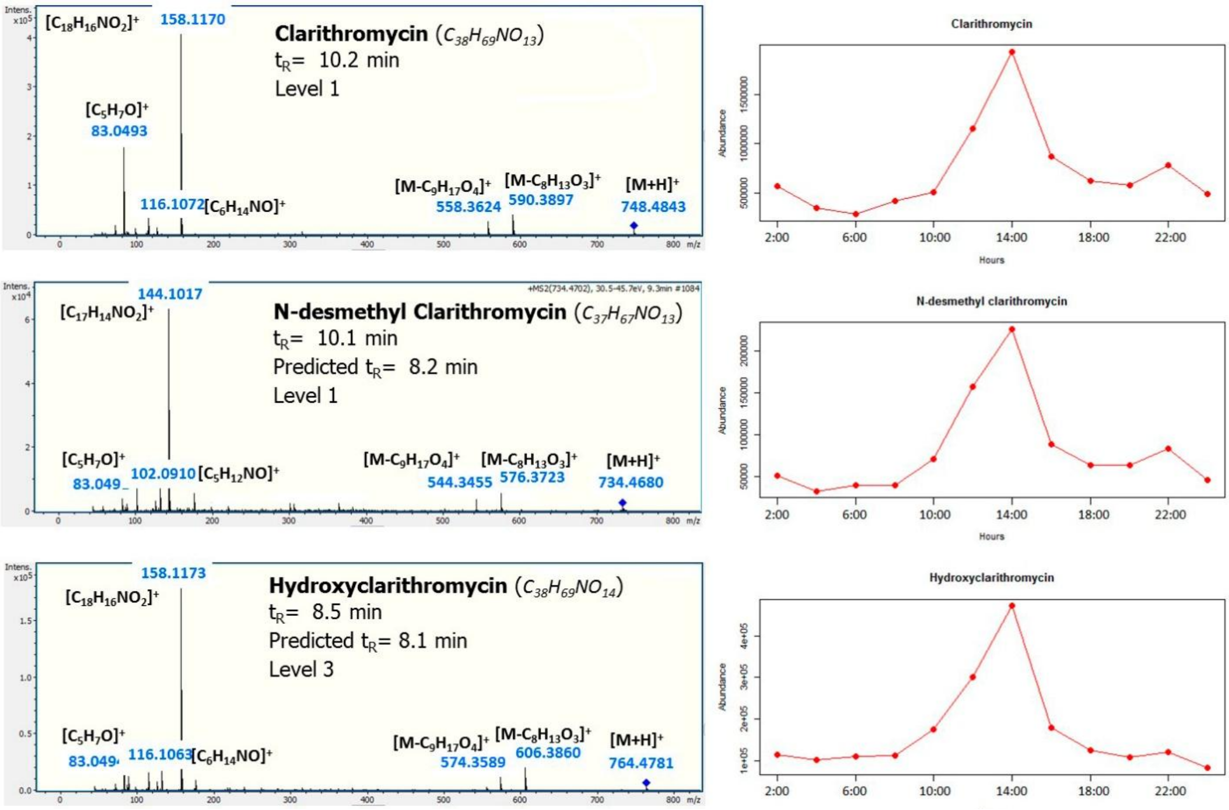

Figure 3. MS/MS spectra and intra-day concentration profiles of clarithromycin and its metabolites N-desmethyl clarithromycin and hydroxyclarithromycin.

This procedure was also used in the case of venlafaxine and its two metabolites N-desmethyl venlafaxine and O-desmethyl venlafaxine, as it is shown in Figure 4. An excellent interrelation in the intra-day profiles among parent/metabolites was observed. A good intra-day interrelation between the two confirmed metabolites cotinine and hydroxycotinine (Figure 5) was also observed, but these profiles did not match the profile of the parent compound, nicotine, well. This indicates that this new strategy can provide valuable additional evidences for the identification, but the results should be interpreted with caution. The absence of interrelation does not imply a false positive.

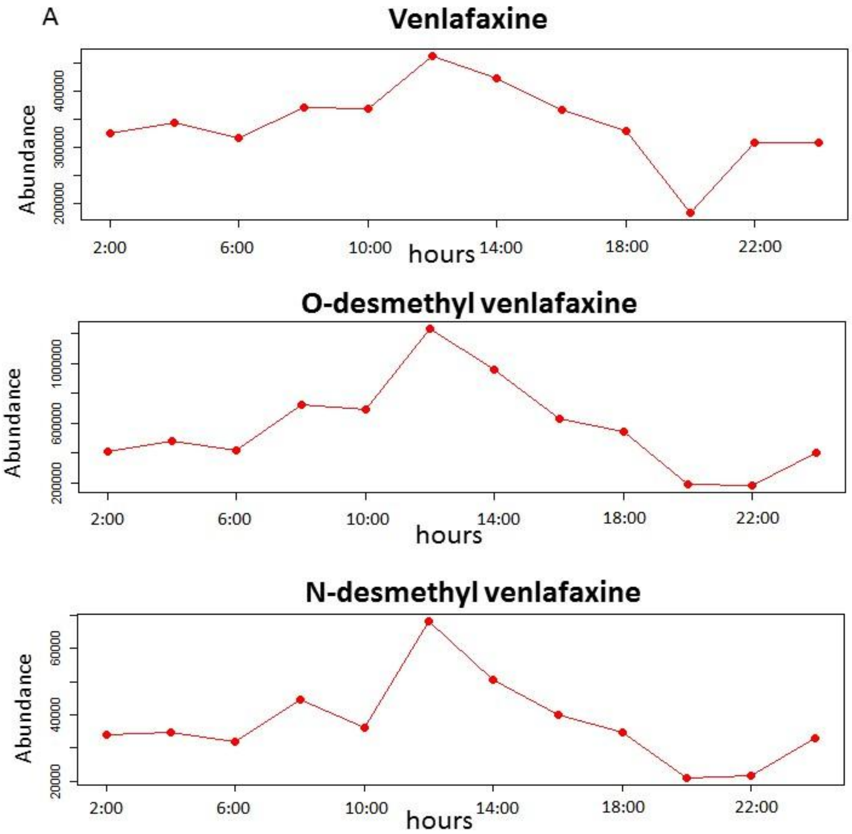

Figure 4. Intra-day concentration profiles of venlafaxine and related metabolites

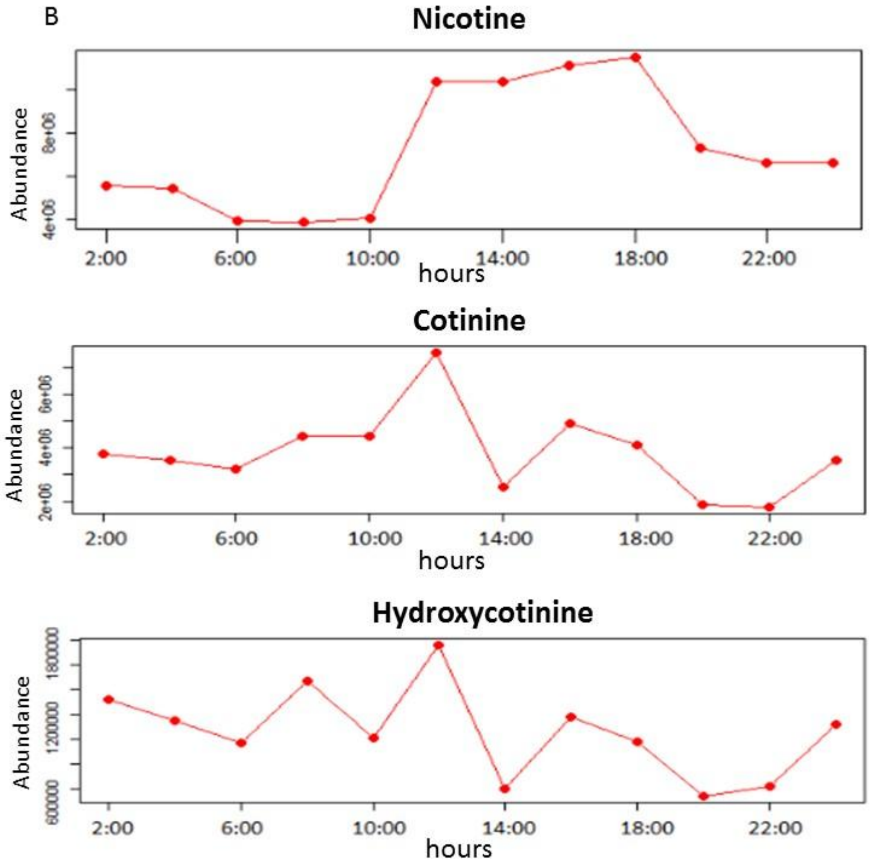

Figure 5. Intra-day concentration profiles of nicotine and related metabolites.

When comparing the intra-day trends between a week day and Saturday (weekend), there are interesting facts, concerning the use of some compounds during the weekdays and the weekends.

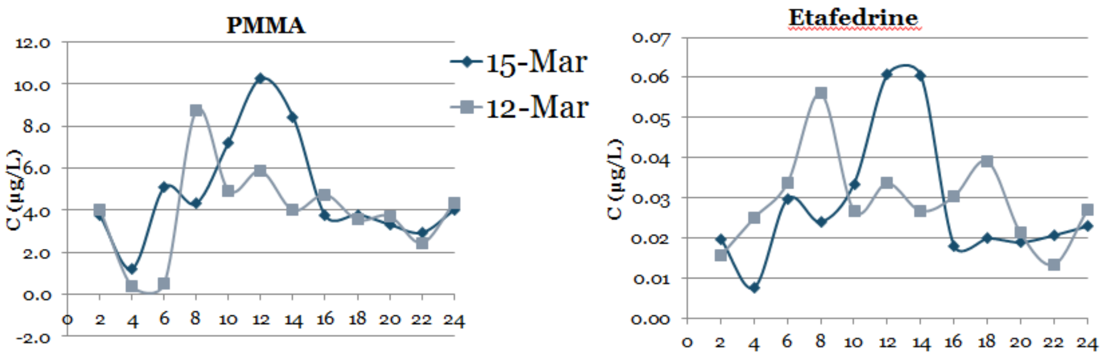

Figure 6. Intra-day concentration profiles of PMMA and etafedrine during a weekday (12-Mar) and Weekend (Sat. 15-Mar)

In Figure 6, we can observe that the peak concentration for PMMA and etafedrine, both stimulants, is on the weekday at 8 am, whereas on Saturday, the maximum concentration is observed at midday.

Similar conclusions were reached from the comparison of the intra-week concentration profiles among parent compounds and related metabolites. Very similar profiles were observed for the aforementioned metabolites of clarithromycin and venlafaxine and their corresponding parent compounds during 7 consecutive days (Figure 7a and 7b). Also good interrelations were found for the metabolites ranitidine-S-oxide and tramadol-N-oxide with their parent compounds (data not shown).

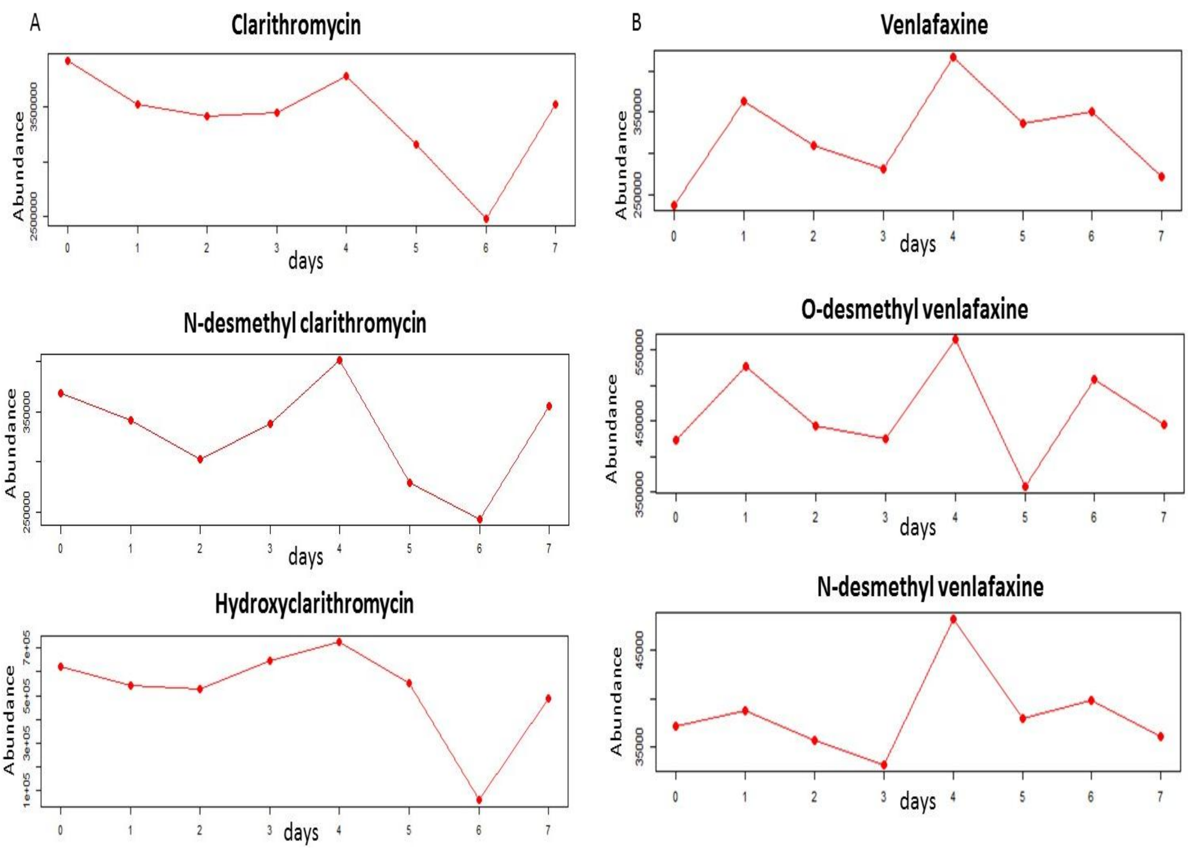

Figure 7. Intra-week concentration profiles of **(a)** clarithromycin and related metabolites and **(b)** venlafaxine and related metabolites

However there are cases, where no significant trend is observed among the days of the week, but it applies the same for the parent compounds and the metabolites, as in the case of carbamazepine (Figure 8).

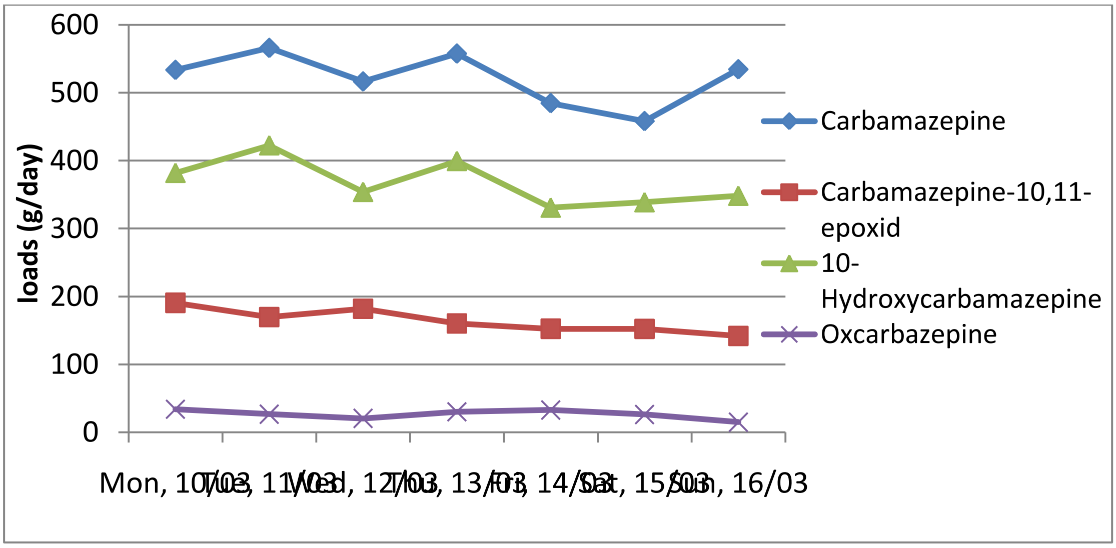

Figure 8. Intra-week concentration profiles of carbamazepine and related metabolites

Increased values were observed during the weekend, or on Friday for some analytes (Figure 9), such as for 2 C-D, a psychedelic drug, acting as a stimulant and a drug which serves as a supporting measure in treating depressant overdose, bemegride.

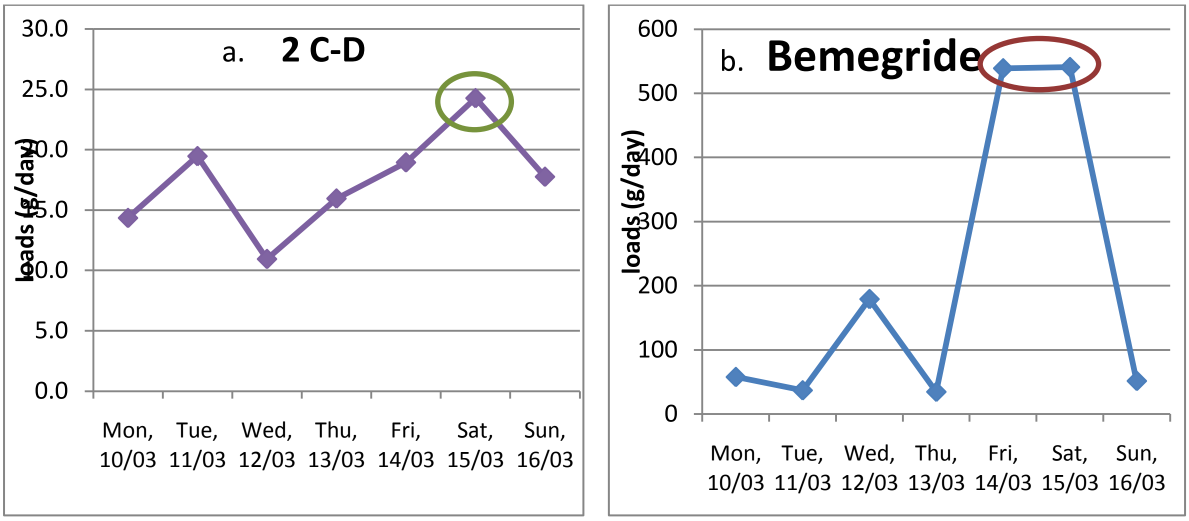

Figure 9. Intra-week loads profiles of **(a)**2 C-D and **(b)** bemegride

Monitor use of parent compounds and evaluation of metabolites as biomarkers

Population size and dynamics are important parameters in many human activities, including material-flow analysis in different environmental matrices and the per capita contributions of different pollutants to the environment "pollutant load per capita". This is particularly relevant for the "wastewater-based epidemiology" approach, which involves analysis of urban wastewater for the combined excretion products of different substances to track human habits and lifestyle. This approach was originally developed for the estimation of illicit drug consumption through wastewater analysis but can be extended to other applications, such as alcohol and nicotine. The rationale for this approach is based on the fact that almost everything we consume is excreted unchanged and/or as a mixture of metabolites in our urine and feces and ultimately ends up in the sewage network. Thus, the concentrations of metabolic residues in raw municipal wastewater can reflect the collective consumption of a substance in a community (Senta et al., 2015).

The use of caffeine, nicotine and some major metabolites was investigated by wastewater analysis and their suitability was tested as qualitative and quantitative biomarkers for assessing population health status.

These compounds were found widely at the mg/L level and mass loads were calculated by multiplying concentrations by the wastewater daily flow rate and normalized to the population served for the plant.

The criterion for selecting metabolites is the percentage of excretion of each metabolite. Thus for caffeine, paraxanthine, theophylline and theobromine were examined. The outcome is that in Athens, the consumption of coffee reaches 6.5 g caffeine /day/ 1000 inhibitants, whereas the upper limit is <200 mg/day, according to EFSA guideline. In Figure 10, the intra-day and intra-week trend of the consumption of caffeine is presented.

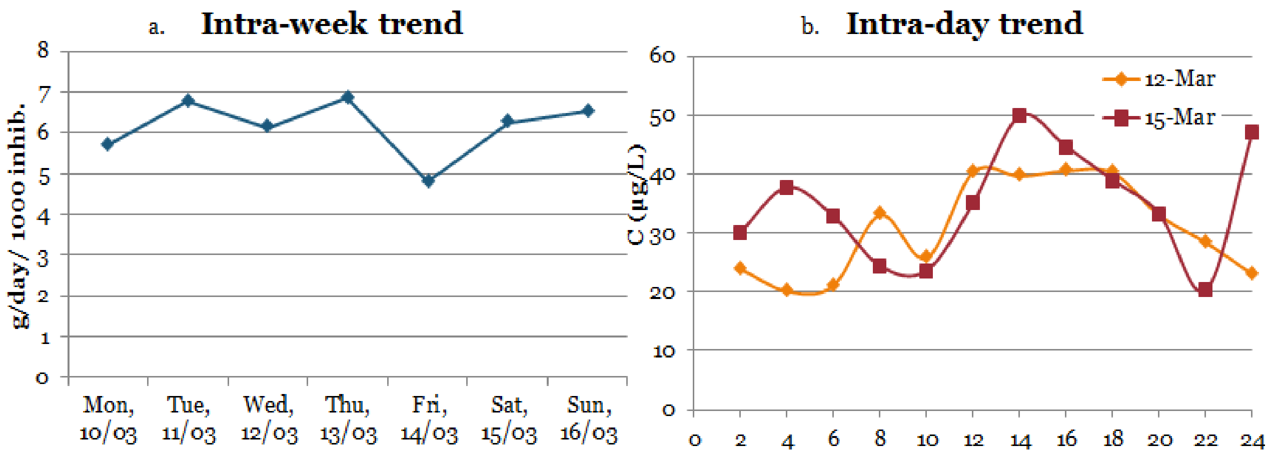

Figure 10. (a) Intra-week trend and **(b)** intra-day trend for a weekday (12-Mar) and weekend (Sat. 15-Mar) of caffeine

For nicotine, we selected two main metabolites excreted in urine: cotinine (30%) and 3-hydroxycotinine (44%). The profiles of cotinine and 3-hydroxicotinine in wastewater were very similar and reflected their human excretion profiles. From the mass loading of the metabolites and according to excretion rates, we come up to nicotine equivalents and by taking into consideration the mean content of nicotine absorbed from one cigarette, we can exclude the number of cigarettes smoked per day in Athens, that reach the number of 13.6 million. If we count for the average number of cigarettes smoked per smoker (21.4 cigarettes per day) (http://livingingreece.gr/2010/05/31/smoking-greece/), we can conclude in the number of 640,000 smokers in Athens.

Conclusions and Future Research

The sewage epidemiology application for illicit drug consumption monitoring could be used prospectively in a multi-disciplinary approach. It can give valuable information on using updated drug profile analyses to rapidly identify emerging hot spots of drug abuse. It can be of great help to test in real time the efficacy of different counter-measures such as prevention through education, enforcement, and global concerted actions against illicit drug consumption. Moreover it can cross-validate population surveys versus wastewater monitoring programs and it can assess the actual amount of illegal money involved in drug trafficking. If applied to other public health issues, this approach could potentially extract useful epidemiological data from qualitative and quantitative profiling of biological indicators entering the sewage system and might reopen the debate on what type of surveillance approaches are ethically and politically acceptable (van Nuijs et al., 2011).

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