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Reflection of socio-economic changes in wastewater: licit and illicit drug use patterns

Nikolaos S. Thomaidis[1,*], Pablo Gago-Ferrero[1], Christoph Ort[2], Niki C. Maragou[1], Nikiforos A. Alygizakis[1], Viola L. Borova[1], Marilena E. Dasenaki[1]

[1] Laboratory of Analytical Chemistry, Department of Chemistry, National and Kapodistrian University of Athens, Panepistimiopolis Zografou, 15771 Athens, Greece

[2] Eawag, Swiss Federal Institute of Aquatic Science and Technology, Überlandstrasse 133, CH-8600 Dübendorf, Switzerland

***Corresponding author:**
Tel: +30 210 7274317
Fax: +30 210 7274750
E-mail: ntho@chem.uoa.gr

ABSTRACT: The economic crisis plaguing Greece was expected to impact consumption of pharmaceuticals and illicit drugs – *a priori* to an unknown extent. We quantified the change of use for various classes of licit and illicit drugs by monitoring Athens' wastewater from 2010 to 2014. A high increase in the use of psychoactive drugs was detected between 2010 and 2014, especially for antipsychotics (35-fold), benzodiazepines (19-fold), and antidepressants (11-fold). This directly reflects the perceived increase of incidences associated with mental illnesses in the population, as a consequence of severe socio-economic changes. Other therapeutic classes, like antiepileptics, hypertensives and gastric and ulcer drugs also showed an increase in use (from 2-fold increase for antiepileptics to 13-fold for hypertensives). In contrast, the overall use of antibiotics and NSAIDs decreased. For mefenamic acid, an almost 28-fold decrease was observed. This finding is likely related to the reduction in drug expenditure applied in public health. A 2-fold increase of methamphetamine use was detected, associated with a cheap street drug called "*sisa*" (related to marginal conducts), which is a health concern. MDMA (5-fold) and methadone (7-fold) use showed also an increase, while cocaine and cannabis estimates did not show a clear trend.

Keywords: wastewater-based epidemiology, pharmaceuticals, illicit drugs, use patterns, wastewater analysis, socio-economic changes, crisis, Greece.

INTRODUCTION

The official ways to obtain figures about the use of licit drugs or pharmaceuticals and the use of illicit drugs are surveys integrated with medical records and drug production data. Although these tools provide a useful approximation, accurate estimates of use rates and drug use prevalence are difficult to perform. Furthermore, these approaches have several limitations, like the inclusion of substantial time lags that negatively impact the reliability, validity and utility of such data.[1] Another disadvantage is that, 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.[2]

Recently, an advanced approach based on data obtained from influent wastewater, has been proposed in order to get information on drug use. Parent compounds and metabolites, excreted after drug use are collected rapidly and pooled by the sewage systems. Determination of these substances in the influent of wastewater treatment plants (WWTP) provides data on the amount and type of drugs used by a population.[3] These data are used to back-calculate the mass loads of the parent drugs and/or metabolites and subsequently to estimate the drug use, provided that the drug metabolism and excretion patterns are known. 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. This methodology has been applied in several studies for back calculation of drug use.[4-9] Although the presence of these compounds has been widely studied, limited number of studies examines the correlation between the determined concentrations and social phenomena.[10-15]

Greece is amongst the most affected countries by the severe economic crisis plaguing Europe since 2008. In 2010, the Greek government requested a loan from the EU, which was the first request for a resave due to the impossibility of limiting the public debt. The financial crisis in Greece directly affected its population, in terms of growing unemployment rates (up to 30%), substantial decline in per capita income and significant increase in poverty. Another consequence was the significant decrease in the financing of the public sector, including

healthcare, which is now less than any of the other pre-2004 European Union members.[16,17] Some studies point to a change in the use of drugs, especially since in 2012 the Greek government cut drug spending.[16,18,19] 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 sewage-based epidemiology and quantify the effects of the economic crisis on the use of various classes of licit and illicit drugs. In particular this study aims to identify trends in the use patterns of licit and illicit drugs during the last 5 years (2010 – 2014) and investigate the potential correlation between these trends and the social parameters such as the increased unemployment rate, the decrease in the public health investments (drastically reduced by capping it at 6% of GDP) and the increase of mental illnesses attributed to the stress generated by the Greek crisis. So far, extensive studies of this type lasting such a long time period, including a large population and that high number of analyzed substances as in the present work have not been carried out.

In order to achieve the aforementioned objective, representative classes of the licit drugs used in modern society such as antidepressants, anxiolytics, antipsychotics, antibiotics, antiepileptics, analgesics, non-steroidal anti-inflammatory drugs (NSAIDs), diuretics, antihypertensives, antiulcers and steroids, as well as the main illicit drugs and their metabolites were considered. The concentrations of these substances in influents were monitored from 2010 to 2014 in Athens. It should be emphasized that the studied WWTP serves more than one third of the population of Greece and it is located in the largest metropolitan area where the effects of the crisis are most noticeable due to the higher cost of living and the weaker family support network. Applying methods of back-calculation to each of the substances, daily use values were estimated and results were discussed and correlated with the severe socio-economic changes.

MATERIALS AND METHODS

Chemicals and Reagents. 148 substances (licit and illicit drugs and related metabolites) were determined in this study using liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS). Target analytes include drugs of the following classes: antibiotics, NSAIDs, antilipidemics, antiepileptics, analgesics, antihypertensives, diuretics, steroids, antiulcers, anesthetics, benzodiazepines, antipsychotics, barbiturates, antidepressants (tricyclic, tetracyclic, selective serotonin re-uptake inhibitors (SSRIs), serotonin–norepinephrine re-uptake inhibitors (SNRIs)) and sympathomimetics, as well as illicit drugs such opiates, opioids and related metabolites, cocaine and related metabolites, amphetamines and hallucinogens (cannabinoids, lysergic acid diethylamide (LSD) and derivatives). Target analyte names, CAS numbers, molecular formulas, molecular weights, pKa and logKow values are listed in Table SI-1 (supporting information, SI-1). Procedures and used chemicals are also specified in section SI-1.

Sampling. 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. In each campaign, 24-hour composite volume proportional samples from influent wastewater were collected during consecutive days. In 2010 sampling was conducted during 7 days: from 5th of December to 11th of December, in 2011 during 8 days: from 3rd of April to 10th of April, in 2012 during 8 days: from 2th of April to 9th of April, in 2013 during 11 days: from 6th of March to 16th of March and in 2014 during 8 days: from 11th of March to 18th of March. This allows investigation of drug use trends throughout the week and over the five-year study period. It is considered that one-week periods are representative, mainly for substances that are consumed by a large proportion of the population and do not show high seasonal variation. Moreover, it is assumed that the sampling period does not affect the overall results, especially for antibiotics that show seasonal variation, since in Greece the period of flu and illnesses extents from November to April with peaks in December and February-March (http://www.keelpno.gr/, the report in Greek can be found here). Therefore, sticking to the same sampling period every year seems to be appropriate for comparing changes between years.

All wastewater samples were collected in pre-cleaned high-density polyethylene (HDPE) bottles. Untreated and treated wastewater was immediately filtered with glass fiber filters (pore size 0.7 μm) after arrival at the laboratory. Samples were acidified with a 1M HCl solution to pH 2.5 ± 0.10 to inhibit transformations and stored in the dark at -20°C until analysis (within one week at most). The conditions of the storage are according to the recent recommendations of a review article on the stability of illicit drugs in wastewater samples.[20] The stability was also checked by adding the labeled internal standards prior to the filtration and storage of the samples, in order to compensate for any loss and transformation.

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 rate (Q) 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.

Analytical Method. An analytical method for the determination of 148 substances based on solid phase extraction and further analysis by LC-MS/MS was used in this study. The method was developed by merging two previous existing methodologies for licit and illicit drugs developed in 2010 and 2011, respectively.[21,22] The samples from the first sampling campaign was analyzed with the first methodology[21] while all other samples were analyzed with the combined one. A detailed description of the analytical methodology, as well as the analytical performance details, is included in supporting information (SI-2). The method has also been validated through a continuous successful participation in an inter-laboratory study (SCORE 2015).[23]

Estimation of Drug Use. The approaches used to back-calculate drug use are based on the one developed by Daughton in 2001,[3] and put into practice for the first time by Zuccato et al., in 2005.[24] These methodologies use the levels determined for some selected compounds (parent or metabolites) in the WWTP influent water.

Licit drug uses were back-calculated following the procedure proposed by Yan et al.[25] The drug usage, back-estimated from the influent wastewater samples, was calculated according to Equation 1.

$$U\left(g\ day^{-1}\right) = \frac{C_{\text{influent}} \times Q \times 10^{-6}}{R_{abs} \times R_{excreted} - R_{abs}} \times \frac{P_T}{P_S} \qquad \text{(Equation 1)}$$

where U represents the back-estimated usage of the target drug (g day^{-1}); $C_{influent}$, (ng L^{-1}) is the measured concentration in the influent and Q (m^3 d^{-1}) is the flow rate of wastewater measured each day; $R_{excreted}$, and R_{abs} refer to the percentage of the parent drug excreted and the absorption rate of the drug (bioavailability), respectively; P_S and P_T refer to the served population by the studied WWTP and the total population of Athens, respectively.

The use rates of all licit drugs were calculated from the concentration of the parent compounds. This procedure was applied for the substances with bioavailability (R_{abs}) and excretion-rate ($R_{excreted}$) data available, listed in Table SI-3A (supporting information SI-3). In the same table the typical oral doses, D (g $dose^{-1}$) are provided. The typical oral doses (D) were used in order to calculate and express the drug use as U (doses day^{-1}), according to Equation (2). The use rates generated using the typical doses and expressed as doses day^{-1} are subjective to a higher degree of uncertainty compared to the use rates expressed as g day^{-1}, since multiple values of doses of a pharmaceutical may be prescribed. However, it is noted that the relative patterns to compare different years are the same irrespective of using loads (g day^{-1}) or consumption (doses day^{-1}).

$$U\left(doses\ day^{-1}\right) = \frac{U\left(g\ day^{-1}\right)}{D\left(g\ doses^{-1}\right)} \qquad \text{(Equation 2)}$$

In the case of illicit drugs, different drug residues were selected as analytical targets to back-calculate consumption. Table SI-3B (supporting information SI-3) shows the selected target analytes for each compound as well as the relation of this compound with the parent drug, the excretion information and the molar mass ratio between the parent drug and the selected analyte. The procedure used to back-calculate the use of these substances is described in detail by Zuccato et al.[5] For all illicit drugs, the compound with highest excretion was selected for back-calculation. For amphetamine, methamphetamine and MDMA (ecstasy), this is the parent compound itself. In other cases the major metabolite was selected, like benzoylecgonine for cocaine, 11-nor-9-carboxyl-Δ^9-tetrahydrocannabinol for THC and morphine for heroin. For morphine, it is necessary to consider that other sources can contribute to the total load, such as the therapeutic use of morphine itself and codeine. For the calculations of heroin use in the present study, codeine-derived morphine was subtracted from the total morphine, assuming that 10% of the used codeine is excreted as morphine.[26] The therapeutic use of morphine was not considered and this may contribute to an overestimation of the absolute values of heroin use. A more detailed discussion is presented in Section "Weekly trends and variations in the use of illicit drugs from 2010 to 2014".

The following assumptions were made for the back-calculation: i) no sewage loss in the sewer system, ii) no relevant transformation and negligible adsorption to particulate matter of targeted substances, iii) no direct drug disposal into the sewage system and iv) no substantial changes of population over the study period. Absolute numbers could change if some of these assumptions were not entirely valid, i.e. systematic shifts (scaling), but no changes in relative patterns. This implies that the statistical analysis of trends is not affected. Furthermore, random uncertainties – from e.g. sampling – are included in the daily variability of the data (error bars in Figures 1 and SI-4A).

RESULTS AND DISCUSSION

Use of Pharmaceuticals During 2010-2014. Measured concentrations including limits of detection (LOD) and number of samples > LOD are listed in Tables SI-4A-E (Supplementary Information SI-4). 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 some cases above 200 μg L^{-1}. Figure 1 shows the calculated consumption as number of doses day^{-1} for different classes of drugs, along with the associated daily variability of each investigated year. This variability is caused by natural variability (true variability) and sampling uncertainty. Uncertainty due to chemical analysis can be found in Table SI-2B (% RSDs, typically less than 25% for the majority of the compounds and it is included in the overall variability). Uncertainty from other sources (flow measurements, back-calculation) are not reflected. However, they can be assumed to be constants (i.e. not changing from one year to another) and, therefore, they affect the results in a systematic manner, which does not impact the trend assessment over the years. Only random uncertainties seem important for the trends analysis and these are included in the daily variability represented in both figures (Fig.1 and Fig.SI-4A) and considered in the statistical tests (Table SI-4F and G). Only drugs for which data on bioavailability and excretion rates were available were included (see Table SI-3A). For all the substances, Figure SI-4A shows the changes in mean loads measured in the sewer in g day^{-1}. Only substances that were detected in over 80% of the samples in at least two of the sampling periods were considered for evaluating trends. The patterns of uses for the various therapeutic classes of monitored compounds between the different sampling periods are discussed in detail.

Benzodiazepines, Antidepressants and Antipsychotics. Variations in the use of benzodiazepines anxiolytics and various types of antidepressants such as Tetracyclic Antidepressants (TeCAs), Selective Serotonin Reuptake Inhibitors (SSRIs) and Serotonin–Norepinephrine Reuptake inhibitors (SNRIs) are shown in Figure 1A. A pronounced increase of the sum of the 4 antidepressants and the 2 benzodiazepines was observed: from approx. 36,000 in 2010 to 500,000 doses day^{-1} in 2014 for the six substances, with a remarkable increase for all

six compounds individually (oxazepam and citalopram 10-fold). The observed increase of paroxetine, a widely used antidepressant is also noteworthy (reaching values up to 96,833 doses day^{-1} (1,937 g day^{-1}). For compounds of these therapeutic classes for which data on excretion and increased bioavailability were not available (e.g. sertraline/norsetraline, amitriptyline, bromazepam, temazepam, alprazolam or doxepine), an increase of loads in the same order of magnitude was observed (Figure SI-4A). The detected levels for the studied antipsychotics (e.g. clozapine/norclozapine, olanzapine) followed the trend of the other psychiatric drugs and showed a substantial increase every year from 2010 to 2014 (Figure SI-4A, data on excretion and bioavailability were not available).

Antihypertensives and gastric and ulcer drugs. Another class of drugs with an increase during the period studied were the antihypertensives (Figure 1B), mainly caused by a large increase of valsartan from 25,988 doses day^{-1} (8,316 g day^{-1}) in 2011 peaking in 2013 with over 625,000 doses day^{-1} (>200 kg day^{-1}) followed by a slight decrease in 2014. A similar but less pronounced pattern was found for the gastric and ulcer drugs (Fig. 1C). A significant increase in use of ranitidine during the study period was observed, from 7,607 doses day^{-1} (1,141 g day^{-1}) in 2011 to values >20,000 doses day^{-1} (3,040 g day^{-1}) in 2013 and 2014 (Figure 1C).

Antiepileptics. Regarding the use of antiepileptics, an overall increase was observed (Figure 1D). Examining the individual compounds of this class, a decrease in use of carbamazepine was noted. Carbamazepine is one of the most widely prescribed and very important compound for the treatment of epilepsy, neuralgia and some psychiatric diseases, such as bipolar affective disorder. However, this was more than compensated by the increase of valproic acid use, a compound with very similar applications, 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 significant increase was also observed for lamotrigine, reaching 15,180 doses day^{-1} (3036 g day^{-1}) in 2014.

Antibiotics. The use of antibiotics, which are among the most frequently prescribed drugs for humans in modern medicine, showed about a 25%-decrease during the study period (Figure 1G). More than a ten-fold decrease was 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 a 30% decrease 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 agreement with other investigations conducted in raw wastewaters in other countries.[27] Reduced loads of norfloxacin, sulfamethoxazole and trimethoprim were also observed, although to a lesser extent. Only in the case of metronidazole, an increase in use was observed in 2014 with 3,834 doses day^{-1} (1,917 g day^{-1}) comparing to the three previous years (1,784 doses day^{-1} or 892 g day^{-1} in 2011). This overall decrease in the loads of antibiotics could be attributed to the significant decrease in the financing of the public health and to an attempt to reduce the excessive use of this class in Greece, comparing to the other countries.

Non-steroidal anti-inflammatory drugs (NSAIDs). NSAIDs were the substances detected at the highest concentrations and with the highest use rates (Figure 1H). Most of the compounds of this class did not demonstrate a noticeable trend, except for mefenamic acid, for which a high decrease was observed in its use, contributing to the overall decrease of NSAIDs during the studied period. Ibuprofen and ketoprofen also showed a decrease in use whereas an increase was only observed for naproxen, indicating a change in the prescription and sales of these substances (Figure 1H and Table SI-4G). 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), favoring its high use.[28] As in the case of antibiotics, the observed decrease in non-steroidal anti-inflammatory drugs could be associated with the limited financing of the public health and to the reduced income of consumers.

Diuretics and antilipidemics. The sum of antilipidemics, atorvastastin and the diuretic hydrochlorthiazide show a noticeable increase in 2014, even though the variations of use of the individual compounds are not consistent (Figure 1E). 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 also observed for hydrochlorthiazide, 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. Furosemide presented a trend similar to hydrochlorthiazide, as demonstrated by the loads graph (Figure SI-4A).

Figure SI-4A also demonstrated the increase in use of methylprednisolone, lidocaine and the overall increase of theophylline between 2010 and 2014, a drug associated with the cure of asthma. No specific trend is observed for paracetamol, ephedrine and caffeine.

Weekly Trends and Variations in the Use of Illicit Drugs during 2010-2014. Figure 1F shows the calculated consumption as grams day^{-1} for the investigated illicit drugs, along with the associated daily variability of each investigated year. Figure SI-4B illustrates the daily use (g day^{-1}) of the investigated illicit drugs for each day of the week between 2010 and 2014. Table 1 summarizes the weekly drug use (kg) and the percentage of users within the population per year for the same period, based on the average dose and for a population of 3,700,000 people.

Cocaine, ecstasy and amphetamine. In the case of ecstasy (Figure SI-4B), a sharp increase in use during the weekend was detected in all the monitored periods, 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 use of ecstasy experienced a significant increase during the studied years (Figure 1F). The estimated use of this substance for 2014 is 0.17 kg $week^{-1}$, while for 2010 the corresponding value is only 0.039 kg $week^{-1}$.

A similar weekly pattern was observed for cocaine (Figure SI-4B), 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, increased use during weekdays was also detected. Regarding this substance, it is noteworthy that high values observed on Mondays, reflects a very high intake of this substance also on Sundays. No particular trend over the studied years was observed for cocaine use (Table 1). 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}). The weekend use of these substances is supported by the European Monitoring Centre for Drugs and Drug Addiction.[29]

Regarding amphetamine (Figure 1F), daily variations throughout the week were not noticeable in the last four sampling campaigns. However, a weekly pattern close to that of ecstasy and cocaine is observed for the first sampling period of 2010 (Figure SI-4B). It is speculated that this could be related to the recreational use of this drug in 2010, which has decreased in the following years. 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. The highest weekly use for amphetamine was estimated for 2010 (2.73 kg week^{-1}) while lower values were estimated for the following years (Table 1).

Cannabis. The case of cannabis use is noteworthy, without any particular trend among the years (Figure 1F and Table SI-4F). Although some studies showed an increase in its use during the weekends,[11] most of them did not report weekly patterns.[7,30] In the present study, a correlation between use and the specific day of the week was not clearly observed (Figure SI-4B). Use of THC was not constant but varied significantly without following a specific pattern; a reason behind its high variability maybe the known analytical difficulties and problems with its stability and, therefore, the cannabis data should be used with caution.[20] An exception was the results for Friday and Saturday in the monitoring campaign of 2013 (Figure SI-4B). The high values reported in those days could be probably attributed to the proximity of the Carnival of Athens, which may be strongly related to a higher use of this substance. This assumption is in agreement with the suggestion that the pattern of use of illicit drugs could vary considerably from one country (or city) to another.[7,29] A slight increase of cannabis use is observed over the studied years, from 211.6 kg week^{-1} in 2011 to 265.5 kg week^{-1} in 2014 (Table 1), however it was not statistically significant (Table SI-4F). The higher estimated use in 2013 (382.9 kg week^{-1}), could be related to the proximity of the Carnival of Athens, as already mentioned.

Heroin, methamphetamine and methadone. The "street" drugs of abuse, heroin and methamphetamine are more related to marginal conducts. These illicit drugs, as well as methadone, exhibited a more constant use throughout the week (Figure SI-4B), with patterns clearly different from that of recreational drugs whose use strongly increases during weekends (e.g. cocaine, ecstasy).

A noticeable increase of methamphetamine and of methadone use is observed over the years 2010 – 2014 (Figure 1F). The weekly estimated use of methamphetamine increased from 0.14 kg week^{-1} in 2010 and 2011 to values above 0.28 kg week^{-1} in 2012 and onwards (Table 1). Methadone use increased from 0.14 kg week^{-1} in 2010 to 0.97 kg week^{-1} in 2014 (Table 1).

On the contrary, no particular trend over the years can be defined for heroin abuse (Table 1). As already reported the amount of morphine due to codeine use has been taken into account for the calculation of heroin use. The therapeutic morphine itself, which may represent a significant portion of the total amount of this substance in wastewater, was not taken into account due to unavailable prescription data. In order to address this issue, back-calculation from a minor metabolite (6-MAM) was suggested, however concentrations obtained for this compound were below or near the limit of quantitation in many cases (due to its instability in wastewater and very small excretion rate), and finally was not be used. However, detection frequency and the levels of 6-MAM increased steadily over the years, as depicted by the loads trends (Fig. SI-4A), which is indicative of a marginal increase in the use of heroin. Not considering the therapeutic use of morphine contributes to an overestimation of the absolute values of 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%). Furthermore 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).

Effects of Severe Socio-economic Changes on the Estimated Use Patterns of Licit and Illicit drugs. The effects of the economic crisis in Greece during the studied period

were very sharp and had a direct impact on its population. Figure SI-5A (supplementary information, SI-5) shows the variation in Gross Domestic Product per capita (GDP) from 2010 to 2013, where a significant decrease in this indicator is observed throughout this period. This drop in GDP involves a significant loss of purchasing power of citizens and, in the case of Greece, an important increase in public debt that has resulted in significant cuts in the public sector. Among the most important cuts was the reduction of public health expenditure, which can be viewed in Figure SI-5B (supplementary information, SI-5). The public hospital budget was reduced by 26% between 2009 and 2011[31] and one stated aim was to reduce the drug expenditure from 4.37 billion euro in 2010 to 2 billion euro by 2014.[16,32] An additional adverse consequence was the steady increase in unemployment, which is shown in Figure SI-5C, which is over 25% in 2013. Significantly severe unemployment is recorded for young people, with rates close to 60% (Figure SI-5D). These socio-economic indicators were correlated by non-parametric Spearman Rank Order Correlations with the sum of each licit drug class and the individual illicit drug use data (Table SI-5).

All the socio-economic indicators were highly correlated with the sum of antidepressants and benzodiazepines (Table SI-5). Unemployment, the worsening economic situation and the impact of high indebtedness of the population can cause or aggravate mental health problems,[33] which is transformed into an increase in the use of the associated drugs. This seems evident from the sharp increase of the use of benzodiazepine anxiolytics and various types of antidepressants. The cases of oxazepam or citalopram are noteworthy, with use rates more than twenty times higher in both cases, as the crisis gets more severe. Equally important is the overall increase in the use of antidepressants during the same period. Results obtained for benzodiazepine anxiolytics and antidepressants as well as for the detected antipsychotics and other psychiatric drugs are in agreement with findings from population surveys suggesting a high increase of the prevalence of major depression from 2008 to 2011, with economic hardship being a major risk factor.[34] Another study reported an increase of 36% in the number of people attempting suicide in the month before the survey.[35]

Economic crisis may also be the main factor in the increase of the use of both antihypertensives and anti-ulcer drugs. Both sums were highly correlated with all socio-economic indicators of the study period (Table SI-5). Hypertension and gastric ulcer are strongly associated with an increased level of stress, even though there are more factors associated. Therefore the increase in the use of these drugs could be related to socio-economic changes experienced by the studied population.

An overall increase of the use of antiepileptics is also observed and it is highly correlated with the socio-economic indicators (Table SI-5). It is noted, however, that the individual substances present different trends over the years. Based on these findings it could be assumed that modifications in the prescription inside one therapeutic class might have occurred.

Results showed a decrease in the use of antibiotics and NSAIDs. This change seems to be highly related with the reduction of public health spending (Table SI-5), especially to that related to the drug expenditure cuts that Greece has experienced recently. It is known that the Greek public health system has suffered a severe and continued cut in drug expenditure during the studied period. The exception to this trend can be found in metronidazole. The higher use of this compound during the studied period may be related with the overall increased incidence of *Clostridium difficile* infections.[36,37]

In view of the results obtained, changes in socio-economic conditions seem to have affected the use patterns of illicit drugs to a minor degree, even if some strong correlations are recorded in the Spearman ranking correlation. An example is cocaine, which use remained relatively constant. Using the data obtained in the present study, the estimated percentage of population under use per year was 0.124 % (Table 1), which is in agreement with the value of 0.2 % that is published in the European Drug Report for the estimation of population between 15 and 34 years old.[29] Possibly, the reported decrease in the use of amphetamine may be related to its use as a medicine to treat various diseases. Nevertheless, in terms of its use as a recreational drug, the decline in use may be related to the increase in the use of ecstasy, a recreational drug used in similar environments and circumstances which seemed to gain popularity in recent years.

Cannabis use did not show any particular trend over the studied years. The estimated percentage of population under use per year was 10.9 % in 2014, far from the value published in the European Drug report, 3.2 %.[29] This high discrepancy may be attributed to analytical uncertainties and difficulties in the determination of THCA. However, another factor that may have had great influence on the increase in the use of this substance in 2014 (and other recreational drugs) is the change in legislation that occurred in 2011. Until then, the Greek legislation was one of the most severe, where drug use and possession for personal use, even in small amounts, was punishable by up to five years in prison. It is likely that this legislation was associated with lower use of this substance compared to most European countries.[29] Currently, the legislation is less strict and probably it has an effect on the increase in cannabis use. Overall, the results show that the use of recreational drugs has not been severely affected by the effects of the economic crisis, since the use of most substances was kept generally constant (cocaine) or increased (MDMA). However, the obtained data does not allow us to draw sound conclusions.

Regarding the drugs associated to marginal conducts, whose use is governed by factors other than those for recreational drugs, the high increase in the use of methamphetamine is remarkable, associated with the spread use of "sisa". "Sisa" is a street methamphetamine preparation found in Greece and originating in Athens. Its use has increased significantly due to its low price, which can range from three to five euros per dose, well below other drugs such as heroin, making it more accessible to marginalized groups with few resources. Increased use of this drug seemed closely linked to socio-economic changes (Table SI-5), resulting from the economic crisis in the country. The use of this drug is of particular concern, because of the serious and rapid effects it has on health, which may lead to death of the user in three months. The high increase observed in the methadone use seems to be strongly related to a change in legislation in 2011, establishing the administration of methadone in hospitals and not in small independent treatment centers.

In conclusion, the results of this study denoted indirectly an increase of mental illnesses in the studied 3.7-million population, since significant increase of psychiatric drugs have observed

during the years of sever socio-economic changes in Greece. The overall use of antibiotics and NSAIDs (mostly mefenamic acid) has decreased, probably due to the cuts applied in the public health spending. Also the increase in the use of illicit drugs associated to marginal conducts (e.g. methamphetamine) was strongly correlated to the adverse socio-economic changes in the country during the study period. These conclusions are in line with the arguments of other studies claiming that although the adverse economic effects of austerity were miscalculated, the social costs were ignored, with harmful effects on the people of Greece.[16]

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ASSOCIATED CONTENT

*Supporting Information: Additional information as noted in the text (chemicals; sample preparation and instrumental parameters; validation data; Detailed analyses results for 5 consecutive years; load graphs for all the substances; statistical analyses results; and Greek socio-economic indicators from 2010 to 2014). This material is available free of charge *via* the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Phone: +30-210-7174317; Fax: +30-210-7274750; E-mail: ntho@chem.uoa.gr

Notes

The authors declare no competing financial interest.

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Table 1: Weekly drug use and percentage of users within the population 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.*

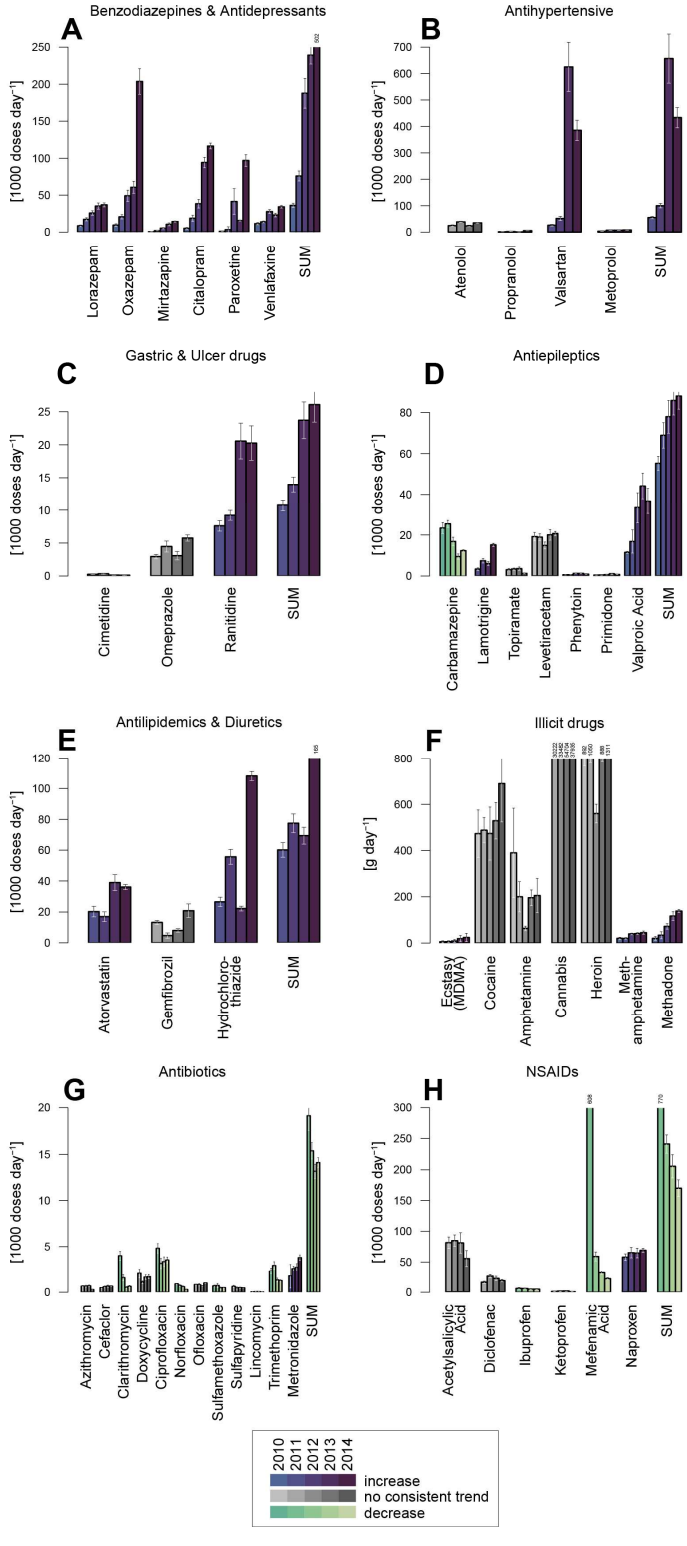
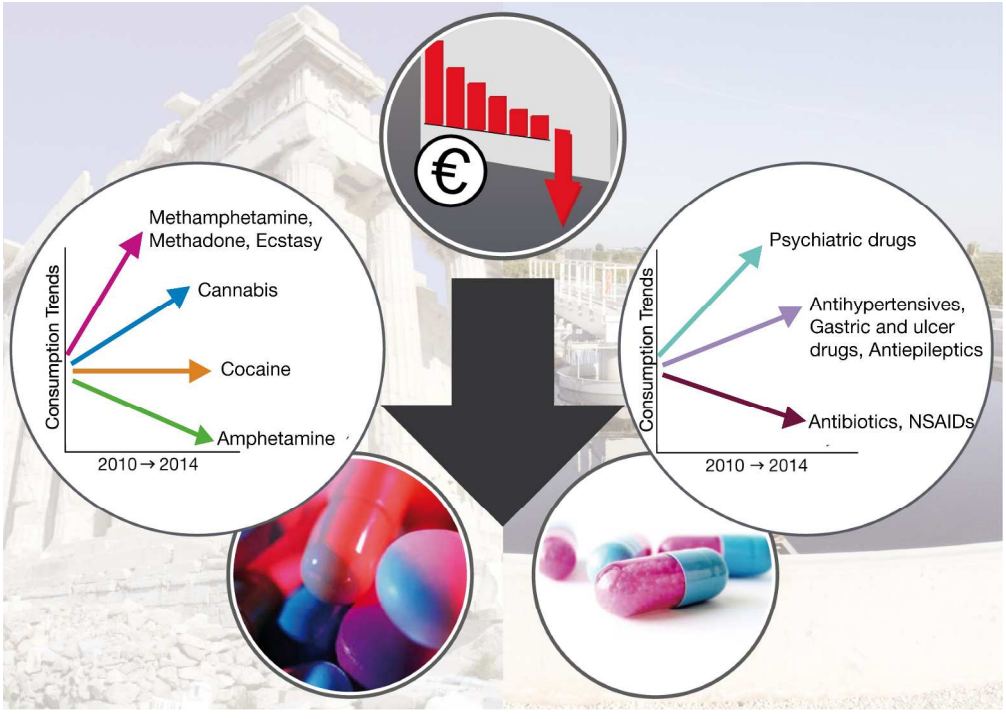


FIGURE 1: Estimated use (doses day^{-1}, ×1000) in Athens for selected pharmaceuticals through the five years investigation (2010-2014). Error bars represent variability of daily values within the x-day period investigated per year.



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