Application of functional data analysis (FDA) to weekly wastewater data

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TABLE OF CONTENTS

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ABBREVIATIONS

ADHD	Attention Deficit Hyperactive Disorders
AIC	Akaike's Information Criterion
ANOVA	Analysis of Variance
AUC	Area Under the Curve
BOD	Biological Oxygen Demand
CI	Confidence Interval
COD	Chemical Oxygen Demand
EMCDDA	European Monitoring Centre for Drugs and Drug Addiction
FANOVA	Functional Analysis of Variance
FDA	Functional Data Analysis
FPC	Functional Principal Component
FPCA	Functional Principal Component analysis
GAM	Generalized Additive Models
GLM	Generalised Linear Models
GCV	Generalized Cross Validation
GDP	Gross Domestic Product
LOD	Limit of Detection
LOQ	Limit of Quantification
NPS	New Psychoactive Substances
PC	Principal Component
PCA	Principal Component Analysis
POCIS	Polar Organic Chemical Integrative Sampler
SD	Standard Deviation
STP	Sewage Treatment Plant
WBE	Wastewater-based Epidemiology
WPC	Wavelet Principal Component
WPCA	Wavelet Principal Component Analysis
WWA	Wastewater Analysis
WWTP	Wastewater Treatment Plant

24h	24 hours

SUMMARY OF THESIS

Background: Wastewater-based epidemiology (WBE) is a novel approach in drug use epidemiology, which may provide more objective estimates of illicit drug use in a community. Simple summary statistics and specification tests have typically been used to analyse WBE data, comparing possible differences between weekday and weekend loads. Such standard statistical methods may, however, overlook important nuanced information in the data. Functional data analysis (FDA) is a statistical framework specifically developed for analysing curve data, and could potentially increase information extraction from temporal WBE data.

Aims: The overall aim of this thesis was to explore the possibility and usefulness of applying advanced statistical methods to WBE data to extract more information on the weekly temporal pattern of drug loads in European cities compared to more traditional statistical methods. We also compared various advanced statistical methods and investigated the possibility of using FDA to distinguish between what could be considered the proper medical use and the recreational use of prescription drugs.

Methods: Raw sewage samples were collected from sewage treatment plants (STPs) for each city over the observational period. Samples were time- or volume-proportional and the concentrations of the investigated drugs were measured by analysing the selected drug excretion residues (target residues) in wastewater. In all papers, the main temporal features of the selected drugs were extracted using functional principal component (FPC) analysis. In papers I and II the individual cities' scores on each of the temporal FPCs were then used as outcome variables in multiple linear regression analysis with various city and country characteristics as predictors. In paper III the weekly temporal patterns of the drug were further investigated comparing FPCA using both Fourier and B-spline basis functions with three different smoothing parameters, along with traditional principal component analysis (PCA) and wavelet-PCA (WPCA) with different mother wavelets and shrinkage rules. The stability of FPCA was explored through bootstrapping and analysis of sensitivity to missing data. In paper IV the weekly component for each drug over the month was extracted using generalized additive models (GAM) with

cyclicity modelled by trigonometric functions, before applying functional principal component analysis (FPCA).

Results: The three first FPCs explained more than 99% of the temporal variation in all analyses. The first component (FPC1) represented the level of the drug load, while the second and third temporal components represented the level and the timing of the weekend peak/peaks. AUC was highly correlated with FPC1, but other temporal characteristics were not captured by simple summary measures. Functional analysis of variance (FANOVA) was less flexible than the FPCA-based regression, and even showed concordance results. The extracted temporal features using PCA, FPCA and WPCA were consistent, but FPCA with Fourier basis and common-optimal smoothing was the most stable and least sensitive method to missing data. The second FPC (FPC2) was the most important temporal feature when investigating the recreational use of prescription drugs.

Conclusions: The findings show that using FDA on WBE data extracts detailed information about drug load patterns during the week which are not identified by more traditional statistical methods. Regression based on FPC results is a valuable addition to FANOVA for estimating associations between temporal patterns and covariate information. Moreover FPCA is a flexible and analytically tractable method for analysing temporal changes in WBE data, and is robust to missing data, but FPCA with Fourier basis functions and common-optimal smoothing parameter is the most accurate approach when analysing WBE data.

SAMMENDRAG [Norwegian]

Backgrunn: Epidemiologi basert på avløpsvann (EBA) er en ny metode innen forskning på bruk av narkotiske stoffer, og kan bidra til mer objektive beregninger av mengden rusmidler som benyttes i et område. Enkel deskriptiv statistikk og signifikanstester har vanligvis blitt brukt til å analysere data fra EBA, f.eks. gjennom å sammenligne mengder målt i avløpsvannet på henholdsvis ukedager og i helgene. Slike enkle statistiske metoder kan imidlertid overse viktig nyanser i dataene. Funksjonell dataanalyse (FDA) er et statistisk rammeverk spesielt utviklet for å analysere kurvedata, og kan potensielt øke informasjonsmengden fra EBA-data.

Formål: Det overordnede målet med avhandlingen var å undersøke hvorvidt mer avanserte statistiske metoder, spesielt FDA, kunne hente ut mer informasjon fra EBA-data. Vi brukte FDA for å avdekke mønstre i de ukentlige data for mengden narkotiske stoffer målt i avfallsanlegg ulike steder i Europa, og sammenlignet dette med resultatene fra mer tradisjonelle statistiske metoder. Ulike avanserte statistiske metoder for temporale data ble også sammenlignet. Til sist har vi brukt FDA for å undersøke om vi kan påvise rekreasjonsbruk (misbruk) av reseptbelagte legemidler.

Metode: Prøver fra kloakken ble samlet inn fra kloakkrenseanlegg for ulike byer i løpet av en observasjonsperiode. Prøvene var tids- eller volumproporsjonale, og konsentrasjonene av de undersøkte medikamentene ble målt ved å analysere utskilte rester eller metabolitter av utvalgte medikamenter i avløpsvannet. I alle artiklene har de viktigste tidsmessige trekkene ved de utvalgte legemidlene blitt hentet ut ved hjelp av funksjonell prinsipalkomponentanalyse (FPCA). I artikkel I og II ble de enkelte byenes skårer på hver av de temporale hovedkomponentene, de funksjonelle prinsipalkomponentene (FPCene) brukt som avhengig variabel i multippel lineær regresjonsanalyse med diverse by- og nasjonsegenskaper som forklaringsvariabler. I artikkel III ble de temporale ukemønstrene i bruken av stoffene undersøkt videre ved å sammenligne resultatene fra FPCA ved å benytte både Fourier og B-spline basisfunksjoner med tre forskjellige glattingsparametere, sammen med tradisjonell prinsipal komponentanalyse (PCA) og wavelet-PCA (WPCA) med ulike mor-wavelets og krympingsregler. Stabiliteten til FPCA på avsløpsvanndata ble utforsket ved hjelp av

bootstrapping og sensitivitetsanalyse for missing. I artikkel IV ble den gjennomsnittlige ukekomponenten for flere legemidler i løpet av en måned hentet ut ved hjelp av en generalisert additive modell (GAM) med ukemønsteret modellert ved hjelp av trigonometriske funksjoner, og FPCA benyttet videre på dette.

Resultater: I alle artiklene forklarte de tre første FPCene mer enn 99% av den temporale variasjonen mellom de ulike byene i dataene. Den første komponenten (FPC1) representerte i hovedsak nivået av medikamentbelastningen, mens den andre og tredje komponenten representerte nivået for og tidspunktet til en topp i løpet av helgen. Arealet under kurven (AUC) var sterkt korrelert med FPC1, mens andre temporale karakteristika (FPC2 og 3) ble ikke fanget opp av enkle statistiske analyser. FPC2 var det viktigste temporale karaktertrekket når man undersøkte rekreasjonsbruk av reseptbelagte legemidler. Funksjonell analyse av varians (FANOVA) var mindre fleksibel enn den benyttede FPCA-baserte regresjonsanalysen, men viste ellers tilsvarende resultater. De temporale mønstrene hentet ut ved hjelp av PCA, FPCA og WPCA var i overenstemmelse med hverandre, men FPCA med Fourier basis og felles glatting var den mest stabile metoden.

Konklusjon: Arbeidene viser at ved å bruke FDA på data fra EBA henter man ut langt mer detaljert informasjon om bruk av rusmidler i løpet av en uke enn det som kan identifiseres med mer tradisjonelle statistiske metoder. Resultatene viser videre at regresjonanalyser basert på FPC-komponenter gir verdifullt tilleggsinformasjon utover det FANOVA kan gi for beregning av assosiasjoner mellom tidsmønstre og forklaringsvariable. FPCA er en fleksibel og analytisk metode for å analysere temporale variasjoner i data fra EBA, og er robust ved missing. FPCA med Fourier basisfunksjoner og felles optimal glattingsparameter anbefales når man skal analysere data fra EBA.

LIST OF PAPERS

I. Salvatore S, Bramness JG, Reid MJ, Thomas KV, Harman C, Røislien J (2015). *Wastewater-Based Epidemiology of Stimulant Drugs: Functional Data Analysis Compared to Traditional Statistical Methods.* PLoS ONE 10(9): e0138669. DOI: *10.1371/journal.pone.0138669*

II. Salvatore S, Frøslie KF, Røislien J, Zuccato E, Castiglioni S, Bramness JG. *A nuanced picture of illicit drug use in 17 Italian cities through functional principal component analysis of temporal wastewater data.* Journal of Public Health, 24(3), 165-174. DOI: *10.1007/s10389-016-0717-8*

III. Salvatore S, Bramness JG, Røislien J: *Exploring functional data analysis and wavelet principal component analysis on ecstasy (MDMA) wastewater data.* BMC Medical Research Methodology (accepted).

IV. Salvatore S, Røislien J, Baz-Lomba JA, Bramness JG: *Functional principal component analysis (FPCA) for analysing wastewater data as a method of investigating whether medicinal drugs are abused* (submitted).

The following papers are not included in the thesis, but are based on the same material:

- van Wel JHP, Kinyua J, van Nuijs A, Salvatore S, Bramness JG, Covaci A, van Hal G: *A comparison between sewage-based epidemiology and epidemiological research in a selected community*. International Journal of drug Policy. [Early Online]. DOI: *http://dx.doi.org/10.1016/j.drugpo.2016.04.003*

- van Wel JHP, Gracia-Lor E, Kinyua J, van Nuijs ALN, Salvatore S, Castiglioni S, Bramness JG, Covaci A, van Hal G: *A comparison between wastewater-based epidemiology and epidemiological survey research on alcohol and tobacco use in a selected community*. Drug and Alcohol Dependence 162 (2016) 170–175. DOI: *doi:10.1016/j.drugalcdep.2016.03.002*

- Baz-Lomba JA, Salvatore S, Garcia-Lor E, Ryu Y, Castiglioni S, Bramness JG, Thomas KV: *Comparison of pharmaceutical, illicit drug, alcohol, nicotine and*

caffeine levels in wastewater with sale, seizure and consumption data for 8 European cities (submitted).

1. INTRODUCTION

1.1 Background

According to the EMCDDA's latest report on the trends and developments of illicit drugs in Europe [1], approximately a quarter of the adult European population has, at some point in their lives, tried an illicit drug. It has been estimated that 78.9 million Europeans have tried cannabis at least once during their lifetime, 15.6 million cocaine, 12.3 million MDMA and 12.0 million amphetamines. The use of heroin and other opioids is relatively rare, but these drugs continue to be associated with most of the morbidity and mortality in Europe.

However, drug use is also characterized by different patterns of consumption associated with different types of harm; from single experimental use to habitual and dependent use. Some of today's illicit drugs were used as medicines for their therapeutic potential when first discovered and introduced on the market [2]. This may be one of the reasons why medicinal drugs are also a public health concern and are monitored by epidemiologists to identify any abuse potential in addition to their therapeutic effect.

1.2 The epidemiology of illicit drug use

In the European Union and in Norway, cannabis is the most used illicit drug and is the most frequently reported drug among patients who enter into drug treatment for the first time [1]. The high level of use, especially in south Europe [3] may be a direct consequence of the more liberal view in recent years on the drug in some European countries. Cannabis is mostly associated by users with feelings of mild euphoria, relaxation and intensification of ordinary experiences such as engaging in sex, eating, watching movies and listening to music [4], making it the preferred drug among adolescents and young adults for recreational purposes [5]. The drug is associated with increased risk of psychiatric disorders, such as schizophrenia [6] and psychotic symptoms [7], creates anxiety and panic among naïve users and dependence over time for chronic users [5].

Cocaine is the second most used illicit drug in Europe and the first among stimulants, followed by amphetamines (amphetamine and methamphetamine) and ecstasy. While cocaine is produced from the leaves of the coca bush in Bolivia, Columbia and Peru, and imported in Europe mainly as cocaine powder, amphetamines are synthetic stimulants mainly produced in small laboratories in Belgium, the Netherlands and the Baltic States. Ecstasy in Europe has been mainly MDMA which is chemically related to amphetamine and is produced almost exclusively in the Netherlands and Belgium. Cocaine, amphetamines and MDMA are central nervous system drugs that cause euphoria, increase confidence, sociability and energy, making them appealing for abuse especially in night life-settings [8-10]. These drugs are associated with a wide range of adverse effects such as depression, panic attack, mood swing, feelings of paranoia and anxiety problems. Moreover, stimulant drug users are more likely to combine the use of these drugs with anti-depressants such as opiates or benzodiazepines due to the sleeping and eating problems which they usually suffer as consequence of stimulant use [11, 12].

Even though heroin is less prevalent than other drugs in Europe, harmful consequences follow its use [13]. Heroin remains the most commonly used opiate and the most addictive substance currently available on the illicit market [14]. In the European Union the number of heroin users was estimated at approximately 1.3 million in 2013 [1]. Injection remains the preferred route of administration of this drug leading to the greatest share of drug-related morbidity and mortality in Europe [1]. Injecting heroin users are among those at highest risk of overdose, violence and infections such as HIV and hepatitis C [15].

1.3 The epidemiology of medicinal drug use

Many medicinal drugs can also be abused and used for recreational purposes. The increasing use of prescription drugs for diseases like non-malignant pain [16, 17], anxiety disorders [18] and attention deficit hyperactive disorders (ADHD) [19] lead to the increase of the abuse of such drugs. The abuse of prescription drugs can roughly be divided into therapeutic overuse of the drugs by patients, or use by others than patients for recreational purposes.

Methadone is a synthetic opioid medication used to treat chronic pain [20]. First synthesized before World War II [21], today, methadone is mostly known as affective medication for the treatment of opioid dependence [22]. However, a number of studies have shown that methadone can also be abused [23, 24]. There are patients who try to forge prescriptions in order to sustain their dependence and abusers without a prescription who buy the drug on the black market or have direct access to a patient's prescriptions.

Benzodiazepines are sedative drugs introduced in the late 1950s to treat anxiety and related disorders [25]. However, benzodiazepines have different potency, and the dosage can impact on the use of such drugs as anxiolytics, hypnotics or antiepileptics [2]. Benzodiazepines are popular among drug users to prolong intoxication, to prevent withdrawals [26], to help with sleep, to counteract feelings of anxiety, and to limit the quantity of their main substance of abuse [27].

Prescription stimulants are also abused. Individuals may use stimulants to increase cognitive abilities and performances, eg, at work or studying [28]. Among those drugs, methylphenidate is the mostly known central nervous system stimulant within the amphetamine-like drugs [29], used to treat patients with ADHD and narcolepsy [19, 30].

There are several reasons which may explain the abuse of prescription drugs. Prescription drugs may be perceived as being more socially acceptable and safer than illicit drugs [31], they avoid the stigma associated with the use of illegal substances, and they are easier to obtain especially for those who are uncomfortable with the risks of obtaining illicit substances from a drug dealer [32].

1.4 Epidemiological methods for drug use research

Drug use epidemiology is a challenging area of epidemiology. The main challenge for epidemiological research is that substance use is a stigmatised and hidden activity, some individuals do not like to report their use of drugs and even if they do, the extent of the use may be inaccurate. Others are unwilling to report their use because of possible negative consequences and illegal implications associated with the use of illegal drugs. A

drug-related offence can lead to significant time in prison, probation and a number of legal implications that can follow the users for the rest of their life.

There may also be some definition and terminological challenges. The distinction between licit and illicit substances could be problematic when comparing different countries; some substances are illegal world-wide, while others are illegal only in some countries. The boundaries between use and abuse may also be difficult to define; a drug may not be recognised as a substance of abuse until it is shown to create levels of abuse and dependence similar to other substances of abuse [33].

When it comes to epidemiological or health-related research, population-based surveys have been considered the principal method of investigation, against which other methods are compared [34, 35]. They have the advantage of using generally standardised methodology, and can provide information on large populations. However population surveys targeting different population i.e. general population, students, young people, drug users, and surveys carried out in different settings such as communities, schools, prisons, drug-related treatment centres and on the street, have a number of limitations. They may have poor response rates due to the illegal nature of illicit drug use and the social stigma often associated with it. Many respondents may have motivations to under-report deliberately, or deny use of these substances and withdraw consent for further studies [36]. Privacy, face-saving and possible legal implications are also major causes of reporting bias and low response rate. Survey questions are prone to recall bias, due to inaccuracies related to users' memory on the frequency of substance use or on their ability to distinguish between different substances available on the illicit market. Furthermore, questionnaires often used in surveys data may use different wordings when referring to a specific drug compared to those in common use in the local community [37] causing bias of unknown direction and magnitude. Lastly, population surveys can be expensive, time-consuming, since the results can be available only after several years the survey is conducted and may miss marginal groups of drug users such as patients in hospitals and prisoners [34]. Therefore they may miss information on the extent of drug use problems in particular communities

including the type of substances and the population groups where more harm is being produced or is likely to be produced.

Routinely collected data from different agencies including hospital emergency departments, social agencies, treatment programmes, general and psychiatric hospitals, health services and law enforcement data [38-42] are also widely used in drug use epidemiology. These data give essential information on more problematic groups of drug users, but are not always easy to access since they usually require ethical approval from dedicated agencies, may be expensive, may be based on routine questions and may differ considerably from country to country making geographical comparisons difficult. They may also be highly subject to policy changes, for example, an increase in the law enforcement on drug-related offences in a city may yield an increasing number of reported drug-related crimes which may not necessarily indicate an increase in drug use. Further, such surveys are not population-based since they are targeted towards selected groups, leading to selection bias. Prevalence rates of drug use for those groups are likely to be higher than those for the general population. Also they may include persons who may have used the drug once or they could have multiple records for the same person as result of multiple crimes or multiple visits to emergency departments. Furthermore data gathered from treatment facilities and drug-related programmes may also underestimate prevalence because of limited places in treatment and the fact that results depend on the existence of the treatment programme itself [34, 35].

Crime statistics such as drug-related offences, drug production and seizures data are also used in drug use epidemiology [42-44], but in the same way as data gathered from treatment facilities, these data are targeted toward selected populations, they are prone to selection bias and may overestimate prevalence of use [35].

When conducting epidemiological research it is important to consider that the use of a drug is not spread evenly across a population. There are individuals who take the drug on a regular basis, while others may only use the drug on particular occasions. For this reason, a single epidemiological method may not be appropriate to investigate the extent of the drug use in a community, while a combination of several methods may

provide a more complete picture of the extent of the drug problem [34]. In the light of the above, there is need for new epidemiological methods in drug-related research which can overcome problems related to traditional methods and may be objective, less expensive, easy to access and available in a short-time.

1.5 Wastewater based epidemiology

The term “wastewater-based epidemiology” (WBE) was coined in recent years following the use of wastewater analysis (WWA) to identify the collective drug use in a specific community [45, 46]. WWA is based on the chemical analysis through mass spectrometry of wastewater samples typically collected from the inlet of sewage treatment plants (STPs), looking at the presence of drugs or their metabolites in those samples [47, 48].

Mass spectrometry is a powerful analytical chemistry tool, used to identify compounds and their concentration in a specific sample, and has played an important role in environmental forensics to identify previously unknown pollutants in the environment [49]. However as in many other research fields, the real potential of mass spectrometry as a basic tool to be used in other research areas was not entirely understood. The idea of ‘wastewater analysis’ using mass spectrometry derived from Daughton’s research on monitoring the environmental pollution of surface and sewage water caused by the use of pharmaceutical products and therapeutic drugs by humans. The idea was that most of the chemicals we swallow daily are metabolized by our body and are excreted unchanged or as a mixture of metabolites in urine and faeces, thus ending up in the STP (Sewage treatment plant). Daughton hypothesized the possibility of screening sewage samples collected from an STP for estimating the collective drug consumption in a specific community by measuring the concentration of drug metabolites found in those samples [49, 50].

In 2005 Zuccato and his group in Italy [45] implemented the approach using cocaine as a model drug. They measured the amount of cocaine and its main metabolite, i.e. benzoylecgonine, in the largest Italian river (the Po) in Pavia and found that an average of 4 kg of cocaine per day was found during the sampling period. Later the methodology was extended to other drugs such as opiates, cannabis and amphetamines [46, 47],

comparing the concentration of the drugs in three different cities located in three different countries i.e. Milan (Italy), Lugano (Switzerland) and London (United Kingdom). In 2007 the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) held the first European meeting on WWA opening up to this new and challenging approach for drug use epidemiology. The WBE era had just started and the approach had been quickly implemented not just for illicit drugs [45, 46, 51-60], but also licit substances [61-64] and prescription drugs [65-69] with promising results at local, national and international levels. Reviews of the methodology and the analytical challenges have also been addressed [48, 70-72]. Several efforts have been made to improve the reliability of the method by addressing all the uncertainty factors [73, 74]; the sampling procedure, the frequency of sampling and the sampling mode [70, 75, 76]; the stability of drugs and their metabolites in the wastewater during their way to the place of sampling and during the storage of the collected samples [77-79]; the back-calculation of drug use [73, 80], the estimated population contributing to the wastewater treatment plant (WWTP) [81, 82] and the issues related to the chemical analyses such as the estimation of the limit of detection (LOD), the limit of quantification (LOQ) and the excretion rate of each substance [73].

WBE has been viewed by researchers as a potentially valuable alternative to standard epidemiological methods to evaluate the extent of drug problems in a specific community. By measuring the target drugs or metabolites in wastewater it is possible to estimate the amount of drug consumed by the population served by the WWTP [46-48]. WWA provides close to real-time estimates, in the 24 hours before the sampling is carried out, overcoming the costs and long times often associated with standard surveys. Moreover, as the use of illicit drugs is linked with a high-risk lifestyle and is perceived by the general population as socially unacceptable, users tend to underreport their use or decline to answer truthfully [83, 84]. Self-report bias and low response rate, which are the main flaws of standard surveys, are overcome by the objectivity of WWA.

WBE also allows for spatial comparisons at national or international level. In Europe, WBE has been applied to compare the use of illicit drugs such as cannabis, cocaine, amphetamine, methamphetamine and ecstasy (MDMA) in different European cities

simultaneously [85, 86]. The first monitoring campaign conducted in 2012 included 19 European cities in 11 countries [85] and two years later a monitoring campaign of about 42 European cities from 21 countries was carried out [86]. These comparisons have provided a whole picture of drug use problems in Europe. However, standard specification tests and simple summary measures which are often the preferred statistical methods when analysing WBE data are rather simplistic and, since they do not take the intra-correlation structure of the data sets into account, may lead to wrong conclusions. As in many other research fields [87], there is a need for statistical methods able to use the data properly, and extract underling features which may be lost with traditional statistical methods.

1.6 The use of advanced statistical tools

Case-control, cross-sectional, time-series or longitudinal studies are among the most common study designs in epidemiological research. Nowadays researchers have access to multiple sources of data, building up data sets with complex correlation structures. How to analyse such types of data best is, however, not always clear and several comparisons between traditional statistical methods and more advanced statistical approaches, as well as improvements of the latter, have been published [87-96].

Even though statistical methods are continuously evolving, their application into medical research is often slow. This may be because in many medical research fields the analyses are performed by researchers whose primary field of expertise is outside of statistics. While simple statistical methods may, in some cases, work well and have the advantage that they are easily understood and used by most quantitative scientists, such methods are problematic if they do not interpret the data properly or, worse, lead to the wrong conclusions. Inappropriate statistical methods are often chosen for the complex correlation structures of many data sets.

The advantage of using more advanced, and more adequate, statistical methods has recently been shown in the analysis of 2-h glucose test data [87, 88] and foetal movement chart data [89-91]. A study on pregnant women demonstrated that more advanced statistical methods, such as functional data analysis (FDA), of glucose curves in

early pregnancy was superior to traditional analyses of oral glucose tolerance test data, in providing important temporal information in terms of differentiating between women who did and did not develop gestational diabetes mellitus later in pregnancy [87]. In a following study, the authors demonstrated that the glucose curve characteristics extracted by FDA, and interpreted clinically as general glucose level during pregnancy, postprandial glucose peak, and third trimester glucose level, were found to have significant impact on birth weight, neonatal percentage of fat and C-peptide in cord blood, whereas this important information would be lost in traditional simple summary measures [88]. Another study on a cohort of pregnant women aimed to identify the main individual temporal patterns in foetal movement counting data using FDA. FDA successfully captured clinically meaningful individual temporal patterns between women and indicated that a decrease in foetal movement data was a potential marker of risk for the baby [89]. In two following studies the authors explored the temporally more flexible wavelet principal component analysis (WPCA) on the same data set, and were able to detect the presence of spikes around the time of decreased foetal movement data which were missed by functional principal component analysis (FPCA) [90, 91]. These studies have demonstrated how appropriate statistical analysis is crucial for extracting important clinical information from temporal data that would be lost when using traditional statistical methods. Standard statistical methods were not able to differentiate between women who did and did not develop gestational diabetes mellitus later in pregnancy, were not able to show that general glucose level during pregnancy, postprandial glucose peak, and third trimester glucose level had significant impact on birth weight, neonatal percentage of fat and C-peptide in cord blood or identify that a decrease in foetal movement data during pregnancy was a potential marker of risk for the baby.

FDA is a statistical framework developed for analysing data representing curves [97-100]. Introduced by Ramsay, FDA has since been applied in different research fields with promising results. When applying FDA, instead of single data points, the entire temporal curve i.e. the observed time period of each drug, is considered as a single unit of observation allowing for feature extraction from a continuous process. Within the

medical research field FDA has given novel insights of clinical relevance in the analysis of magnetic resonance imaging [101], renal anaemia [102], human movement [103], foetal movement [89], glucose regulation [87, 88] and behavioural processes [104] data. A review on the advantages of this statistical approach has been published [105].

Thus, we decided to explore the suitability of FDA for investigating temporal patterns of drug use detected from WWA. We hypothesized that while some information, such as the general level of a drug in the wastewater, can be extracted using traditional statistical measures such as the area under the curve (AUC) and the mean load throughout the observed period, they cannot help to identify temporal features of the use of the drugs throughout the time period so those features may be lost when using standard statistical approaches.

Within the FDA framework several advanced statistical methods, such as FPCA and functional analysis of variance (FANOVA) [97-99], are available and the best FDA method to analyse WBE data is unclear. Technical aspects of the FDA approach, such as the choice of the basis functions to model the underlying process properly, the choice of the smoothing parameter to remove random noise and the robustness of the approach to missing data might have an impact on the analytical results and thus needs to be investigated.

2. AIMS OF THE THESIS

2.1 General aim

The overall aim of this thesis was to explore the possible usefulness of using advanced statistical methods on WBE data, in order to extract more information on the weekly temporal pattern of daily drug loads in Europe.

2.2 Specific aims

Specifically, the aims of the studies included in this thesis were:

1. To study the suitability of FDA in the analysis of WBE data, by comparing results obtained using FDA with those obtained using more traditional statistical methods. [Paper I]

2. Use FDA to uncover the main temporal features of the pattern of use of drugs throughout the course of the observed period, looking at the concentration of the parent compounds or metabolites ending up in the wastewater treatment plant. [Paper I, Paper II, Paper IV]

3. To compare different potentially suitable statistical methods for the analysis of WBE data. Further, to investigate the stability of FPCA regarding choice of basis functions and missing data. [Papers I and III]

4. To apply the FPCA approach to prescription drugs, particularly to see if the method could distinguish between what could be considered the proper medical use and the recreational use of those drugs. [Paper IV]

3. MATERIAL AND METHODS

3.1 Source of data

The papers presented in this thesis are all based on wastewater data sets.

3.1.1 Paper I

The paper was based on a WBE data set from 42 European cities [86]. Raw sewage was collected from the inlet of 47 STPs in 42 cities from 21 European countries, servicing a combined population of approximately 24.7 million inhabitants. Samples were collected from each location over seven consecutive days, in March 2013. For the purpose of the study only the concentrations of ecstasy (MDMA) and amphetamine in wastewater were considered. The daily mass loads were expressed in mg/10 000 people/day. Concentrations for each drug below the LOQ were replaced by LOQ/2 [70] if at least one day in the week had a concentration value above the LOQ. Cities with no measurements above LOQ were excluded. Four cities (9.5%) were excluded for MDMA and nine cities (21.4%) were excluded for amphetamine. The chemical analyses were conducted at each lab location.

3.1.2 Paper II

The study was based on wastewater data from 17 Italian cities collected during a one week monitoring campaign. Raw sewage was collected from the inlet of 17 STPs in 17 Italian cities. Samples were collected repeatedly from each location over seven consecutive days, in November 2013. The concentration of cannabis, cocaine, heroin, MDMA, methamphetamine and ketamine were measured by analysing the selected drug excretion residues (target residues) in wastewater. The daily mass load was based on the original concentration (ng/L), and normalized by flow rate (L/day) and the population size of the catchment area (mg/10 000 people/day). The daily mass load over one week, of a specific drug, in a specific city i.e. 17 cities per six drugs, constituted the 102 separate temporal data sets for the analysis. Cities with drug concentrations below the LOQ were excluded from the analysis. This resulted in a study sample of 92 separate temporal data sets for the study. All the chemical analyses were conducted at the Mario Negri Institute in Milan.

3.1.3 Paper III

The paper was based on a WBE data set from 42 European cities [86]. Raw sewage was collected from the inlet of 47 STPs in 42 cities from 21 European countries, servicing a combined population of approximately 24.7 million inhabitants. Samples were collected from each location over seven consecutive days in March 2013. For the purpose of the study only the concentration of MDMA in wastewater was considered. The daily mass loads were expressed in mg/10 000 people/day. Four cities (9.5%) had no values above the LOQ and were excluded from the analysis. The resulting study sample for further statistical analysis consisted of 38 cities. The chemical analyses were conducted at each lab location.

3.1.4 Paper IV

The sewage samples were collected from an STP in Oslo, Norway. This STP processes sewage from a metropolitan and suburban population of approximately 580 000 people. An automatic wastewater sampler was used to collect 8-h composite samples during weekdays (Mon-Thu) and 6-h composite samples during weekends (Fri-Sun), starting on Monday 3rd February 2014 and ending on Sunday 2nd March 2014, resulting in a total of 97 samples. The concentration of cocaine, amphetamine, methamphetamine, heroin, atenolol, paracetamol, metoprolol, citalopram, carbamazepine, methadone, oxazepam and methylphenidate was estimated by analysing the selected drug excretion residues (target residues) in wastewater [46, 71]. The calculation of the mass load was based on the original concentration (ng/L), and normalized by flow rate (L/day). The mass load over one month of a specific drug constituted the 12 separate temporal data sets for the analysis. All the chemical analyses were conducted at the Norwegian Institute for Water Research (NIVA) in Oslo.

3.2 Ethical considerations

This study did not require any specific permission. The use of wastewater data to estimate the use and abuse of licit and illicit drugs in a specific community does not raise any major ethical issues [106, 107]. Even though wastewater samples are collected from STPs without the consent of the individuals living in that particular area and contributing

to the STPs, the samples collected are composite samples, and individuals cannot be identified. The STPs used for monitoring purposes usually include 10 000 or more people and the risk of harm is negligible. Moreover, it would not be possible to request informed consent from all individuals, residents and visitors who contributed to the wastewater samples.

However, WWA can also be performed in different setting such as prisons, schools, entertainment venues (festivals, dance parties or pubs) and work places. In these scenarios the results of the research findings could stigmatize all individuals belonging to that particular institution or taking part at that specific event and therefore ethical concerns could arise. In such cases, researchers could avoid those risks by not disclosing the location and details of study sites when publishing results [107].

3.3 Funding

This study was funded by the European Union-International Training Network SEWPROF (Marie Curie-FP7-PEOPLE Grant #317205) and the Norwegian Centre for Addiction Research (SERAF), University of Oslo. The analytical campaign in Italy ("Aqua Drugs" Project) was supported by Dipartimento Politiche Antidroga (Presidenza del Consiglio dei Ministri, Rome, Italy). The analytical campaign in Oslo was supported by the Norwegian Institute for Water Research (NIVA, Oslo, Norway).

3.4 Statistical analyses

The main statistical method in all papers was FDA. FPCA was used in all papers, while FANOVA was used in paper I only. Multiple regression analysis on the functional principal component (FPC) score variables was used in papers I and II. Traditional principal component analysis (PCA) and WPCA were used in paper III, while generalized additive models (GAM) with trigonometric functions to describe cyclic temporal patterns were used in paper IV.

3.4.1 Data description (papers I-IV)

Descriptive statistics were presented as median and quartiles (Q1, Q3), because of heavily skewed distributions. All data were log-transformed before proceeding with further statistical analyses.

The unit of observation in paper I was a seven day week starting Wednesday and ending Tuesday. For six (14.3%) cities, the data sampling started later in the week. Missing data for MDMA and amphetamine across all the 42 cities ranged from 1.7% to 2.2%.

The unit of observation in paper II was a seven day week starting Monday and ending Sunday. Two out of 92 separate temporal data sets had one missing data point, otherwise the 92 data sets were complete.

The unit of observation in paper III was a seven day week starting Wednesday and ending Tuesday. For six (14.3%) cities, the data sampling started later in the week. Missing data across all the 38 cities was 2.2%.

The unit of observation in paper IV was a month, i.e. approximately four week cycles. Missing data for the 12 drugs under study ranged from 0% to 4%. For two of the drugs, i.e. amphetamine and methamphetamine, only three weeks during the sampling campaign were available, while for methylphenidate only two weeks were available. Missing data for these three drugs ranged from 2% to 4%.

For all the studies included in this thesis, the missing data was below 5%. Due to the low amount of missing [108], single imputation [109, 110] was performed in each study before proceeding with further statistical analyses.

3.4.2 Traditional data analysis

In paper I, simple summary measures of WBE data included the overall mean throughout the week, the AUC and the difference *d* between weekdays and weekends. The Wilcox test was used to assess whether there were significant differences between the load of the drugs on weekdays and at the weekend. In paper II, the Kruskal-Wallis' test followed by Mann-Whitney tests with Holm correction were used to test differences in all

pairwise comparisons between the median-week load of two different drugs. The level of significance was set at 0.05 for all tests.

3.4.3 Generalised additive models (paper IV)

GAM is an extension of the generalised linear model (GLM) to allow for non-linearity [111]. In paper IV for each of the 12 separate temporal data sets, an optimal GAM with trigonometric functions to model cyclic behaviour was estimated. Instead of fitting a linear term of time t for each drug to model the possible long-term change during the course of a month, we fitted a smooth function $\mathrm{f(t)}$ using splines, i.e. a set of higher-order polynomials. The optimal spline was found using the Generalized Cross Validation (GCV) criterion [111]. The weekly pattern is an additional, cyclic, temporal component in the data, repeating every seven days. Using the Fourier series expansion theorem, we used linear combinations of sine and cosine functions to fit this weekly component [112, 113]. The Gamma regression model with the natural logarithm as the link function [111] for the weekly drug load at time t, $\mathrm{y_t}$, and each drug is

$$\ln[E(y_t)] = c + f(t) + \sum_{k=1}^{K}[a_k \cos\left(\frac{2\pi kt}{T}\right) + b_k \sin\left(\frac{2\pi kt}{T}\right)] ,$$

with period T=7, c a constant, and $\mathrm{f(t)}$ the long-term non-linear trend. Note that a further cyclic within-day component could also be added if of interest. From the above model, the estimated weekly temporal component, based on the four week sampling period, was extracted for further statistical analyses.

3.4.4 Functional data analysis (papers I-IV)

FDA is a mathematical framework especially developed for analysing curves. Instead of single time points, each function, estimated from a series of consecutive observations, is considered as a single unit of observation allowing for extraction of information from a temporal process as a whole.

The first step in FDA is to fit a mathematical function to each set of temporal data. Among all the possible choice of basis functions, Fourier and B-spline basis functions are the most commonly used [98]. Each individual WBE temporal data set in each study was converted into a continuous smoothed curve, forming the basis for the subsequent FDA

[98, 99]. The optimal smoothing of the functions, for removing the random day-to-day variation, e.g. non-systematic error, measurement error, was estimated using the GCV criterion [114]. In paper I, B-splines basis function with common-optimal smoothing parameter was used. In paper II Fourier basis function with common-optimal smoothing parameter was used. In paper III both B-splines and Fourier basis functions with no smoothing, individual-optimal smoothing and common-optimal smoothing were investigated. In paper IV, Fourier basis with seven basis functions and no smoothing parameter was used.

3.4.4.1 Functional principal component analysis

Traditional PCA is used to reveal the internal structure of the data in order to explain variability [115]. FPCA is the functional extension of PCA to curve data [98] and was used to explore the temporal variation in the fitted WBE curves. FPCA leads to FPC curves, which are new functional curve variables uncorrelated by construction which describe the main modes of the temporal variation in the sample of WBE curves [98]. A common practice before applying the FPCA is to normalize the data in order to remove the temporal mean. However in our study we did not subtract the mean before performing FPCA, since we were also interested in the shape of this main temporal mode. The first FPC thus took almost all the variability as it to some extent represented the normalization of the data. The percentage of explained variation for each FPCs thus cannot be interpreted in the same way as for FPCA on normalized data. FPCA also leads to FPC scores for each WBE curve showing the degree to which that particular pattern is represented in that specific WBE curve. By applying FPCA it is thus possible to study how WBE curves for different drugs vary between different cities. Further, an important step in FPCA is to find a meaningful interpretation of each FPC accordingly to the pattern it exhibits.

In paper I, the association between the traditional statistical measures of wastewater drug loads and the FPCA was assessed by calculating the Pearson correlation coefficient (r) between the FPC scores, the overall mean of the log-transformed data, AUC and the difference *d* between weekdays and weekend means.

FPCA was used in all four papers, while traditional PCA was used only in paper III as a comparison with FPCA.

3.4.4.2 Functional analysis of variance (paper I)

FANOVA is the extension of traditional analysis of variance (ANOVA) to functional data [98]. We used FANOVA in paper I to analyse the effect of five possible predictors on the shape of the wastewater drug load curves: latitude; longitude; gross domestic product (GDP) of country; relative size of the city and density of the city [99]. We dichotomized each of the continuous explanatory variables and compared the mean curves in the two groups. The impact of choice of cut-off point was explored by selecting cut-off points across the whole observed range of the covariates. Functional confidence intervals (95%) and p-value curves, as well as an overall p-value, were calculated for each covariate using a functional permutation F-test [99].

3.4.4.3 Multiple regression analyses (papers I and II)

In paper I, in order to explore multiple predictors such as latitude; longitude; GDP of country; relative size of the city and density of the city simultaneously, without the need for dichotomization, we used the cities' scores for the estimated FPCs as outcome variables in multiple linear regression models. The optimal sub-model was chosen using Akaike's Information Criterion (AIC) [116]. The results were compared with those from FANOVA analysis.

In paper II, the multiple linear regression was conducted considering five covariates: location (north, centre or south Italy); size of the city (large: >350 000 inhabitants, medium: 120 000-350 000 inhabitants, or small: <120 000 inhabitants); migration rate (the difference between the number of people coming into the city minus the number of people leaving the city, measured per 100 000 inhabitants), gender ratio (female/male) and drug type. We fitted both univariate regression models and a full multiple regression model including all covariates. The optimal sub-model was then chosen using AIC.

3.4.4.4 Wavelet-based principal component analysis (paper III)

WPCA is an extension of traditional PCA to the wavelet domain [91]. Wavelets is a mathematical framework developed for analysing high-dimensional data [117]. Wavelet

basis functions are localized in both time and space, allowing for proper modelling of less smooth temporal data, even spikes [118, 119] and therefore they can be an alternative to Fourier or B-splines basis functions. WPCA leads to new variables in wavelet domain, and these variables back-transformed to time domain become the wavelet principal components (WPCs). Also, each WBE curve is provided with a score on each WPC indicating the intensity with which the WPC pattern is present in that specific temporal curve. Similarly to FPCA each WPC is interpreted according to the temporal pattern it exhibits.

3.4.4.5 Robustness analyses

In paper I, to explore whether the FDA results of temporal patterns would emerge purely by chance due to the nature of the curve fitting process, we also performed the FDA on a dataset obtained by randomly sorting of the original data.

In paper II, supplementary analyses were performed on standardized data, where standardization of each separate temporal data set was done by subtracting the mean and dividing by the standard deviation.

In paper III, the sensitivity of FDA to the choice of basis functions, to missing data and to the imputation of the values below the LOQ was explored. A non-parametric bootstrapping procedure was used to construct confidence intervals (CIs) [120, 121] in order to compare the results from the different FDA approaches. The 1000 re-samples obtained by random sampling with repetition from the original 38 temporal data sets, was used to calculate the 95% CI for each FPC. The CIs were calculated for both Fourier and B-splines basis functions, and both no smoothing, individual-optimal smoothing and common-optimal smoothing parameter. The sensitivity to missing data was evaluated for both Fourier and B-splines basis functions and for the three choices of smoothing parameters by randomly deleting an increasing number of observations; 5, 10, 15 and 20% of original values. Finally two approaches for imputing values below the LOQ were compared; the common practice of replacing the values below the LOQ with LOQ/2 [86], and replacing those values by a random draw from a uniform distribution on the interval [0, LOQ] [122].

In paper IV, supplementary analyses were performed for all possible pairwise combinations of the three groups of drugs; prescription and illicit drugs, prescription and licit drugs, illicit and licit drugs.

3.5 Software

The analyses in all papers were performed in R [123]. The imputation was performed using Amelia II and the *amelia* package [124], and FDA, FPCA and FANOVA using the *fda* package [99]. Since no R package for WPCA currently exists the WPCA in paper III was performed by building on features of package *wavethresh* [119]. The GAM model in paper IV was fitted using the *mgcv* package [111].

4. RESULTS

4.1 Paper I: Wastewater-Based Epidemiology of Stimulant Drugs: Functional Data Analysis Compared to Traditional Statistical Methods.

The smoothed WBE curves for both drugs i.e. MDMA and amphetamine showed large variation between the 42 European cities. The first three FPCs explained in total more than 99% of the temporal variation between these cities and were interpreted as "general level" (FPC1, 91.8%-98.9%), "weekend peak" (FPC2, 1%-6.2%) and "weekend peak timing" (FPC3, 0.1%-1.5%). Curves with a negative FPC1 score had a lower general level of drug than the overall mean, while curves with a positive FPC1 score had a higher general level of drug than the overall mean. Curves with negative FPC2 scores had a lower difference between the weekend peak and the midweek level, while curves with positive scores on FPC2 had a large difference between the weekend peak and the midweek level with high load of the drug at the weekend. Finally curves with negative FPC3 scores showed an early peak moved towards Saturday, while curves with positive FPC3 scores showed a late peak moved towards Monday.

The simple summary measures led to similar interpretation of the FPCs. The mean week load and the AUC statistics were almost perfectly correlated with FPC1 ($r = 0.999$, for both drugs), while the difference between weekday and weekend loads was moderately correlated with FPC2 ($r = 0.762$ for MDMA) and FPC3 ($r = -0.497$ and $r = -0.760$ for MDMA and amphetamine respectively).

The standard statistical analysis found the level of amphetamine in the wastewater to be higher compared to MDMA. While for MDMA the median load increased significantly at the weekend ($p<0.001$) as compared to weekdays. This increase did not occur for amphetamine ($p = 0.369$).

The FANOVA and the multiple linear regression using FPC scores as outcome showed similar results. From the multiple regression results, latitude and longitude were associated with MDMA load curve patterns throughout the week, while only latitude was associated with the load curve patterns for amphetamine. However the FANOVA results showed that for some of the predictors, choice of cut-off value for the

dichotomization had a major impact on the estimated significance of the difference between the mean of the corresponding groups.

4.2 Paper II: A nuanced picture of illicit drug use in 17 Italian cities through functional principal component analysis of temporal wastewater data.

The smoothed 92 temporal WBE curves showed large variation across different drugs in the Italian peninsula. The first three FPCs explained 99.5%, 0.4% and 0.1% of the temporal variation respectively and were labelled “general level”, “weekend-midweek discrepancy” and “weekend peak timing”. Similarly to FPC2 and FPC3, the first and second FPC of the supplementary analyses (SFPC1, 61.7% and SFPC2, 37.7%) were labelled “weekend-midweek discrepancy” and “weekend peak timing” respectively. Moreover, in this analysis, the third FPC distinguished between curves with a peak moved towards Saturday, and curves with a late peak moved towards Tuesday.

The multiple linear regression using the FPC1 scores as outcome showed that the “general level” of the drugs in the Italian wastewater was significantly related to the type of drug, location and size of the city. Cannabis was the drug with the highest general level during the observed week in the Italian wastewater followed by cocaine and heroin, while higher level of the drugs were found in central and larger cities.

The multiple linear regression on the FPC2 and SFPC1 scores (“weekend-midweek discrepancy”), showed that higher temporal peak of the drug, i.e. high discrepancy between weekend peak and midweek level, were associated with the type of drug, the size of the city and the migration rate. MDMA was the drug with the highest discrepancy between the weekend peak and the midweek level, followed by cocaine and ketamine. Large-size cities were associated with higher discrepancy between the weekend peak and the midweek level compared to medium-size cities, while high number of immigrants to the city was associated with low discrepancy between the weekend peak and the midweek level.

The results of multiple regression analysis on the FPC3 and SFPC2 (“weekend peak timing”) scores’ indicated that in small cities, the peak shifted towards Saturday, that is,

earlier in the week when compared to larger cities. A similar pattern occurred in cities with high immigration rate and in northern and southern Italy compared to central Italy.

4.3 Paper III: Exploring functional data analysis and wavelet principal component analysis on ecstasy (MDMA) wastewater data.

The first PC, FPC and WPC resulting from traditional PCA, FPCA and WPCA analysis, explained 86.9%, 87.5-92.1% and 81.5-82.6% of the total variation between cities, respectively. The second and third PCs, FPCs and WPCs explained 7.0%, 5.8-6.9% and 7.3-12.8%, and 2.4%, 1.7-2.9% and 1.9-3.2% of the total variation, respectively.

The temporal patterns extracted by the first three PCs, FPCs and WPCs were consistent. Their interpretation was drawn from the FPCA results by plotting the mean of the fitted curves together with how the shape of an individual curve differed from the mean curve if a multiple of the functional principal component curve was added to, or subtracted from, the mean curve. The first three FPCs were labelled as “general level in wastewater”, “discrepancy between the weekend peak and midweek level”, and “weekend peak timing” respectively.

The bootstrapping procedure showed that the FPCs were quite stable for each choice of smoothing parameter, while the common-optimal smoothing was the best choice both when Fourier and B-splines basis functions. Moreover, when using Fourier basis functions the FPCs were stable up to 15% of missing data, while using B-splines basis functions the FPCs were stable up to 10% of missing data. Overall, Fourier basis functions with common-optimal smoothing parameter was the most suitable approach for this type of data.

4.4 Paper IV: Assessing medicinal drug abuse using functional principal component analysis (FPCA) of wastewater data.

The 12 smoothed temporal WBE curves showed large variation in the Norwegian city of Oslo’s wastewater. The dominating FPC (FPC1) explained 99.7% of the temporal variation between temporal curves and was labelled “general level”, while the second and third FPCs accounted for a small amount of the temporal variability and were labelled “weekend-midweek discrepancy” and “timing of peaks” respectively.

In this paper, the third FPC (FPC3) was labelled “timing of peaks”. All temporal curves showed two peaks on this component; one peak around Monday/Tuesday, and one peak around Friday. For curves with a positive score on FPC3 the first peak was shifted towards Monday and the second peak shifted towards Thursday – Friday, while for curves with a negative score on FPC3 the first peak was shifted towards Tuesday and the second towards Friday – Saturday.

FPC1 scores indicate that among the licit drugs paracetamol had the highest general level in the wastewater, while methamphetamine and oxazepam had the highest general level among illicit and prescription drugs, respectively. The FPC2 scores showed that cocaine followed by methylphenidate were the two drugs with the strongest weekend peak, that is, the largest difference between weekend peak and midweek level, with a high load of the drug at the weekend, while oxazepam followed by heroin (using morphine as target residue) were the two drugs with the strongest opposite pattern that is the strongest weekend low with two high loads of the drugs during the week.

The patterns shown by the first three FPCs of the analyses when considering only two out of three groups of drugs were in agreement with results from the analyses on all the fitted curves. The only exceptions were the patterns shown by the second and third FPCs when the FPCA was carried out on prescription and licit drugs only. In this analysis the second FPC did not show a strong weekend peak as for the other two analyses, while the third FPC captured an opposite pattern between Monday and Friday.

5. DISCUSSION

5.1 Discussion of main findings

By using FDA on the WWA data we were able to extract more detailed information about drug load patterns during the week. These data would to a lesser extent be identified by more traditional statistical methods. General level of drug load, weekend-midweek discrepancy and weekend peak timing were the three most important temporal features extracted by the use of FPCA. Contrary to our initial worry that FPCA would cause over-smoothing of the data, the analysis did not smooth away essential information in the temporal wastewater data which were captured by the temporally more flexible WPCA. On the contrary, the FPCA approach was robust and not particularly sensitive to the choice of basis function or to missing data. The second FPC (FPC2) resulting from the FPCA was interpreted as the weekend-midweek discrepancy. This component was the most striking temporal feature extracted from WBE data and because of its close connection with drugs of abuse, was interpreted as a sign of weekend recreational use. The appearance of this phenomenon for some prescription drugs as well indicated to us that WWA followed by FPCA could be used to identify recreational use of medicinal drugs with an abuse potential.

5.1.1 FDA vs standard statistical methods

Using FDA, and in particular FPCA, we were able to extract valuable, nuanced temporal information on the use of ecstasy (MDMA) and amphetamine throughout the week that simpler statistical methods missed. Using traditional statistical methods and specification tests we were not able to identify any weekend pattern for amphetamine throughout the week, but using FPCA we were able to demonstrate this. The AUC statistics, however, carried both valuable and precise information about the level of the drug, which was supported by the almost perfect correlation between the AUC and the scores on the first FPC interpreted as the level of drug in wastewater.

A number of studies conducted on WBE data have shown an increasing use of illicit drugs at the weekend [53, 55, 56, 85]. However, to assess whether the use of those drugs was significantly higher at the weekend when compared to weekdays, standard

statistical tests have been used. When using statistical tests, is important to define which days of the week constitute the “weekend”. Defying the “weekend” a priori, before running the statistical tests, may introduce bias in the analysis and may significantly impact the results. Different drugs are characterized by different elimination half-life. There are drugs excreted from the human body within hours and drugs with long elimination half-life which can be excreted in several days [125-127]. The elimination half-life of a drug will therefore affect when the drug load peak is visible in wastewater. The first paper demonstrated that the “weekend” is a somewhat less well defined time period than the traditional cultural understanding of it. Using FPCA one may estimate what constitutes the “weekend” for each city and each drug, without having to define it a priori, as is needed when applying standard statistical tests.

5.1.2 Temporal information in wastewater curves

FPCA decomposes the variation between curves into a set of uncorrelated temporal features [98], but the usefulness of this analysis depends on how the FPCs are interpreted. For all papers, the first FPC represented mainly the general drug level in the wastewater, accounting alone for more than 80% of the variability between individual temporal curves. The level of the drugs in the wastewater was in accordance with previous studies on the same material [86, 128]. The second and third FPCs roughly represented how pronounced a weekend peak was and the timing of such a peak, while only in paper IV, the third FPC was interpreted as the timing of a two-peak pattern throughout the week.

5.1.2.1 Temporal pattern: level of drug in wastewater (FPC1)

In paper I, performing multiple regression analyses using FPC scores as outcome variables, we found that the level of drug in wastewater was associated with the geographical position of the city; in line with previous findings [129, 130], the load of ecstasy increased significantly in north-west Europe, while the load of amphetamine increased in a northerly direction.

At a national level (paper II), the predominant drug in the Italian wastewater was cannabis, which is consistent with previous reports on the use of illicit drugs in south

Europe [3] and also in Italy [131]. The level of such drugs was lower in southern Italy, where the underground economy connected to illicit drug demand has been shown to be lower [132]. High levels of drugs have been found in large cities, which might indicate a higher use of illicit drugs. The European market sees an estimated consumption of cannabis reaching 2500 tonnes annually [133]. The high level of use may be explained by a shift in public opinion towards a more liberal view on the drug, but cannabis has also become the most prominent drug among those who entered into drug treatment for the first time in Europe. While cocaine and heroin are less prevalent than cannabis in the Italian wastewater, harmful consequences follow their use and they are often involved in drug related deaths [13, 134, 135]. Monitoring the level of such drugs in the wastewater could be helpful in detecting levels and changes in the illicit drug market, thus permitting preventive actions. Historically Italy does not have high use of methamphetamine [51], but because it is relatively cheap and available it can be an alternative to cocaine or heroin [136].

In paper IV, the first FPC revealed that paracetamol was the predominant drug in the Oslo wastewater, followed by methamphetamine and oxazepam for illicit and prescription drugs respectively. A high level of paracetamol could be related to high over-the-counter sales worldwide [137] and how those drugs are perceived by the general population [138]. A high methamphetamine result could possibly also be in line with what found previously [85] and with our knowledge of the high levels of use of this drug in western Europe [130].

5.1.2.2 Temporal patterns: weekend high (FPC2) & weekend timing (FPC3)

Besides the general level of the drug load extracted from FPC1, the most nuanced result from our study was the information captured by the second and third FPCs interpreted as weekend-midweek discrepancy and weekend peak timing respectively. Particularly, the second FPC was the most important temporal feature extracted from WBE data and because of its ability t identify possible weekend recreational use was also the most important finding of this work. This information is lost when using traditional statistical methods, as demonstrated by our first paper; using traditional statistical methods and

specification tests, we were not able to identify any weekend pattern for amphetamine throughout the week, but we were able to detect this using FPCA.

In the Italian study (paper II), the second FPC showed that MDMA was the drug with the most prominent weekend-midweek discrepancy and all the Italian cities were clustered by their FPC2 score on MDMA, which suggested that MDMA may be a drug preferred in night-life settings. The weekend-midweek discrepancy was lower in medium-size cities than in larger cities, while different night life settings between small and large cities have been also shown by the third FPC. The third FPC showed an interesting reversed pattern in the two major tourist cities of Rome and Florence. Rome appeared to be a Friday-Saturday party city regardless of the drug in focus, while Florence showed a peak moved toward Tuesday which could indicate different night life settings.

The ultimate goal of this investigation was to see whether applying FPCA to wastewater data would improve WBE. More specifically, we wanted to see whether a weekend peak could demonstrate recreational use of medicinal drugs. In paper IV, the second FPC was interpreted as a sign of weekend recreational use and distinguished between drugs with a high peak at the weekend; cocaine, methylphenidate and carbamazepine, and drugs without such a peak like heroin, oxazepam and methadone. Drugs with a negative score on the second FPC showed a lower level than the mean during weekdays and a higher level at the weekend, while drugs with a positive score on the second FPC showed no weekend peak, but two peaks during the week, a first stronger peak of the drug at the beginning of the week followed by a second peak midweek and a drop at the weekend. Beside the weekend high for cocaine, which has been reported from previous studies [85], FPC2 showed, unexpectedly, a weekend peak for the medicinal drug methylphenidate, which may indicate possible recreational use of the drug.

5.1.3 Comparing advanced statistical methods

With paper I, we showed that performing multiple regression analyses using FPC scores as outcome variables to explore whether the various temporal patterns of wastewater drug loads throughout a week were associated with basic characteristics of the city, could be a better approach as compared to FANOVA. Usually, FANOVA is the suggested

way to analyse the association between functional data and covariates [98]. However, FANOVA needs dichotomous explanatory variables, and most of the predictors that we investigated were continuous. Categorizing continuous predictors in regression models has been thoroughly examined in the statistical literature, and repeatedly argued against, as it reduces power and introduces bias of unknown direction and magnitude [139-141]. In our study applying FANOVA would introduce bias in the analysis, due to the arbitrary choice of cut-off points and resulting number of cities in the groups [139]. The significance of the F-test strongly depended on the chosen cut-off level of the explanatory variable. Moreover, FANOVA cannot adjust for further covariates and it only looks at the mean temporal pattern. While this will verify differences between cities, it will not identify the mode of the difference. Even though our suggested multiple regression is not part of the original FDA framework, it opens for more flexibility. It has been proposed previously for the analysis of 2-h glucose test data and foetal movement data [87, 89].

In paper III, we compared traditional PCA, FPCA with different basis functions and smoothing parameters, and WPCA with different mother wavelets and shrinkage rules. FPCA extracted all temporal features discovered by the temporally more flexible wavelet approach, overcoming the initial concern of over-smoothing. Even though the patterns extracted by PCA, FPCA and WPCA were qualitatively consistent, the interpretation of the PCs and WPCs can be difficult to compare to the FPCs. In PCA, individual days are assumed to be independent variables, and the method does not take the temporal nature of the data into account. In this sense the method is fundamentally wrong for this type of data. Further, WPCA is an extension of traditional PCA, but less direct than FPCA. In the version of WPCA applied here, PCA is performed on the smoothed wavelet coefficients in wavelet domain, where the wavelet coefficients constitute independent variables for subsequent PCA in wavelet domain, before back calculating each WPC to time domain. As a result the patterns of the WPCs do not have the same scale as the PCs and FPCs, making direct comparison between the methods difficult.

Using Fourier basis functions, the patterns shown by each FPC were consistent regardless of the choice of smoothing. A common optimal smoothing parameter did

however lead to an increase in the total variation explained by the first FPC. Using B-splines, the extracted temporal patterns were mainly the same as those found using a Fourier basis. However, the third FPC appears less capable of modelling the difference between weekday and weekend loads, and there were larger differences between the different choices of smoothing parameter. Overall common-optimal smoothing seemed to perform better than no smoothing or individual-optimal smoothing, where some spurious variability was detected. Moreover, epidemiological interpretation of the FPCs is often easier as the FPC curves can be illustrated by plots showing how an individual curve differs from the mean curve if the FPC scores are high or low, rather than mere plotting of the FPC curves [99].

In paper III, we also investigated the stability of the FPCA results to the choice of the basis functions and the smoothing techniques by a bootstrapping procedure, and sensitivity of FPCA to missing data, and compared two methods for imputing values below the LOQ. However, we found that using FPCA with Fourier basis functions and common optimal smoothing is a precise, flexible and stable method for analysing WWA data.

Through bootstrapping we found that using Fourier basis may be a better approach when investigating patterns in WWA data since the empirical CIs are narrower than all the other cases, and when exploring sensitivity to missing data, results are stable even with 15% missing data.

5.1.4 Proper vs recreational use of prescription drugs

In paper IV, the second FPC was the most important temporal feature extracted by FPCA and was also able to identify a weekend peak for some prescription drugs with known potential for abuse. This pointed to actual recreational use of these drugs, especially methylphenidate, because of its high weekend peak, as shown by its high negative score on FPC2. Earlier studies have shown that drugs of abuse may have a weekend high indicating recreational use [57]. In this paper, the second FPC distinguished between drugs with a high peak at the weekend; cocaine, methylphenidate and carbamazepine, and drugs without such a peak like heroin, oxazepam and methadone. The weekend

high for methylphenidate was unexpected. Methylphenidate is a central nervous system prescription stimulant within the amphetamine-like drugs that is mainly used for treating ADHD and narcolepsy. For this reason, if any difference between weekdays and weekend were to be expected for methylphenidate it would be that less methylphenidate would be used at the weekend because of weekend holidays in the treatment of ADHD [142]. However the high negative score of this drug on the second FPC indicated that it may be used recreationally at the weekends, confirming previous concerns [131, 132].

While a “weekend high” may be an indicator of non-medical recreational use of a drug, one cannot immediately conclude that the absence of such a peak excludes the abuse of the drug. Some of the drugs investigated in this study with known abuse potential lacked this weekend high. Some drugs even had a positive score on the second FPC characterising no weekend peak, but rather two peaks during the week, a first stronger peak of the drug at the beginning of the week followed by a second peak at the midweek and a drop at the weekend. Oxazepam, followed by heroin, was the drug with the strongest positive pattern on the second FPC. A possible explanation is that oxazepam, which is the metabolite of several benzodiazepines, may be used for sedation and sleeping disorders right after the weekend, when the use of simulants may be preferred especially during night life settings [80]. The high level of heroin during the weekdays may be explained by the use of morphine as heroin metabolite in wastewater [48], which also comes from other sources such as surgery.

5.2 Challenges of interdisciplinary research

WBE is an interdisciplinary research field which integrates information, data, concept, tools and theories for an advanced understanding of the drug use problem in a specific community. The first conference on WBE namely ‘Testing the waters’ was held in 2013 by the EMCDDA uniting diverse disciplines [74]. It brought together experts from different fields i.e. analytical chemistries, health science, epidemiology and statistics, speaking different discipline languages [143]. In this way, WBE is an example of much of modern science. Conducting interdisciplinary research is challenging. One researcher

cannot master all these fields, but all involved researchers need to have a basic understanding of adjacent fields, filling the gap between their own expertise and that of other scientists in the network. All these types of knowledge are important since data can be misunderstood, underused or even over-interpreted. In WBE, the combination of experts, knowledge and tools from different fields has overcome problems of a single method and opened up for new ideas and new challenges in drug use epidemiology. A practical example is the use of mass spectrometry in WBE research. Mass spectrometry is a powerful tool, which has been used in analytical chemistry to identify compounds and their concentration in a specific sample, and used among others in environmental forensics to identify the identity of previously unknown pollutants in the environment [49]. The idea of using mass spectrometry as a basic tool for drug use epidemiological research has given new insight into the problem and opened up for new ways of thinking.

5.3 Methodological considerations

5.3.1 Wastewater analysis

In WBE research the sample collection has a central role. There are currently two different types of sampling techniques; active sampling, on which the present work is based, and passive sampling. Active samples are collected at a specific point in time and are usually time- or volume- proportional [70, 71, 75]. Active samples give snapshots of the drug use problem in a specific community usually based on a short-time monitoring campaign. They are time dependent and usually more expensive than passive sampling devices. Passive sampling devices also called polar organic chemical integrative sampler (POCIS) are devices which consist of a semi-permeable membrane and a solvent. The set of POCIS are deployed in the wastewater over a defined period of time and left to accumulate target compounds [144-146]. The advantage of POCIS over active sampling devices are: lower detection limits, less fluctuating concentrations due to the integration over time and higher stability for the target compounds.

However, unlike active sampling devices, POCIS are not able to detect rapid changes in the concentration of drugs in wastewater from one day to another since they provide an

integrated average of the drug concentration in the wastewater over the deployed time-period in wastewater (usually two weeks). Moreover, they are affected by saturation problems and need to be calibrated prior to usage [145]. Thus different sampling method can give information with different resolutions. In our study we looked at a weekly series of 24h composite samples, enabling us to investigate weekly temporal variability for each drug of interest.

The occurrence of specific events such as festivals during a monitoring campaign can impact the results drawn from wastewater analysis, as it is known from the literature that the use of drugs is likely to increase during such events [147-149]. WBE campaigns are usually carried out during a time period in which no particular events are known to take place in order to give as true a picture as possible of the community under study.

On the other hand, those events need to be monitored and used as a first screening for new drugs, which are constantly synthesized and sold on the illicit market, the so-called new psychoactive substances (NPS) [150-153]. The great advantage of WBE compared to traditional epidemiological methods is its almost real-time monitoring and cost-effectiveness. After the occurrence of a particular event WBE can provide results within 24 hours allowing for immediate intervention.

5.3.1.1 Level of drug use (FPC1) estimates' bias

The estimation of drug use in a community by measurements of drug residues in wastewater requires several steps. The general approach for the back-calculation of drug consumption is performed by calculating the daily loads of target residues (g/day) by multiplying the daily concentrations of the measured residues (ng/l) by the daily flow rate of sewage (m3/day); then the total consumption is estimated by applying a specific correction factor that takes into account the excretion rate of the target residue of the drug and the molecular mass ratio of the parent drug to its metabolite. By dividing the daily loads by the estimated number of people served by the STP (mg/day/1 000 inhabitants) is possible also to compare results between different cities [46, 71, 74]. All these parameters may be influenced by external factors, and together with those can

affect directly or indirectly the drug consumption estimates from the back-calculation. Therefore WBE results need to be interpreted with caution [154].

Low potency drugs may require higher doses to give the same pharmacological effect compared to high potency drugs. Low potency drugs may therefore be present in higher concentrations in wastewater. Also drugs that are metabolized to a lesser degree, and thus excreted unchanged for detection, may be present in higher concentrations. To overcome these difficulties, correction factors have been introduced in the back-calculation of the estimates of drug consumed in a population by looking at the concentration of their metabolites or parent compounds in wastewater [46, 61, 155]. The correction factors are based on published urinary excretion kinetic variables for each drug. Also, the route of drug administration is important, since it may also influence the excretion of some drugs. Knowledge on the preferred route of administration of a particular drug in a specific community is therefore important for the choice of the excretion rate in the back-calculation of drug use estimates. Such information needs to be supplied by using traditional epidemiological methods before proceeding with the use of WBE. A minor point is that poly-drug use is common in drug-using communities and therefore the interaction of different drugs to some extent impacts on the excretion of the considered drugs and thus on the estimated level of drug use in a specific community.

The daily fluid flow rate in the wastewater is another important parameter which influences the concentration of drugs and metabolites, and needs to be provided from each STP for each day of the monitoring campaign. For example, during rainy days the concentration of drugs and their metabolites in wastewater is likely to be lower due to an increase in the volume of water coming to the STP, therefore the low concentration does not necessary correspond to a decrease in drug use. By contrast, the concentration of drugs and their metabolites in sewage is likely to be higher during dry days when the volume of wastewater in each STP is lower.

Furthermore, the stability and the choice of the most appropriate metabolite or parent drug in the wastewater of the drug of interest plays an important role in the estimation

of the level of use [47, 156-158]. Some drugs or metabolites are more stable than others in wastewater [159]. For example, cannabis' metabolite (TCH-COOH) is a *sticky* compound and tends to stick on the surface of the pipes in the WWTP leading to an underestimation of the actual use. On the other hand, estimates of drugs like amphetamine, methamphetamine and MDMA based on the parent drugs in the wastewater may lead to an overestimation of the actual use, since they may result from disposal of unused drugs [74]. While it is reassuring that these compounds are stable in wastewater, it introduces the challenge that it is difficult to differentiate between drugs actually consumed in the population and drugs just discarded in wastewater. To overcome this problem, enantiomeric profiling of sewage samples need to be taken [160, 161]. An example of this effect was striking in the results of the first monitoring campaign on the use of illicit drugs across Europe [85]. The levels of amphetamine and MDMA in Belgium and the Netherlands were found to be extremely high as result of the manufacture of these substances in clandestine laboratories in the catchment area and a raid by the police on one of such laboratory during the sampling period.

However, by simply measuring the amount of drug metabolites in wastewater just normalised by flow rate over time without the effort of back-calculating the drug-use estimates, WBE still provides good insight into the patterns of drug use in a specific community, monitoring possible change over time and capturing information on emerging substances. Spatial differences within and between countries can be drawn by normalising the drug loads for the estimated population served by each STP.

5.3.1.2 Height and timing of the weekend peak (FPC2 and FPC3) bias

The second and third FPCs resulting from the FPCA, represented two of the most important weekly temporal features of the use of drugs detected from wastewater analysis in our studies.

The second FPC labelled a "weekend-midweek discrepancy", i.e. the discrepancy between the weekend peak and the midweek level of the drug, may be influenced by changes in the structure of the population served by the WWTP. During the weekends there may be more people coming to the city from neighbour towns leading to an

increase of the affluent population to the STP, rather than merely the increasing use of drug by the local community.

The third FPC was labelled “weekend peak timing”, representing the timing of the weekend peak of the drug. This component may be influenced by two main factors, one related with the characteristics of the WWTP and the second related to the characteristics of the specific drug. Differences in the timing of the weekend peak may reflect not only dissimilarities in drug use pattern, but may result from different retention time of the drug into the sewer system as a result of diverse structure of WWTPs and pipe’s length between different cities. Moreover each drug has a distinctive elimination half-life, there are drugs like cocaine which are excreted from the human body after a short time and other drugs such as cannabis characterised by long elimination half-life [125-127].

5.3.1.3 Spatial comparisons’ bias

As in standard epidemiological research where the main assumption is that the study sample is representative of the entire population under study, also in WBE research the sample collection has a central role and the major assumption is that the wastewater sample collected is composed by a pooled urine sample representative of the entire population served by the WWTP. This aspect is even more important when comparing wastewater results between different countries or even between cities served by different WWTPs in the same country. Understanding the dynamics of the wastewater flow, catchment size, population served by the WWTP and sewer type, is important when choosing the appropriate sampling setup and collecting representative samples which allowing for spatial comparison. Choosing an inappropriate sampling setup can lead to over-interpretation of data and totally wrong conclusions [70]. However, in our studies from different European cities (papers I and III) all the included cities had to provide information on the WWTP, such as sampling procedure, flow rate, estimated population served by the WWTP and its variability (commuters), and structural state of sewers, filling out a specific tailored questionnaire [85]. The cities which did not comply with the minimal requirements were excluded from the studies.

The estimation of the population served by each WWTP is also a source of bias when it comes to spatial comparisons between populations with different sizes. Currently each WWTP provides an estimation of the population served by the plant by using census data or variables such as chemical oxygen demand (COD), biological oxygen demand (BOD), total nitrogen and phosphorus or ammonium [162-164]. However these factors are strongly influenced by the composition of the wastewater, i.e. industrial, domestic or mixed and researchers are currently trying to identify more suitable biomarkers which only account for human metabolism. An ideal biomarker should be unique to human metabolism and have no or minimal exogenous sources, have a stable excretion ratio with minimal variability, be stable in wastewater and be easily determined in environmental samples. Nicotine and caffeine have been explored for this in Italy, and nicotine seemed to be a good candidate for this purpose because of the agreement between the census population and the results obtained by using it as human biomarker [62]. However, while the use of nicotine as a population biomarker may work well in Italy since most of nicotine in wastewater comes from smoking cigarettes, in west Europe the high use of “snus” at least in Nordic countries, makes nicotine an unsuitable biomarker.

5.3.1.4 Comparison between WBE and standard data sources

A number of comparisons between WBE data and standard data sources have been investigated in recent years. Sales data have been used to investigate the most suitable alcohol biomarker from wastewater analysis, looking at agreement between the two data sources [165]; comparison between prescription data and WWA results have been drawn on for pharmaceuticals [166-168] as well as comparing survey data or crime statistics and WWA data for illicit drugs [169-171], while in other cases WBE data have been used as complementary source of information to traditional data sources [172, 173].

These comparisons are also challenging. The comparison needs to be made on the same population in the same time period, and while WBE gives results within 24 hours, a population survey needs longer before the results are available. However, wastewater samples have the advantage that they can be stored in huge databases and investigated

retrospectively when new research questions arise. A study comparing WBE and population survey on the same population was carried out in a small Belgian city [174, 175], but unfortunately, the response rate for the survey was very low and the comparison between the two data sources was inconclusive.

5.3.1.5 Confounding (papers I and II)

Confounding is a bias of the estimated effect of an independent variable on an outcome of interest due to the presence of a confounding factor which associates with both the outcome and the independent variable [176]. Confounders may lead to underestimation, overestimation or changes in the sign of the estimated effects of the exposure on the outcome, and thus need to be accounted for by proper adjustments [177].

In paper I, we estimated the effect of longitude, latitude, density, relative size of the city and GDP on the FPC's scores in multiple regression analyses, as well as on the mean temporal wastewater curve by FANOVA. In the FANOVA analyses we did not adjust for covariates. Dimension of the WWTP, retention type of the drug into the sewage, area covered by the WWTP and population variation may have influenced the results, but could not be adjusted for due to the lack of information and the sample size of the data sets. These and other unknown potential confounders may have biased the results.

In paper II, we estimated the effect of location (north, centre and south), size of the city (large, medium and small), migration rate, gender ratio, and type of drug on the FPC's scores in multiple regression analyses. Also in this case, characteristics of the WWTP and population variation during the week period may have influenced the results as possible confounders. We did not adjust for the pharmacokinetics and elimination half-life of the drugs due to collinearity problems in the analyses.

5.3.2 Statistical analysis

WBE studies on the use of drugs throughout a specific time period are usually presented as mean and standard deviation (SD) and the comparisons between the drug loads are usually carried out using specification tests [3, 46, 51-57, 61, 62, 85]. We were

concerned that these traditional statistics were insufficient for optimal, or even proper, analysis for WWA data, and wanted to explore more advanced statistical methods specifically developed for analysing curve data.

5.3.2.1 Functional data analysis (papers I-IV)

FDA is extra beneficial when large amounts of data are available. In papers I and III we only had seven measurements per each temporal data set. This is quite few, but still enough to extract interesting temporal features. More detailed temporal features could be detected by each FPC when more measurements per day are available. In paper III, besides the low number of measurements per day, also the number of cities in the study was relatively small. Larger sample size would have allowed us to use more explanatory variables in the subsequent multiple regression analysis carried on the FPC's scores, allowing for a better understanding of possible predictors of patterns of drug use.

The choice of basis functions in the FDA may also affect the results. The most commonly used basis functions in FDA are Fourier and B-spline basis functions, for cyclic and non-cyclic data, respectively, even though other alternatives also exist [97-99]. Choosing Fourier basis functions is suitable when the process under study is periodic and thus repeats over time, while B-spline basis functions are primarily used for non-periodic data, thanks to their great flexibility. Papers I-III were based on a one-week-period temporal data set, where the week was the cyclic component of interest. Since we did not have repeated weeks, the temporal process under study was on the boundary between a periodic and non-periodic temporal process. A concern with FDA is that Fourier and B-spline basis functions may over-smooth the underling temporal process if there are rapid changes from one day to another. Wavelet bases may thus represent a useful alternative. Wavelet functions are localized in both time and space, allowing for modelling of less smooth temporal data, even spikes [117-119], and has recently been applied successfully to analysis of foetal movement data [90, 91]. We investigated the effect of three choices of basis function on the WBE results of FPCA in paper III, as well as the impact of the choice of different mother wavelets in the WPCA.

The choice of the smoothing parameter used for removing the random day-to-day variation may also affect results. In papers I and II we used a common-optimal smoothing, estimated using the GCV criterion [114, 178], while in paper III we investigated the results when using different choices of smoothing parameter; common-optimal smoothing, individual-optimal smoothing and no smoothing for FDA, and Bayes, universal and no shrinkage for the wavelets [179-181]. In paper IV no smoothing was used, as smoothing is implicit in the GAM fitting procedure preceding the FPCA.

A simpler alternative to the FPCA may be traditional PCA using each day of the week as independent separate explanatory variables. As the measurements were taken at the same time point for each drug or city, this method would be expected to extract similar information to the FPCA. However, as pointed out in paper III, the interpretation of the results would be difficult since the temporal nature of the underlying process is not taken into account, and the results thus rely on an assumption that is most likely violated.

5.3.2.2 Interpretation of FPCs

FPCA decomposes the variation between curves into a set of uncorrelated temporal features called FPCs, but the usefulness of this analysis depends on how the FPCs can be interpreted. This interpretation is usually drawn by plotting the mean curve and seeing how the shape of an individual curve differs from the mean curve if a multiple of the principal component curve is added to or subtracted from the mean curve [99]. However, this interpretation may be challenging and requires a priori knowledge of the temporal process under study. In all papers, the first FPC mainly represented the general level of drug load in the wastewater, while the second and third FPCs roughly represented the high of the weekend peak and timing of such a peak, respectively.

5.3.2.3 Categorisation of continuous variables (papers I and IV)

In paper I, to compare the results between FANOVA and the regression analysis on the scores of the FPCs we dichotomised the explanatory variables, i.e. longitude, latitude, density, relative size of the city and gross domestic product (GDP), in the FANOVA analysis, as FANOVA can only take categorical covariates. Such dichotomisation is

generally not recommended as it may introduce bias of unknown direction and magnitude [139-141, 182]. Problems with dichotomization were supported by the results of our exploratory analyses. In paper II, to ease the presentation of the results, to be consistent with publications on the same material [128, 183] and to be able to compare with previous studies in the same area [3, 51] we chose to categorise the variables location and size of the city.

5.3.2.4 FANOVA vs multiple regression on FPCs' scores (papers I)

Usually, FANOVA is the suggested way to analyse the association between functional data and covariates [98]. However, FANOVA needs dichotomous explanatory variables, and most of the predictors that we investigated were continuous. In our study applying FANOVA would thus introduce bias in the analysis, due to the arbitrary choice of the number of cities in the various groups [139]. The significance of the F-test was strongly dependent on the chosen cut-off level of the explanatory variable. Moreover, FANOVA cannot adjust for other covariates in a multiple regression model and it only looks at the mean temporal pattern. While this will verify differences between cities, it will not identify the mode of the difference. The suggested multiple regression on the FPCs' scores is not part of the original FDA framework, but opens for more flexibility. It has been proposed previously for the analysis of glucose and foetal movement data [87, 89] with promising results.

5.4 External validity

The generalisability of our study is limited to the cities and countries investigated and needs to be discussed. In paper I, the study was conducted in 42 European cities, covering a total of 21 European countries [86]. In paper II, 17 Italian cities were investigated [128] while in paper IV we looked only at the Norwegian capital, Oslo. However, these analyses may still not represent the total inhabitants of each city we looked at, since the interpretation is limited to the population served by each of the WWTPs.

The findings are limited to the community under investigation and to the observed time-period, and therefore some of our results may be different in other studies. Structural

changes of the population covered by the WWTP or changes in social life, life style factors or economy of a country may have an influence on the resulting drug use patterns throughout the course of a week.

However, the use of standardised methods such as FPCA as a main methodology to investigate those patterns, make it possible to compare results and monitor the extent of the use. In particular the methodology was extended to different areas of interest and was generalizable to different groups of drugs. The investigation of patterns of use of illicit drugs as well as prescription drugs and medicinal drugs enriched the data set and opened up for the generalisability of the statistical methodology to different settings.

6. CONCLUSIONS

In this thesis, we have used FDA to gain insight into the temporal patterns of drug use in a specific community by looking at drug metabolites and parent compounds ending up in the wastewater system. FDA represented a novel statistical approach for analysing WBE data and has shown promising results. The detailed conclusions from our work are the following:

- FDA of WBE data was superior to traditional statistical methods. FDA and in particular FPCA identified important temporal features of the patterns of drug use throughout the week period which was lost when using traditional statistical approaches.
- The first three most important FPC's resulting from the FPCA provided epidemiological interpretation of the extracted WBE temporal patterns on general level of drug load, weekend-midweek discrepancy and weekend peak timing respectively.
- Regression based on FPC results was superior to FANOVA for estimating associations between temporal patterns and covariate information. FPCA extracted all temporal features discovered by the temporally more flexible wavelet approach and the traditional PCA. The FPCA approach was robust and not particularly sensitive to the choice of basis function or to missing data. However Fourier basis functions with common-optimal smoothing parameter generally performed better.
- The second FPC, resulting from the FPCA pointed to possible recreational use of some prescription drugs with known abuse potential.

7. IMPLICATIONS AND FUTURE RESEARCH

WBE is a novel approach in drug use epidemiology which has shown promising results as an alternative to traditional epidemiological methods for investigating the extent of drug use in specific communities. WBE is widely used and provides researchers with large amount of data each year. However there is still a lack of advanced statistical approaches applied to these data and FDA is a new tool for WBE research.

The results from paper I imply that there is a need in this field for statistical methods which can extract information from curves rather than single time points. Currently too many studies restrict the analysis to traditional statistics and specification tests [45-58, 61-68] losing important information about the temporal pattern of drug use, throughout a week period. We thus recommend using FDA for further analysis on WBE data and in particular FPCA.

Moreover the FDA results also demonstrated that the “weekend” is a somewhat less well defined time period than the traditional cultural understanding of it. Using FDA we could estimate what constitutes the “weekend” for each city and each drug, without having to define it a priori, as is needed when applying standard statistical tests. Defining the weekend a priori may introduce bias in the results, since each drug has different elimination half-life [125-127] and each WWTP may have different dimension and pipe length [70, 75].

When the research question is limited to the level of drug use detected from WWA, we found that the AUC is a good statistical indicator because of the high correlation between the AUC and the scores of the first FPC i.e. general level of drug load in the wastewater.

Even though the patterns shown by the most important FPCs were consistent when using different basis functions and smoothing techniques, we recommend using Fourier basis function with common-optimal smoothing parameter, when fitting the curves for the subsequent FPCA. Fourier basis function with common-optimal smoothing parameter showed stable results up to 15% of missing data and narrower empirical CIs for each FPC.

The current methodology has shown promising results also when investigating the abuse potential of prescription drugs. Understanding temporal drug use patterns is a key element in evaluating whether a drug may be abused. The methodology should be explored on a wider range of drugs and the results should be combined with those from other data sources in order to provide epidemiologists, health professionals and policy makers with the knowledge needed for immediate or long-term interventions.

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