Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

Science of the Total Environment 799 (2021) 149230

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Change in the chemical content of untreated wastewater of Athens, Greece under COVID-19 pandemic

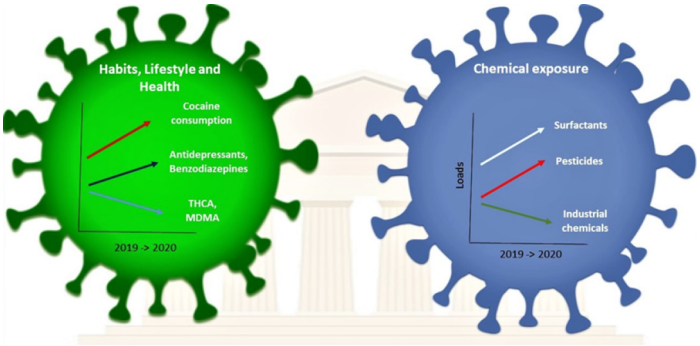
Nikiforos Alygizakis [a,*], Aikaterini Galani [a], Nikolaos I. Rousis [a], Reza Aalizadeh [a], Meletios-Athanasios Dimopoulos [b], Nikolaos S. Thomaidis [a,*]

[a] Laboratory of Analytical Chemistry, Department of Chemistry, National and Kapodistrian University of Athens, Panepistimiopolis Zografou, 15771 Athens, Greece
[b] Department of Clinical Therapeutics, School of Medicine, National and Kapodistrian University of Athens, 15528 Athens, Greece

HIGHLIGHTS

- Findings reflect the effects of pandemic and social distancing.
- Wastewater-based epidemiology was used to assess the exposure to various chemicals.
- Significant changes in illicit drug consumption were observed.
- Surfactants and biocides showed a notable increase 196% and 152%, respectively.
- Significant reduction of the industrial chemicals (52%) due to business closure

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 2 March 2021
Received in revised form 19 July 2021
Accepted 20 July 2021
Available online 30 July 2021

Editor: Damià Barceló

Keywords:
Wastewater-based epidemiology
High resolution mass spectrometry
Wide-scope screening
Illicit drug consumption changes
Surfactants

ABSTRACT

COVID-19 pandemic spread rapidly worldwide with unanticipated effects on mental health, lifestyle, stability of economies and societies. Although many research groups have already reported SARS-CoV-2 surveillance in untreated wastewater, only few studies evaluated the implications of the pandemic on the use of chemicals by influent wastewater analysis. Wide-scope target and suspect screening were used to monitor the effects of the pandemic on the Greek population through wastewater-based epidemiology. Composite 24 h influent wastewater samples were collected from the wastewater treatment plant of Athens during the first lockdown and analyzed by liquid chromatography mass spectrometry. A wide range of compounds was investigated (11,286), including antipsychotic drugs, illicit drugs, tobacco compounds, food additives, pesticides, biocides, surfactants and industrial chemicals. Mass loads of chemical markers were estimated and compared with the data obtained under non-COVID-19 conditions (campaign 2019). The findings revealed increases in surfactants (+196%), biocides (+152%), cationic quaternary ammonium surfactants (used as surfactants and biocides) (+331%), whereas the most important decreases were estimated for tobacco (−33%) and industrial chemicals (−52%). The introduction of social-restriction measures by the government affected all aspects of life.
© 2021 Elsevier B.V. All rights reserved.

1. Introduction

The coronavirus SARS-CoV-2 was firstly reported in the Chinese city of Wuhan in December 2019. Since then, the coronavirus disease (COVID-19) spread all over the world and caused millions of deaths (WHO, 2021). On 11th of March 2020, the World Health Organization

* Corresponding authors.
E-mail addresses: nalygizakis@chem.uoa.gr (N. Alygizakis), ntho@chem.uoa.gr (N.S. Thomaidis).

https://doi.org/10.1016/j.scitotenv.2021.149230
0048-9697/© 2021 Elsevier B.V. All rights reserved.

(WHO) declared a pandemic and COVID-19 became a public health threat that affects and harms the stability of societies (Gu et al., 2020; Holshue et al., 2020). The wastewater-based epidemiology (WBE) community responded rapidly to the urgent need for public health monitoring, by validating new WBE methodologies for SARS-CoV-2. Untreated wastewater samples were collected from many cities worldwide and analytical methods were developed for the detection of SARS-CoV-2 produced by infected asymptomatic and symptomatic people (Ahmed et al., 2020; Alygizakis et al., 2020; La Rosa et al., 2020; Medema et al., 2020). WBE is an advanced approach for measuring endogenous and exogenous biomarkers of humans in untreated wastewater. The applications of WBE have increased rapidly during the last decade and the findings provided important information objectively and in real time about public health, drug abuse, chemical exposure and population lifestyle (Choi et al., 2018; Diamanti et al., 2019; Gracia-Lor et al., 2018; Lai et al., 2018; Ort et al., 2018; Rousis et al., 2020).

Liquid chromatography tandem mass spectrometry is the most widely-used technique in WBE for the identification and quantification of human biomarkers through target screening (reference standards available) (Ort et al., 2018; Thomaidis et al., 2016). Recently, high resolution mass spectrometry (HRMS) methods have gained popularity and played a key role in the identification of overlooked compounds in complex matrices, such as wastewater, through suspect and non-target screening (Aalizadeh et al., 2019; Gago-Ferrero et al., 2020). Application of these new HRMS methods can lead to the tentative identification of hundreds of overlooked emerging contaminants and their transformation products (TPs) and thus provide better insights (Alygizakis et al., 2019a).

So far the majority of the WBE researchers have focused on the detection of SARS-CoV-2, with a few studies evaluating substances related to population habits and life style changes during the COVID-19 pandemic (Bade et al., 2020a; Reinstadler et al., 2021; Wang et al., 2020). Changes in alcohol consumption due to social distancing measures activated by COVID-19 were investigated in South Australia. Wastewater analysis showed that alcohol consumption decreased following enforced restrictions. Moreover, the usual weekend peak that is typical for alcohol consumption was also flatter than usual (Bade et al., 2020a). Likewise, a study in Austria observed a similar trend where the alcohol consumption declined around 20%. The same study investigated licit and illicit drug consumption. No variation before and during COVID-19 lockdown on the consumption of long-term pharmaceuticals was found, but significant changes were estimated for short-term pharmaceuticals and some recreational drugs. No statistically significant difference was found for caffeine and nicotine consumption (Reinstadler et al., 2021). In New York, a research group compared the quantity of SARS-CoV-2 in wastewater with the overall COVID-19 positive tests in humans and the consumption of drugs estimated by WBE analysis. It was concluded that high quantities of SARS-CoV-2 in wastewater and many positive clinical tests were strongly correlated to higher consumption rates of antidepressants, antiepileptics, antihypertensives, antihistamines, opioids, and stimulants (Wang et al., 2020). Finally, the stress of the population in Kentucky and Tennessee during the first period of the pandemic was assessed by determination of isoprostanes in wastewater. The elevated levels of these specific human stress biomarkers in wastewater were attributed to the oxidative anxiety induced by COVID-19 uncertainties (Bowers and Subedi, 2021).

The objective of this study was to investigate the presence of different classes of chemical compounds in influent wastewater before and during the pandemic that could be related to measures taken by the authorities, such as social distancing and lockdown. Therefore, novel HRMS methods such as wide-scope target and suspect screening were used to screen for the occurrence of emerging substances in combination with WBE to estimate the exposure and use of the detected substances. Critical evaluation and comparison of the results under lockdown conditions and under non-pandemic conditions were performed and differences in the consumption were revealed for (i) antipsychotic drugs including benzodiazepines and antidepressants, (ii) illicit drugs and (iii) markers of human habits and activities, such as food additives, dietary supplements, tobacco compounds, biocides and surfactants. For the first time, this study examined and estimated the loads of such a high number of compounds (11,286 compounds were screened) from various chemical classes that might be associated with the COVID-19 pandemic situation in Athens, Greece. This work can be used as an indicator for further studies and could potentially be extended to other countries.

2. Material and methods

2.1. Chemicals and reagents

Acetonitrile, methanol, hydrochloric acid (37%) and ethyl acetate (99.9%) were purchased from Merck (Darmstadt, Germany), 2-propanol was purchased from Fisher Scientific (Geel, Belgium), sodium hydroxide monohydrate (NaOH) for trace analysis, formic acid, ammonium formate were purchased from Fluka (Buchs, Switzerland), and ammonia 25% was purchased from Panreac (Barcelona, Spain). Empty polypropylene tubes (6 mL) and the following sorbent materials: Sepra ZT (Strata-X), Sepra ZT-WCX (Strata-X-CW) and ZT-WAX (Strata-X-AW) were purchased from Phenomenex (Torrance, USA). The polar sorbent (hyper crosslinked hydroxylated polystyrene-divinylbenzene copolymer; isolute ENV+) and the frits (20 μm, 6 mL) that separate the cartridge in layers were purchased from Biotage (Ystrad Mynach, UK). Wastewater filtration was realized with glass fiber filters (GF/F, pore size 0.7 μm), purchased from Whatman International Ltd. (Maidstone, England).

Benzodiazepines and antipsychotics antidepressants, further subcategorized into tricyclic, tetracyclic, selective serotonin reuptake inhibitors (SSRIs), serotonin−norepinephrine reuptake inhibitors (SNRIs), were purchased from Alfa-Aesar (Voula, Athens, Greece) and Merck (Chalkidona, Greece). Target compounds were supplied by Bruker Daltonics (in total 675 reference standards of pesticides), the EAWAG (270 standards covering different chemical classes), the Laboratory of the Olympic Sports Center of Athens (142 illicit drugs including new designer drugs). Drugs of abuse, divided in the following sub-categories: i) opiates, opioids and related metabolites, ii) amphetamines, iii) hallucinogens (cannabinoids, lysergic acid diethylamide (LSD), and derivatives)) and iv) cocaine and related metabolites were purchased from LGC Standards (Athens, Greece). Information on the determined compounds is given in Table S1 of the Supporting Information (SI).

2.2. Sample collection

24-h composite flow-proportional influent wastewater samples were collected from the inlet of Psyttalia WWTP in cooperation with Athens Water Supply & Sewerage Company (EYDAP). The WWTP of Athens is designed with primary sedimentation, activated sludge process with biological nitrogen and phosphorus removal and secondary sedimentation. The hydraulic retention time in bioreactors is 9 h, and the sludge residence time is 8 days. The sludge is managed by thickening, anaerobic digestion and mechanical dewatering. The closest connected household is 0.5 km and the most remote 30 km from the WWTP. Seven daily samples were collected in 2019 under non-pandemic conditions (from 13th to 19th of March), in context of the annual monitoring campaign organized by the SCORE action ES1307 (Gonzalez-Marino et al., 2020). Samples were obtained during a 'normal' week, avoiding special events such as public holidays or festivals and heavy rain conditions that can potentially limit the WWTP capabilities. A prolonged sampling of fifteen wastewater consecutive days was executed in 2020 under full lockdown conditions (from 25th of March until 8th of April). The first lockdown involved business closure, school closure, court closure, traveling restriction, interruption of sports, social

distancing measures and strict restrictions in movement of citizens (Hellenic Republic, 2020). The average wastewater flow rate was 794,871 m^3 day^{-1} in 2019 and 788,727 m^3 day^{-1} in 2020. The number of inhabitants was estimated at 3,995,020 for 2019 and 4,009,346 for 2020 (Been et al., 2014). The estimated number of inhabitants between the two campaigns did not change significantly. All the information about the studied WWTP can be found in Section 2 of the supporting information (SI) document. All influent wastewater samples were collected in high-density polyethylene bottles previously cleaned with methanol and Milli-Q water. The wastewater samples were filtered with glass fiber filters (pore size 0.7 μm) upon arrival at the laboratory and the pH was adjusted (6.5 ± 0.1) using formic acid 0.1 M.

2.3. Sample preparation protocol

Extraction of the samples was carried out using a modified protocol (Gago-Ferrero et al., 2015; Kern et al., 2009). Sample aliquots (100 mL) were pH-adjusted, spiked with a mix of internal standards (Table S3E, SI) and then passed through the cartridge made by different sorbent types (Strata-X, Strata-X-CW, Strata-X-AW, isolute ENV+) (Gago-Ferrero et al., 2020). Briefly, the cartridges were activated with 3 mL of methanol, followed by 3 mL of water. Afterwards, the samples were loaded without using vacuum. Then, cartridges were dried for 1 h under vacuum. The elution was performed with a mixture of 4 mL methanol and ethyl acetate (50:50, *v*/v) containing 2% ammonia and a mixture of 2 mL methanol and ethyl acetate (50:50, v/v) containing 1.7% formic acid. The extracts were evaporated to a volume of 50 μL approximately, using a nitrogen stream and were reconstituted to a final volume of 500 μL methanol and water (50:50, *v*/v). Finally, the extracts were filtered using 0.2 μm RC membrane filters (Phenomenex, Torrance, CA, USA).

2.4. Instrumentation and analytical methods

The extracts were analyzed with two instrumental methods: a highly sensitive method for the detection and quantification of drugs of abuse and antipsychotic drugs by liquid chromatography tandem mass spectrometry (LC-MS/MS) (Alygizakis et al., 2016; Thomaidis et al., 2016) and a wide-scope target screening method able to screen compounds of various chemical classes by liquid chromatography quadrupole time-of-flight mass spectrometry (Gago-Ferrero et al., 2020; Gago-Ferrero et al., 2015). The LC-MS/MS method was used for selected target drugs since it yields lower limits of detection (LOD).

2.4.1. LC-MS/MS analytical method

LC-MS/MS analysis was conducted using a Thermo UHPLC Accela system, which was connected to a Thermo TSQ Quantum Access triple-200 quadrupole mass spectrometer (San Jose, USA). The mass spectrometer is equipped with an electrospray ionization source in both positive and negative ionization. The chromatographic column Atlantis T3 C18 (100 mm × 2.1 mm, 3 μm) was used for separation at a constant flow rate (100 μL min^{-1}). The injection volume was 10 μL. Detailed information about the gradient program and the ESI settings are presented in Table S3A.

2.4.2. LC-HRMS analytical method

LC-HRMS analysis was performed on an UHPLC/QTOF-MS system. The system is equipped with a UHPLC device (Dionex UltiMate 3000 RSLC from Thermo Fisher Scientific) and is coupled to the Bruker Maxis Impact QTOF-MS/MS analyzer (Bremen, Germany). The chromatographic column Acclaim RSLC C18 column (2.1 × 100 mm, 2.2 μm) was preceded by an pre-column ACQUITY UPLC BEH C18 1.7 μm (Waters, Ireland). The pre-guard column and the analytical column were thermostated at 30 °C during separation.

The samples were analyzed with four acquisition modes: broadband collision-induced dissociation (bbCID, data-independent) in positive ionization, bbCID in negative ionization, data-dependent MS/MS acquisition (AutoMS) recording the five most-abundant ions per scan in positive ionization and AutoMS in negative ionization. In both cases the scanning range was set to 50–1000 Da with scan rate of 2 Hz. The collision energy was set to 25 eV for bbCID and predefined values based on the mass and the charge state of every ion. More information of the instrumental performance is provided in Tables S3B and S3C.

2.5. Computational resources

2.5.1. LC-MS/MS computational workflow

The Xcalibur software suite (TSQ14, SUR1, XReport 1.0, 2.0 SR2) was used for instrument control, data recording and assessment. The selected reaction monitoring (SRM) mode was used and the transitions of the precursor ion and its two most abundant product ions were monitored. All the peaks were finally integrated using LCquan 2.7 (Thermo Fisher Scientific).

2.5.2. LC-HRMS computational workflow

The raw files of the LC-HRMS analysis (target screening), were processed by Bruker TASQ 1.4 software (Bruker Daltonics, Bremen, Germany). A calibration method ensured mass accuracy below 2 mDa for the whole chromatographic run. Target list of the National and Kapodistrian University of Athens can be found at NORMAN suspect list exchange (https://www.norman-network.com/nds/SLE/).

For wide-scope suspect screening, all files were converted to open-source format (mzML) using Bruker CompassXport 3.0.9.2. (Bremen, Germany). Data files with meta-data were uploaded to NORMAN Digital Sample Freezing Platform (DSFP) (Alygizakis et al., 2019b).

The uploaded mzML samples in DSFP pass from a non-target screening workflow involving processing with centWave peak picker (via xcms R-package) (Tautenhahn et al., 2008) using previously optimized *ppm* and *peakwidth* parameters (Libiseller et al., 2015). The peak picking algorithm searches for consecutive masses within a mass error threshold forming peak shape in chromatographic dimension (Tautenhahn et al., 2008). The next step is componentization, which is a procedure for grouping peaks coming from the same compound (adducts, isotopic peaks, in-source fragments). For this purpose, functions from nontarget R-package are used (Loos et al., 2012). The final output is a component list. This component list can be searched for thousands of compounds by suspect screening. Once the samples are uploaded to DSFP, they can be screened for a 'yes/no' presence of virtually any compound amenable to LC-MS analysis using a combination of information on its (i) exact mass, (ii) predicted retention time window in the chromatogram (iii) isotopic fit and (iv) qualifier fragment ions.

DSFP is part of the NORMAN Database System (NDS) (Dulio et al., 2020). It is connected to the NDS module called NORMAN Substance Database (https://www.norman-network.com/nds/susdat/) (NORMAN Network et al., 2020), in which mass spectrometric information for the contaminants is collected (adducts, fragmentation pattern, preferable ionization, predicted retention time index etc.). DSFP uses this information to screen substances.

Suspect screening of 9150 compounds was performed in this study: 7586 environmentally-relevant compounds with known fragmentation, and the surfactant specific lists S7 EAWAGSURF (410 compounds) and S23 EIUBASURF (1154 compounds).

2.6. Semi-quantification methodology

Detected suspected compounds were semi-quantified based on the standard addition curve of the structurally most similar target compound. For example, the suspected plasticizer diisobutyl phthalate was semi-quantified based on the calibration curve of the diethylphthalate, because the compounds have high structural similarity. To find the structurally most similar target compound, 2D-linear fragment descriptors based on the original definitions of atom pairs and atom

sequences were calculated (Chen and Reynolds, 2002) and Tanimoto coefficient was used as the similarity distance function. The output of the calculation is the structurally most similar target compound, and the similarity percentage between the suspected compound and the compound with the closest structure is reported. The semi-quantification approach was validated to acquire some knowledge on the expected uncertainty. The calibration curves for 778 compounds were generated for positive (681 compounds) and negative (207 compounds) electrospray ionization. These calibration curves were used to semi-quantify compounds, which were previously quantified by target screening analytical methods. The result of this investigation, which was performed in an iterative way, leaving out a known concentration at a time, can be found in Fig. S3D. The percentage structural similarity proved to be a good measure of the uncertainty of the semi-quantification. Overall, structure similarity above 30% indicated a maximum error in semi-quantification of one order of magnitude. This semi-quantification method together with other semi-quantification strategies (Kruve et al., 2021) is currently under comparison within the collaborative trial on semi-quantification organized by the NORMAN network (NORMAN Network, 2021).

2.7. Quality assurance and quality control

For each sampling year, 24-h flow-proportional influent wastewater samples were collected by the WWTP operators and were stored in a freezer (−20 °C) at the laboratory of the water company (EYDAP S.A.) in the Psyttalia island. All the samples were transferred in a portable freezer at the laboratory and were let at room temperature in the fume hood to defreeze. The samples were processed immediately after defreeze and in a single batch. The samples collected from 13th to 22nd of March 2019 were processed immediately after the campaign and were analyzed as soon as the instruments were available (27th and 28th of March 2019). The same methodology was followed for the sampling campaign of 2020 (sample collection from 25th of March to 8th of April 2020), immediate processing and analysis (11th and 12th of April 2020). The reason of the immediate processing was to minimize the likelihood of potential degradation of the samples.

For each year, the batch of the samples was supplemented with an ultrapure Milli-Q water sample serving as procedural blank sample with the aim to identify any contamination originating from solvents and laboratory conditions. Moreover, a pooled sample (mixture of all influent samples) was divided in four fractions and was spiked with target compounds at the following concentration levels 0, 10, 100 and 1000 ng L^{-1}. Quantification was performed by standard addition for the majority of the compounds and by isotopic dilution for selected compounds (internal standard available). An additional quantification experiment was conducted for few detected target substances that were not initially spiked or/and substances that were spiked but proved to be at much higher concentration levels (>1000 ng L^{-1}). All samples (including spike samples) were spiked with a mix of internal standards (Table S3E) at 100 ng L^{-1} level to facilitate precise quantification of these specific target analytes, for evaluation analyte losses (recovery) during the sample preparation and for evaluation of instrumental stability (injection volume, instrument sensitivity and ionization efficiency). Prior to the analysis of the extracts, the electrospray ionization (ion transfer tube and skimmer cone) was cleaned with a mixture of water and isopropanol (70:30) in a thermostated sonication device (50 °C) for 15 min followed by Milli-Q water (15 min, 50 °C) and methanol (15 min, 50 °C). The mass spectrometer was let at operate mode for 45 min, was calibrated with sodium formate (LC-QTOF) and polytyrosine (LC-MS/MS) and was checked regarding sensitivity. The performance of the instruments was continuously monitored through the peak area and the retention time of the internal standards. The performance of the instruments during the chromatographic batch proved to be satisfactory in terms of quality assurance and quality control (Ng et al., 2020; Schulze et al., 2020). The chromatographic system proved to be highly repeatable with drift in retention time less than 0.1 min based on the expected retention time of target compounds. No loss in sensitivity was observed based on the response of the internal standards (Fig. 3F, SI). Principal component analysis (PCA) in positive (Fig. 3G, SI) and negative ionization (Fig. 3H, SI) showed clustering of the QC samples, samples of 2019 and 2020 showed high variation and no specific clustering and blank samples were in both cases isolated from the samples. It is worth stating that the laboratory is accredited (ISO/IEC 17025:2017) for numerous analytical methods using equipment and instruments that were used in this study. Moreover, the laboratory has established standard operational procedures (SOPs) for performance check of the instruments.

All target detections passed the following identification criteria: i) mass accuracy <2 mDa, ii) RT tolerance ±0.2 min, iii) presence of at least two qualifier fragment ions, except substances that do not yield more fragments because of their chemical structure and iv) compliant isotopic fit in case isotopic peaks were available. All suspect identifications were verified manually to reduce the possibility of false findings. The detections were allocated in identification confidence levels (Table S1, SI). Identification confidence 2A was indicated with spectral match between experimental and library spectrum with match more than 90% using OrgMassSpecR (Dodder and Mullen, 2014; Stein and Scott, 1994).

2.8. Calculation of loads, assessment of the consumption of drugs and statistical analysis

Mass loads were calculated by multiplying the concentrations and the daily wastewater flow rate for each daily sample. Afterwards, these loads were multiplied by specific correction factors (Table 3). Finally, population normalized consumption rates were estimated, by dividing by the number of inhabitants (inh.) within the WWTP service area and then multiplying by 1000 (mg/day/1000 inh.). The non-parametric Wilcoxon signed-rank test was utilized to compare the load levels between the two sampling campaigns considering a statistical significance level of $p < 0.05$, given that the data were not normally distributed. The application of the statistical test was realized in R v4.0.3 through the function *wilcox.test* included in the stats R-package.

3. Results and discussion

The Greek government announced the total lockdown due to COVID-19 pandemic on the 23rd of March 2020. The official lockdown lasted more than five weeks according to the national organization of public health (EODY), followed by gradual lifting of restrictions. The pandemic lockdown caused serious economic losses, reduction in trading and drastic lifestyle changes. The results of the present study reflected the change of the chemical universe of untreated wastewater under COVID-19 pandemic.

The detected classes of compounds are summarized in Table 1. The highest increase under lockdown conditions were observed for cationic quaternary ammonium surfactants (+331%), surfactants (+196%) and biocides (+152%), whereas the most important decrease was found for tobacco (−33%) and industrial chemicals (−52%).

The loads for all individual substances are provided at Section 4 (SI). The results of suspect screening are presented as heatmaps in SI at Section 5. The loads (g day^{-1}) and the % change before and during the COVID-19 pandemic for the various detected chemicals are discussed in detail per chemical class.

3.1. Antipsychotic drugs

COVID-19 pandemic and the measures implemented by the authorities had short- and long-term psychosocial and mental health implications (Singh et al., 2020). Studies performed in Greece showed that the citizens were affected by severe anxiety, fear, depressive symptoms and

Table 1
Mean mass loads (g day^{-1}), standard deviations (SD) of the untreated wastewater samples for each year and % change during the study period (2019–2020).

Chemical class	Loads 2019 (g day^{-1})	SD 2019	Loads 2020 (g day^{-1})	SD 2020	Change 2019–2020 (%)	*p*-Value
Cationic surfactants (Biocides)	478	173	2061	476	+331	<2.0E−06
Surfactants	327,063	32,953	968,818	163,259	+196	<2.0E−06
Biocides (used also as pesticides)	62	4.7	156	8.5	+152	<2.0E−06
Food additives and Dietary Supplements	10,394	1852	22,128	1673	+113	<2.0E−06
Illicit drugs	1243	86	1632	73	+31	<2.0E−06
Benzodiazepines	257	21	309	16	+20	4.1E−06
Pesticides only	5174	329	5873	350	+14	2.0E−04
Antidepressants	2156	111	2227	92	+3	8.4E−02
Antipsychotics	481	28	481	11	0	5.1E−01
UV filters	1100	145	886	63.9	−24	2.8E−04
Tobacco	58,929	4582	44,401	3466	−33	<2.0E−06
Industrial chemicals	37,145	5757	24,371	1558	−52	<2.0E−06

uncertainty to this frightening condition due to disrupted social, and family life, education disruption, traveling cancelation, and other aspects of life (Dagklis et al., 2020; Kaparounaki et al., 2020; Parlapani et al., 2020). Wastewater analysis showed which psychoactive pharmaceuticals were used more under pandemic conditions and to what extent. The combination of wastewater results with medical, sociological and psychological studies can give more insight into the mental health of Athenians' and, potentially, other populations worldwide. Our findings showed no increase for antipsychotics, insignificant increase by 3% for antidepressants and increase in benzodiazepines by 20%.

Other pharmaceuticals, not belonging to antipsychotic drugs which are used for therapeutic purposes (e.g. antihypertensive drugs, antiepileptic drugs, analgesics and many other classes) proved to be a class of compounds with high variations in consumption during the first strict lockdown. Given their importance and the need for thorough investigation of their consumption patterns, they were studied separately elsewhere (Galani et al., 2021).

3.1.1. Benzodiazepines

Benzodiazepines are a class of psychoactive pharmaceuticals with anxiolytic and hypnotic action. During COVID-19 pandemic, an overall increase (+20%) was observed (Table 1). In 2019, the detected concentration levels for lorazepam, one of the most widely prescribed anxiolytic drugs (Garcia et al., 2018), were approximately 111 g day^{-1} and a remarkable increase of 77% was observed in 2020. However, the detected loads for few benzodiazepines did not follow the same change of lorazepam and showed a slight decrease in 2020. Oxazepam, the second most widely used benzodiazepine, showed a slight decrease of −16%.

3.1.2. Antidepressants and antipsychotics

The concentration levels of antidepressants showed a non-significant increase of +3% during lockdown. A noteworthy increase was observed only for venlafaxine, a serotonin−norepinephrine reuptake inhibitor (SNRI), from 383 g day^{-1} in 2019 to 1059 g day^{-1} in 2020. On the contrary, 8-OH-Mirtazapine showed a substantial decrease in 2020 (from 653 g day^{-1} to 325 g day^{-1}). Overall, the observations for antidepressants, used to monitor mental health of a catchment, are in agreement with the results of Tyrol region in Austria (Reinstadler et al., 2021). Both Reinstadler et al., and our WBE results did not show significant variations, even though there is evidence that the lockdown and quarantine had an impact on mental health of the communities (Parlapani et al., 2020).

Mass loads of antipsychotics remained steady. The compounds of this pharmaceutical class are used to manage psychosis, schizophrenia, paranoia or disordered thought. Mesoridazine was the only antipsychotic drug that showed noteworthy increase during lockdown (from 18 g day^{-1} to 39 g day^{-1}, p-value<0.05), perhaps due to a change in prescription patterns.

3.2. Illicit drugs

Tetrahydrocannabinol-9-carboxylic acid (THCA) did not show a statistically significant change during quarantine (Table 2), which is in agreement with the report by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) (EMCDDA, 2020). Ecstasy (MDMA) showed a significant decrease during lockdown. The loads in wastewater were reduced from 24 g day^{-1} to 11 g day^{-1}. The loads were back-calculated in consumption (Table 2) and doses per 1000 people (Table 3). The doses for MDMA were calculated to 0.28 per 1000 people in 2019 and 0.13 per 1000 people in 2020. MDMA is a substance largely linked to parties, clubs and generally night life. The stay-at-home measures and the closure of night clubs drastically affected the concentration levels of MDMA.

However, an increasing trend was observed for other stimulants such as amphetamine, methamphetamine and cocaine. Cocaine consumption was monitored through concentrations of benzoylecgonine, which is the major metabolite of cocaine. Amphetamine, showed a noteworthy increase in consumption. Methamphetamine use in Athens continue to increase during the last years (Gonzalez-Marino et al., 2020). This trend continued and loads increased from 45 g day^{-1} in 2019 to 62 g day^{-1} under lockdown conditions in 2020. These mass loads were back-calculated to 0.87 doses per 1000 people for 2020 and 0.59 for 2019. Benzoylecgonine showed a substantial increase from 207 g day^{-1} to 350 g day^{-1}. This translates to an increase of cocaine consumption from 2.0 doses per 1000 citizens to 3.39 doses per 1000 citizens. This remarkable increase in consumption of cocaine, amphetamine and methamphetamine is of concern and highlights that users maintained their access to illicit drugs despite the strict measures in movements. It is also likely that this increase is associated with the increasing depression levels observed during lockdown (Fancourt et al., 2021). Further investigation to confirm the establishment of these mental health conditions is needed to reach robust conclusions.

Despite the increasing trend, cocaine consumption remained lower than the mean consumption estimated in 120 cities (37 countries) between 2011 and 2017 (Gonzalez-Marino et al., 2020). Methamphetamine consumption is close to the average consumption levels,

Table 2
Consumption (mean ± standard deviation) of cocaine, amphetamine, methamphetamine, and MDMA expressed as mg/day/1000 people, using correction factors.

Compound	Consumption 2019	Consumption 2020	*p*-Value	Variation
Amphetamine	20.0 ± 10.4	86 ± 22	1.7E−05	Increase
Cocaine	134 ± 38	216 ± 39	2.1E−04	Increase
Methamphetamine	25.9 ± 8.4	37.9 ± 6.6	5.6E−03	Increase
MDMA (ecstasy)	8.8 ± 3.4	4.1 ± 2.6	7.4E−03	Decrease
THC (cannabis)	7389 ± 2075	5913 ± 1793	9.1E−02	Decrease

Table 3
Correction factors used in the present study and calculated doses per 1000 people under normal (year 2019) and strict lockdown (year 2020) conditions.

Compound	Biomarker	Correction factor	References for correction factors	Doses (mg)	References for doses	Doses per 1000 people in 2019	Doses per 1000 people in 2020
Cocaine	Benzoylecgonine	3.59	(Gracia-Lor et al., 2016; Gonzalez-Marino et al., 2020)	100	(Gonzalez-Marino et al., 2020)	2.0	3.39
Amphetamine	Amphetamine	2.77	(Gracia-Lor et al., 2016; Gonzalez-Marino et al., 2020)	50	(Gonzalez-Marino et al., 2020)	0.36	3.64
Methamphetamine	Methamphetamine	2.44	(Gracia-Lor et al., 2016; Gonzalez-Marino et al., 2020)	50	(Gonzalez-Marino et al., 2020)	0.59	0.87
MDMA (ecstasy)	MDMA	4.4	(Gracia-Lor et al., 2016; Gonzalez-Marino et al., 2020)	100	(Gonzalez-Marino et al., 2020)	0.28	0.13
Nicotine	Cotinine	3.4	(Montes et al., 2020)	1.25	(Montes et al., 2020)	3800	2347
Nicotine	Hydroxycotinine	1.9	(Montes et al., 2020)	1.25	(Montes et al., 2020)	3472	2809
THC (Cannabis)	THCA	0.6	(Zuccato and Castiglioni, 2011)	125	(Zuccato and Castiglioni, 2011)	59.1	47.3

whereas amphetamine is above the average consumption levels revealed by the same multinational study (Gonzalez-Marino et al., 2020). The findings of our study indicate that significant and rapid changes in drug consumption patterns were observed during the early stages of the COVID-19 pandemic. These changes can be attributed to the implementation of confinement and restriction of social interactions (Bergeron et al., 2020; EMCDDA, 2020).

Another interesting observation was the identification using the HRMS target method and corresponding reference standards, of other stimulants, such as amphetamine-p-hydroxy, para methoxy amphetamine (PMA), para-methoxy-*N*-methylamphetamine (PMMA) and amfepramone that were found only in 2020. It has to been noted that these compounds were rarely detected in other studies. PMMA was only identified in Athens and Zurich (Kinyua et al., 2015; Kinyua et al., 2021) and PMA in Athens and Australia (Bade et al., 2020b; Diamanti et al., 2019; Tscharke et al., 2016). Suspect screening analysis resulted in the identification of six stimulants, namely cis-4-methylaminorex, *N*-methyl-2-AI, TDIQ, MMDA-2, 4,4'-Dimethylaminorex and N-hydroxy-MDA (Fig. S5B). Three of them were detected only in 2020. This is the first time that these compounds were detected in influent wastewater, as can be verified from the a published study (Bijlsma et al., 2019). The increasing detection of new stimulants in wastewater is of concern and may indicate that drug users were pushed toward alternative psychotropic drugs due to social isolation and the limited access to detoxification centers (Zaami et al., 2020).

Seven opioids (buprenorphine, morphine, codeine, codeine-6-glucuronide, 6-monoacetylmorphine, methadone, EDDP) were detected in untreated wastewater, including parent compounds and human urinary metabolites. More specifically, the mass loads of buprenorphine, a prescription opioid, were decreased significantly in 2020, but the loads of methadone remained stable. However, the metabolite of methadone (2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine, EDDP) showed increased mass loads (35%) in 2020. The ratio of EDDP/methadone measured in wastewater was 1 (2019 campaign) and 1.5 (2020 campaign) indicating that direct disposal of methadone occurred both years, since a ratio around 2 is used to confirm that the found levels are mainly due to human metabolism (Du et al., 2019). Mass loads of morphine and codeine were increased substantially, but the exclusive metabolite of heroine, 6-acetylmorphine, decreased drastically (90%) in 2020. This metabolite has low stability, can revert to morphine in wastewater and its excretion rate is limited (Gracia-Lor et al., 2016; McCall et al., 2016). The transformation of 6-acetylmorphine to morphine could be a potential reason for the increased mass loads of the latter. Back-calculation of heroin based on morphine (most abundant metabolite) should be done with caution, since morphine is also a metabolite of some pharmaceuticals such as codeine, ethylmorphine and pholcodine and the amount of therapeutic morphine and codeine should be subtracted (Gracia-Lor et al., 2016). Therefore, back-calculation was not performed for heroin.

3.3. Tobacco consumption

Smoking is associated with the risk of respiratory tract infections. A population-based research suggested that youth using e-cigarettes and cigarettes are at higher risk of COVID-19 (Gaiha et al., 2020). However, the harm from the joint effect of tobacco use and COVID-19 is not fully supported (Van Hal, 2019; van Zyl-Smit et al., 2020). In this study, nicotine and two human urinary metabolites, namely cotinine and hydroxycotinine that represent smoking habits of a population were determined in wastewater. According to the findings, a reduction in smoking habits was noticed. The cumulative loads of cotinine, hydroxy-cotinine and nicotine reduced from 58,929 g day^{-1} to 44,401 g day^{-1} in 2020. Furthermore, nicotine consumption was calculated using the WBE approach and specific correction factors (Table 3). The number of cigarettes per 1000 people for the campaign of 2019 were estimated between 3472 and 3800, while for 2020 were calculated between 2347 and 2809. This behavior can be regarded as a compliance of citizens to the studies associating smoking with SARS-CoV-2 (Grundy et al., 2020), which was intensively communicated by the mass media during the first lockdown. It should be stated that some people may found the opportunity to quit smoking, since it was revealed that the pandemic is related to respiratory diseases. On the contrary, some others may presented an increased desire to smoke due to augmented stress of a possibly fatal illness, possibility of unemployment and feelings of insecurity (van Zyl-Smit et al., 2020). The results of this study showed that the general population decided to reduce smoking. Our investigation highlights that WBE can act as monitoring tool to evaluate the effect of decision-making by policy-makers to measure the effect of promotional activities.

3.4. Surfactants

In the present study, various classes of surfactants were examined under normal conditions in 2019 and during lockdown in 2020. An overall significant increase (196%, p-value <2.0E-6) was observed that indicated a wide use for all the compounds with disinfectant activity (Tables 4 and S5C). The study reported anionic detergents and surfactants found in personal care products (soaps, shampoos, and toothpaste), surfactants used as antioxidants, solubilizers, laundry and dish products and anionic surfactants used in dishwashing and laundry, such as sodium lauryl ethyl sulfates (SLES), linear alkylbenzene sulfonates (LAS), sulfophenyl alkyl carboxylic acids (SPAC), nonylphenol ethoxylates (NPEO), nonylphenol ethoxylate sulfate (NPEO-SO_4), secondary alkane sulfonates (SAS), glycol ether sulfates (GES), cationic surfactants and polyethylenoglycols (PEGs).

It is noteworthy, that this is the first study that reported the detection of such long homologue series of PEGs in wastewater of Athens. The heatmap that includes the summary of all detected surfactants, highlights the increase of all classes of surfactants during lockdown

Table 4
Mean mass loads expressed in g day^{-1}, standard deviations (SD), % change during the study period (2019–2020) and *p*-values for various classes of surfactants.

Class	Loads 2019 (g day^{-1})	SD 2019	Loads 2020 (g day^{-1})	SD 2020	Change 2019–2020%	*p*-Value
SLES	20,297	4212	91,975	60,821	+353	2.E−04
LAS	97,229	20,425	358,832	114,268	+269	1.E−07
SPA-C	12,060	4688	32,813	9013	+172	2.E−07
SPC-C	11,994	4820	33,097	9000	+176	2.E−07
STA-C	836	274	1132	292	+35	1.E−02
DATS	2266	520	8495	3668	+275	5.E−06
NPEO and NPEO-SO4	398	160	1583	784	+298	2.E−05
SAS	2778	2049	10,568	5393	+280	4.E−05
GES	37,359	12,660	199,292	94,559	+433	5.E−06
cationic surfactants	429	156	1897	673	+342	2.E−07
PEGs	141,417	20,997	229,135	27,383	+62	5.E−08
Surfactants	327,063	32,953	968,818	163,259	+196	5.E−11

(Fig. S5C). Stay-at-home measures, working from home, social distancing, hand-washing and surface-disinfection advisories may have driven the observed high detection levels for all the aforementioned compounds in 2020 compared to the previous year. More specific, LAS, GES and PEGs were the 3 surfactant classes with the highest detection levels for both years.

3.5. Pesticides and biocides

The European Commission has implemented different regulations for the chemical classes of pesticides and biocides (also referred as plant protection products, PPPs) and it distinguished them according to their use. Pesticides are used to protect plants against harmful organisms, to preserve the crop, and to prevent the growth of competitive plants (European Commission, 2009). Likewise, the European Biocidal Product Regulation (BPR) defines that biocides are used "with the intention of destroying, deterring, rendering harmless, preventing the action of, or otherwise exerting a controlling effect on, any harmful organism by any means other than mere physical or mechanical action" (European Parliament and Council, 2012). Although, distinct regulations were applied, some active ingredients can be used both as pesticides and biocides. Thus, the findings of the present work were categorized in three groups: 1) PPPs used as pesticides only; 2) PPPs used as biocides and pesticides; 3) cationic surfactants with increased biocidal activity.

A slight increase of pesticide amounts was observed in the present work (Table 1). However, high variation was estimated for the compounds; for instance, the fungicides spiroxamine (+364%) and propamocarb (−256%) showed the highest increase and reduction, respectively. It has to be noted that seven pesticides were detected only in the 2020 sampling campaign (Table S4). Nevertheless, the herbicide dinoterb and the plant growth regulator indole-3-acetic acid showed high mass loads in 2019 and both were reduced significantly in 2020. Metolachlor and its transformation product metolachlor-morpholinon presented the same % increase during quarantine. The use of metolachlor is banned in Europe, but its isomers' s-metolachlor is licensed. The occurrence of metolachlor was confirmed by the corresponding standard, but one of the main limitations of the generic HRMS methodology is that usually cannot distinguish isomeric compounds. Furthermore, it is expected that s-metolachlor presents the same characteristics (e.g. retention time and fragment ions) with those of metolachlor. Thus, it cannot be confirmed if it is either metolachlor which has been banned or its isomer. Amitraz was not detected in either campaign, in contrast to its main transformation product N-2,4-dimethylphenylformamide. Furthermore, two pesticides, namely fluazifop-P and thiofanox-sulfoxide were detected only in 2020 sporadically by suspect analysis. It should be stressed that in total twenty pesticides were quantified and the use of nine of them is forbidden in the EU, which is a situation of concern. No specific variations were observed for pesticides according to the obtained results during the pandemic period. It has to be highlighted that the sampling campaigns did not exactly match (13–19 March 2019 vs 25 March–8 April 2020) and some higher loads in 2020 could be due to the fact that April is the beginning of pesticide (i.e. insecticides) application season. However, the impact caused by COVID-19 pandemic (lockdown, travel restrictions, border closures and quarantine) in the flow of pesticides resulted to their limited production and supply. China, which is a key producer and supplier of pesticides worldwide reported a sharp decrease in production (Lamichhane and Reay-Jones, 2021). Finally, the human exposure to pesticides was not evaluated on this work, since no human urinary metabolites were investigated (Rousis et al., 2017).

The presence of biocides in wastewater was increased considerably and the majority of them, ten out of fourteen, were only identified in 2020 (Table S4). These biocides are used as preservatives and for pests control with the exception of mecetronium ethyl sulphate which is used as disinfectant. Indeed, the United States Centers for Disease Control and Prevention (CDC) recommended that cleaning visibly dirty surfaces followed by disinfection is a highly effective way to battle the spread of SARS-CoV-2 in households and community settings (Centers for Disease Control and Prevention, 2020). It was proved that SARS-CoV-2 remained active on hard surfaces for hours and even days (van Doremalen et al., 2020). The population may have applied biocides used in pest control for disinfection purposes by mistake, showing the unawareness of the citizens in the use of chemicals. Additionally, the observed increase in biocides could be attributed to the fact that people remained at homes and engaged with household activities. Athenian citizens, obeying to the authorities' instructions, used various types of biocidal agents for chemical disinfection.

In the present work the highest increases under lockdown conditions, considering all classes of chemicals, were observed for cationic quaternary ammonium surfactants (331%) (Table 1), and more specifically to benzalkonium chloride (BAC), alkyltrimethylammonium chlorides (ATMAC) and diallyldimethylammonium chloride (DADMAC) groups. Furthermore, six out of eleven compounds were identified only in 2020 (Table S4). Many authorities worldwide published documents and official guides recommending disinfecting products with viricidal efficacy. These products included alcohols, quaternary ammonium salts, phenolic compounds and others in order to be used on surfaces and hand hygiene (Li et al., 2020). Wastewater analysis pointed out the change in the population habits during pandemic and showed that citizens followed the recommendations given by the authorities. However, to fully support our findings more research is needed at local level (i.e. houses), to investigate the "real" use of these chemicals using probably questionnaires (i.e. name of used chemicals, frequency of use, way of application and application space).

3.6. Industrial chemicals

Industrial chemicals showed a noticeable decrease from 37,145 g day^{-1} to 24,371 g day^{-1} (Table S4). Benzoic acid and *N*-methyl-2-pyrrolidone were the two compounds with the highest mass loads. Benzoic acid, a compound with many uses in industry (e.g. adhesive, sealant chemical, intermediate in synthesis, lubricant additive and plasticizer), showed a slight decrease in 2020 from 14,519 g day^{-1} to 13,170 g day^{-1}. Additionally, *N*-methyl-2-pyrrolidone showed a 2-fold decrease from 13,537 g day^{-1} to 6448 g day^{-1}. Due to lockdown, strict restrictions and stay-at-home measures, many industries reduced their production as reflected in the results of many chemicals that are strongly correlated with industries. The industrial activity actually changed during the COVID-19 pandemic, since the economic operations reduced, keeping open only the businesses essential to the supply chains. Therefore, positive and negative consequences in the environment and on wastes more specifically

were identified globally, such as higher air quality and increased plastic pollution (Patricio Silva et al., 2021).

Benzotriazoles and benzothiazoles are produced in high volumes and are used in many industrial and consumer products (Asimakopoulos et al., 2013). Generally, the compounds of these classes showed increase in 2020, but benzotriazole and 4/5-methyl-benzotriazole, the compounds with the highest mass loads in 2019, decreased during lockdown (from 186 g day^{-1} to 122 g day^{-1} and from 188 g day^{-1} to 175 g day^{-1} respectively).

Bisphenols and alkyl phenols are widespread chemicals and two of the most used classes of phenols. They are used in many industrial and consumer products and more specifically in production of epoxy resins and polycarbonate plastics, as disinfectants and antioxidants and can be formed as breakdown products of detergents (Senta et al., 2020). 4-tert-Octylphenol (4-t-OP), an endocrine disrupter alkyl phenol, is the most widely used industrial chemical as demonstrated by the concentration levels in both years (2794 g day^{-1} in 2019 and 2100 g day^{-1} in 2020). Among bisphenols, bisphenol A (BPA) is the most widely used for decades and here showed a slight increase during lockdown (from 347 g day^{-1} in 2019 to 382 g day^{-1} to 2020). BPA has been identified as an agent that interferes with natural hormones in the body, which are responsible for the homeostasis, reproduction or behavior (Mohapatra et al., 2010).

Per and polyfluoroalkyl substances is a class of artificial, fully fluorinated organic compounds that are potentially harmful. They are used as polymers, surfactants, friction reducers, and repellents. *Per*- and polyfluoroalkyl substances (PFAS) are also used directly or as technical aids (dispersants and emulsifiers) in many industrial applications and in the synthesis of adjuvants in pesticides (Kwiatkowski et al., 2020). They were detected at low levels both years and most of them (perfluorohexanoic acid (PFHxA), perfluorooctanoic acid (PFOA), perfluorooctanesulfonic acid (PFOS)) showed noteworthy decreases during lockdown, while perfluorobutane sulfonic acid (PFBuS) and perfluoroheptanoic acid (PFHpA) were detected only in 2020. Many organizations and agencies such as the US EPA, Europe's REACH, the Canadian and Australian governments, have taken measures to control or eliminate their use (Choi et al., 2018).

Phthalates and phosphates have widespread use and environmental persistence (Reemtsma et al., 2008). In addition, they are two of the most important industrial chemicals with enormous amounts of production especially in Europe (Clara et al., 2010). The only compounds that showed increase during lockdown were diethyl phthalate and dimethyl phthalate. Di (2-ethylhexyl) phthalate (DEHP), the most studied plasticizer worldwide, showed an 2-fold decrease (from 503 g day^{-1} to 274 g day^{-1} in 2020). It has to be mentioned that the use of DEHP in the European Union is restricted, as referred to REACH regulation (European Commission, 2018).

Suspect analysis was able to detect seventeen compounds without the need of the corresponding reference standards (Fig. S5A), belonging to the general category of industrial chemicals. Three compounds were phosphate esters (tris(2-butoxyethyl) phosphate, trioctyl phosphate and diethyl phosphate), three phthalate esters (isobutyl phthalate, diisobutyl phthalate and dimethyl terephthalate), five amines (diethanolamine, triethanolamine, tetradecylamine, bis-2-ethylhexylamine, and 2-naphthylamine), and six other industrial substances. The estimated loads were lower for 2020, which was in agreement with the results from target screening.

3.7. Food additives and dietary supplements

An increasing pattern was observed on dietary habits based on the investigation of the following seven compounds: acesulfame, cyclamic acid, saccharine, sucralose, triethylcitrate, indole-3-carbinol, sulforaphane. The loads of these compounds cumulatively showed a substantial increase (p-value <2.0E-06) in loads from 10,394 g day^{-1} in 2019 to 22,128 g day^{-1} in 2020. Despite the low number of analytes, the consumption of food may change during lockdowns. The COVID-19 pandemic influenced all parts of human life including food consumption. Staying indoors and working remotely altered the food habits and the food purchased by households (Batlle-Bayer et al., 2020). This change may be depicted in the chemical substances of influent wastewater. A more detailed investigation is needed toward monitoring of food additives and dietary supplements by WBE.

3.8. UV filters

The results of four UV filters, namely benzophenone 3, benzophenone 4, phenylbenzimidazole sulfonic acid and dioxybenzone showed a slight decrease (−24%), mainly attributed to benzophenone 3, which is found in sunscreen lotions, conditioners and cosmetics. UV filters are designed to protect skin against harmful UV radiation. Therefore, they are mainly used in sunscreen lotions and cosmetics, but they are also found in other products, such as plastics, food packages and rubbers. The WBE approach was rarely applied for the evaluation of human exposure to this chemical class and thus the data availability is limited (Senta et al., 2020). The low reduction of these compounds could be attributed to the fact that people staying at home do not need to protect against the sun. However, given the low number of detected UV filters, no robust conclusions can be drawn.

4. Limitations and future perspectives

The present work investigated the presence of 2136 target compounds and 9150 suspected compounds in untreated wastewater sampled in two different years (under lockdown and non-lockdown conditions), using advanced analytical and computational approaches. An attempt was conducted to investigate the effect of COVID-19 pandemic on the use of specific compounds and the human exposure and consumption through the WBE approach. Approximately 3% of the screened compounds were detected. Some of them have been rarely reported in the literature, highlighting the need to constantly create and update environmentally-relevant compound databases. Conducting such wide-scope investigations is not trivial, requires experience and significant time. Furthermore, it is critical to apply strong quality assurance and quality control (QA&QC). QA&QC requires the use of many internal standards with various physico-chemical properties from the beginning of an analysis (sample preparation). It was underlined that reference standards can assist on the semi-quantification of suspect compounds and therefore it is suggested to use chemicals of several classes that present different structures and elute throughout the chromatography. Although a large number of chemicals was identified, only few of them could be related to human consumption. This was due to the fact that the compound lists consisted mainly of parent compounds and not human urinary metabolites, as required by the WBE approach. Hence, new lists should be built containing mainly metabolites. Furthermore, estimation of human exposure to some of the identified compounds could not be performed, since stability data (in-sample and in-sewer), human urinary pharmacokinetic studies and data on interferences from additional sources in wastewater are not available. Future studies could investigate all the mentioned limitations and propose suitable WBE biomarkers. Other WBE issues relate to the population estimation and the refinement of existed correction factors. Finally, we acknowledge the fact that WBE is inevitably accompanied with back-calculation estimations, which must be minimized to reduce the noise in the datasets.

5. Conclusions

The present study investigated the change of chemical universe in untreated wastewater before and during the COVID-19 pandemic in Athens. An advanced methodology was applied combining the advantages of wide-scope screening with the wastewater-based epidemiology

approach. WBE was used as a tool to examine and assess the consumption and use of a wide variety of chemicals. Many classes of compounds were associated with COVID-19 pandemic, reflecting the effects of lockdown and other measures in population health, habits, lifestyle and disposition. The findings of our study indicate that significant and rapid changes in drug consumption patterns were observed during the early stages of the COVID-19 pandemic. These changes can be attributed to the implementation of confinement and restrict social interactions. The Athenian population followed the authorities advisories and restrictions as indicated by the substantial increase of chemical classes such as surfactants and disinfectants. However, the consumption of drugs of abuse showed mixed trends. COVID-19 pandemic represents a global challenge for the whole population at all levels of societies such as safety, accessible health, food security, stability of economy and unemployment.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Nikiforos Alygizakis: Investigation, Formal analysis, Data curation, Visualization, Writing – original draft. **Aikaterini Galani:** Formal analysis, Investigation, Writing – original draft. **Nikolaos I. Rousis:** Formal analysis, Investigation, Writing – original draft. **Reza Aalizadeh:** Formal analysis, Investigation, Writing – original draft. **Meletios-Athanasios Dimopoulos:** Writing – review & editing. **Nikolaos S. Thomaidis:** Conceptualization, Funding acquisition, Writing – review & editing, Supervision.

Declaration of competing interest

All the authors declare no conflict of interest.

Acknowledgements

Authors would like to acknowledge Mr. Spyridon Dimoulas and Mr. Iraklis Karayiannis (EYDAP S.A.) for granting permission to the Athens WWTP and the operators of the WWTP for their support in sample collection.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.149230.

References

Aalizadeh, R., Nika, M.C., Thomaidis, N.S., 2019. Development and application of retention time prediction models in the suspect and non-target screening of emerging contaminants. J. Hazard. Mater. 363, 277–285.

Ahmed, W., Angel, N., Edson, J., Bibby, K., Bivins, A., O'Brien, J.W., et al., 2020. First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: a proof of concept for the wastewater surveillance of COVID-19 in the community. Sci. Total Environ. 728, 138764.

Alygizakis, N.A., Gago-Ferrero, P., Borova, V.L., Pavlidou, A., Hatzianestis, I., Thomaidis, N.S., 2016. Occurrence and spatial distribution of 158 pharmaceuticals, drugs of abuse and related metabolites in offshore seawater. Sci. Total Environ. 541, 1097–1105.

Alygizakis, N.A., Gago-Ferrero, P., Hollender, J., Thomaidis, N.S., 2019a. Untargeted time-pattern analysis of LC-HRMS data to detect spills and compounds with high fluctuation in influent wastewater. J. Hazard. Mater. 361, 19–29.

Alygizakis, N.A., Oswald, P., Thomaidis, N.S., Schymanski, E.L., Aalizadeh, R., Schulze, T., et al., 2019b. NORMAN digital sample freezing platform: a european virtual platform to exchange liquid chromatography high resolution-mass spectrometry data and screen suspects in "digitally frozen" environmental samples. TrAC Trends Anal. Chem. 115, 129–137.

Alygizakis, N., Markou, A.N., Rousis, N.I., Galani, A., Avgeris, M., Adamopoulos, P.G., et al., 2020. Analytical methodologies for the detection of SARS-CoV-2 in wastewater: protocols and future perspectives. Trends Anal. Chem. 116125.

Asimakopoulos, A.G., Wang, L., Thomaidis, N.S., Kannan, K., 2013. Benzotriazoles and benzothiazoles in human urine from several countries: a perspective on occurrence, biotransformation, and human exposure. Environ. Int. 59, 274–281.

Bade, R., Simpson, B.S., Ghetia, M., Nguyen, L., White, J.M., Gerber, C., 2020a. Changes in alcohol consumption associated with social distancing and self-isolation policies triggered by COVID-19 in South Australia: a wastewater analysis study. Addiction 116, 1600–1605.

Bade, R., White, J.M., Nguyen, L., Tscharke, B., Mueller, J.F., O'Brien, J.W., et al., 2020b. Determining changes in new psychoactive substance use in Australia by wastewater analysis. Sci. Total Environ. 731.

Batlle-Bayer, L., Aldaco, R., Bala, A., Puig, R., Laso, J., Margallo, M., et al., 2020. Environmental and nutritional impacts of dietary changes in Spain during the COVID-19 lockdown. Sci. Total Environ. 748, 141410.

Been, F., Rossi, L., Ort, C., Rudaz, S., Delemont, O., Esseiva, P., 2014. Population normalization with ammonium in wastewater-based epidemiology: application to illicit drug monitoring. Environ. Sci. Technol. 48, 8162–8169.

Bergeron, A., Decary-Hetu, D., Giommoni, L., 2020. Preliminary findings of the impact of COVID-19 on drugs crypto markets. Int. J. Drug Policy 102870.

Bijlsma, L., Celma, A., Hernandez, F., 2019. Monitoring new psychoactive substances use through wastewater analysis: current situation, challenges and limitations. Curr. Opin. Environ. Sci. Health 9, 1–12.

Bowers, I., Subedi, B., 2021. Isoprostanes in wastewater as biomarkers of oxidative stress during COVID-19 pandemic. Chemosphere 271, 129489.

Centers for Disease Control and Prevention, 2020. Cleaning and disinfection for households. Avaliable at. https://www.cdc.gov/coronavirus/2019-ncov/prevent-getting-sick/cleaning-disinfection.html Last accessed 23 Oct 2020.

Chen, X., Reynolds, C.H., 2002. Performance of similarity measures in 2D fragment-based similarity searching: comparison of structural descriptors and similarity coefficients. J. Chem. Inf. Comput. Sci. 42, 1407–14014.

Choi, P.M., Tscharke, B.J., Donner, E., O'Brien, J.W., Grant, S.C., Kaserzon, S.L., et al., 2018. Wastewater-based epidemiology biomarkers: past, present and future. TrAC Trends Anal. Chem. 105, 453–469.

Clara, M., Windhofer, G., Hartl, W., Braun, K., Simon, M., Gans, O., et al., 2010. Occurrence of phthalates in surface runoff, untreated and treated wastewater and fate during wastewater treatment. Chemosphere 78, 1078–1084.

Dagklis, T., Tsakiridis, I., Mamopoulos, A., Athanasiadis, A., Pearson, R., Papazisis, G., 2020. Impact of the COVID-19 lockdown on antenatal mental health in Greece. Psychiatry Clin. Neurosci. 74, 616–617. https://doi.org/10.1111/pcn.13135.

Diamanti, K., Aalizadeh, R., Alygizakis, N., Galani, A., Mardal, M., Thomaidis, N.S., 2019. Wide-scope target and suspect screening methodologies to investigate the occurrence of new psychoactive substances in influent wastewater from Athens. Sci. Total Environ. 685, 1058–1065.

Dodder, N.G., Mullen, K.M., 2014. Organic/biological mass spectrometry data analysis. Available at. https://cran.r-project.org/web/packages/OrgMassSpecR/index.html Last accessed 28th April 2021.

Du, P., Thai, P.K., Zhou, Z., Xu, Z., Zhang, X., Wang, J., et al., 2019. Monitoring consumption of methadone and heroin in major Chinese cities by wastewater-based epidemiology. Drug Alcohol Depend. 205.

Dulio, V., Koschorreck, J., van Bavel, B., van den Brink, P., Hollender, J., Munthe, J., et al., 2020. The NORMAN Association and the European Partnership for Chemicals Risk Assessment (PARC): let's cooperate! Environmental sciences. Europe 32.

EMCDDA, 2020. Impact of COVID-19 on patterns of drug use and drug-related harms in Europe.Available at. https://www.emcdda.europa.eu/publications/ad-hoc-publication/impact-covid-19-patterns-drug-use-and-harms_en Last Accessed 23 Oct 2020.

European Commission, 2009. Regulation (EC) no 1107/2009 of the European Parliament and of the council of 21 October 2009 concerning the placing of plant protection products on the market and repealing council directives 79/117/EEC and 91/414/EEC.Available at. http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex:32009R1107 Last accessed 23 Oct 2020.

European Commission, 2018. COMMISSION REGULATION (EU) 2018/2005 of 17 December 2018 amending Annex XVII to Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards bis(2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP), benzyl butyl phthalate (BBP) and diisobutyl phthalate (DIBP).Available at. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32018R2005&from=EN Last accessed 23 Oct 2020.

European Parliament and Council, 2012. REGULATION (EU) No 528/2012 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 22 May 2012 concerning the making available on the market and use of biocidal products.Available at. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32012R0528 Last accessed 23 Oct 2020.

Fancourt, D., Steptoe, A., Bu, F., 2021. Trajectories of anxiety and depressive symptoms during enforced isolation due to COVID-19 in England: a longitudinal observational study. Lancet Psychiatry 8, 141–149.

Gago-Ferrero, P., Schymanski, E.L., Bletsou, A.A., Aalizadeh, R., Hollender, J., Thomaidis, N.S., 2015. Extended suspect and non-target strategies to characterize emerging polar organic contaminants in raw wastewater with LC-HRMS/MS. Environ. Sci. Technol. 49, 12333–12341.

Gago-Ferrero, P., Bletsou, A.A., Damalas, D.E., Aalizadeh, R., Alygizakis, N.A., Singer, H.P., et al., 2020. Wide-scope target screening of >2000 emerging contaminants in wastewater samples with UPLC-Q-ToF-HRMS/MS and smart evaluation of its performance through the validation of 195 selected representative analytes. J. Hazard. Mater. 387, 121712.

Gaiha, S.M., Cheng, J., Halpern-Felsher, B., 2020. Association between youth smoking, electronic cigarette use, and COVID-19. J. Adolesc. Health 67, 519–523.

Galani, A., Alygizakis, N., Aalizadeh, R., Kastritis, E., Dimopoulos, M.A., Thomaidis, N.S., 2021. Patterns of pharmaceuticals use during the first wave of COVID-19 pandemic in Athens, Greece, as revealed by wastewater-based epidemiology. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2021.149014 in press.

Garcia, M.A.F., de Labry, Olry, Lima, A., Ferrer Lopez, I., Bermudez-Tamayo, C., 2018. Analysis of changes in trends in the consumption rates of benzodiazepines and benzodiazepine-related drugs. J. Pharm. Policy Pract. 11, 1.

Gonzalez-Marino, I., Baz-Lomba, J.A., Alygizakis, N.A., Andres-Costa, M.J., Bade, R., Bannwarth, A., et al., 2020. Spatio-temporal assessment of illicit drug use at large scale: evidence from 7 years of international wastewater monitoring. Addiction 115, 109–120.

Gracia-Lor, E., Zuccato, E., Castiglioni, S., 2016. Refining correction factors for back-calculation of illicit drug use. Sci. Total Environ. 573, 1648–1659.

Gracia-Lor, E., Rousis, N.I., Hernandez, F., Zuccato, E., Castiglioni, S., 2018. Wastewater-based epidemiology as a novel biomonitoring tool to evaluate human exposure to pollutants. Environ. Sci. Technol. 52, 10224–10226.

Grundy, E.J., Suddek, T., Filippidis, F.T., Majeed, A., Coronini-Cronberg, S., 2020. Smoking, SARS-CoV-2 and COVID-19: a review of reviews considering implications for public health policy and practice. Tob. Induc. Dis. 18, 58.

Gu, J., Han, B., Wang, J., 2020. COVID-19: gastrointestinal manifestations and potential fecal-Oral transmission. Gastroenterology 158, 1518–1519.

Hellenic Republic, 2020. Urgent measurements to deal with the negative consequences of COVID-19 and the need to restrict its spread. (FEK A 55/2020, Last accessed 28th April 2021, Available at. http://www.et.gr/idocs-nph/search/pdfViewerForm.html?args=5C7QrtC22wHUdWr4xouZundtvSoClrL8ExDiwSIm0cLtIl9LGdkF53Ulxsx942CdyqxSQYNuqAGCF0lfB9HI6qSYtMQEkEHLwnFqmgJSA5WIsluV-nRwO1oKqSe4BlOTSpEWYhszF8P8UqWb_zFijP3fNjb3qKpGxGNtxU_uAtLw56ULsxCc54tGXdl9oyQ1.

Holshue, M.L., DeBolt, C., Lindquist, S., Lofy, K.H., Wiesman, J., Bruce, H., et al., 2020. First case of 2019 novel coronavirus in the United States. N. Engl. J. Med. 382, 929–936.

Kaparounaki, C.K., Patsali, M.E., Mousa, D.V., Papadopoulou, E.V.K., Papadopoulou, K.K.K., Fountoulakis, K.N., 2020. University students' mental health amidst the COVID-19 quarantine in Greece. Psychiatry Res. 290, 113111.

Kern, S., Fenner, K., Singer, H.P., Schwarzenbach, R.P., Hollender, J., 2009. Identification of transformation products of organic contaminants in antural waters by computer-aided prediction and high-resolution mass spectrometry. Environ. Sci. Technol. 43, 7039–7046.

Kinyua, J., Covaci, A., Maho, W., McCall, A.-K., Neels, H., van Nuijs, A.L.N., 2015. Sewage-based epidemiology in monitoring the use of new psychoactive substances: validation and application of an analytical method using LC-MS/MS. Drug Test. Anal. 7, 812–818.

Kinyua, J.P., A.K, Rousis, N.I., Nika, M.-C., Covaci, A., Thomaidis, N.S., Van Nuijs, A.L.N., 2021. Investigation of biotransformation products of p-methoxymethylamphetamine and dihydromephedrone in wastewater by high-resolution mass spectrometry. Metabolites 11, 66.

Kruve, A., Kiefer, K., Hollender, J., 2021. Benchmarking of the quantification approaches for the non-targeted screening of micropollutants and their transformation products in groundwater. Anal. Bioanal. Chem. 413, 1549–1559.

Kwiatkowski, C.F., Andrews, D.Q., Birnbaum, L.S., Bruton, T.A., DeWitt, J.C., Knappe, D.R.U., et al., 2020. Scientific basis for managing PFAS as a chemical class. Environ. Sci. Technol. Lett. 7, 532–543.

La Rosa, G., Bonadonna, L., Lucentini, L., Kenmoe, S., Suffredini, E., 2020. Coronavirus in water environments: occurrence, persistence and concentration methods - a scoping review. Water Res. 179, 115899.

Lai, F.Y., Lympousi, K., Been, F., Benaglia, L., Udrisard, R., Delemont, O., et al., 2018. Levels of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in raw wastewater as an innovative perspective for investigating population-wide exposure to third-hand smoke. Sci. Rep. 8, 13254.

Lamichhane, J.R., Reay-Jones, F.P., 2021. Editorial: impacts of COVID-19 on global plant health and crop protection and the resulting effect on global food security and safety. Crop Prot. 139, 105383.

Li, D., Sangion, A., Li, L., 2020. Evaluating consumer exposure to disinfecting chemicals against coronavirus disease 2019 (COVID-19) and associated health risks. Environ. Int. 145, 106108.

Libiseller, G., Dvorzak, M., Kleb, U., Gander, E., Eisenberg, T., Madeo, F., et al., 2015. IPO: a tool for automated optimization of XCMS parameters. BMC Bioinformatics 16, 118.

Loos, M., Hollender, J., Schymanski, E.L., Ruff, M., Singer, H.P., 2012. Bottom-up peak grouping for unknown identification from high-resolution mass spectrometry data. ASMS 2012 annual conference Vancouver, oral session Informatics: Identification.

McCall, A.K., Bade, R., Kinyua, J., Lai, F.Y., Thai, P.K., Covaci, A., et al., 2016. Critical review on the stability of illicit drugs in sewers and wastewater samples. Water Res. 88, 933–947.

Medema, G., Heijnen, L., Elsinga, G., Italiaander, R., Brouwer, A., 2020. Presence of SARS-Coronavirus-2 RNA in sewage and correlation with reported COVID-19 prevalence in the early stage of the epidemic in the Netherlands. Environ. Sci. Technol. Lett. 7, 511–516.

Mohapatra, D.P., Brar, S.K., Tyagi, R.D., Surampalli, R.Y., 2010. Physico-chemical pre-treatment and biotransformation of wastewater and wastewater sludge–fate of bisphenol a. Chemosphere 78, 923–941.

Montes, R., Rodil, R., Rico, A., Cela, R., González-Mariño, I., Hernández, F., Bijlsma, L., Celma, A., Picó, Y., Andreu, V., de Alda, M.L., López-García, E., Postigo, C., Pocurull, E., Marcé, M.R., Rosende, M., Olivares, M., Valcárcel, Y., Quintana, J.B., 2020. First nation-wide estimation of tobacco consumption in Spain using wastewater-based epidemiology. Sci. Total Environ. 741, 140384. https://doi.org/10.1016/j.scitotenv.2020.140384.

Ng, B., Quinete, N., Gardinali, P.R., 2020. Assessing accuracy, precision and selectivity using quality controls for non-targeted analysis. Sci. Total Environ. 713, 136568.

NORMAN Network, 2021. NORMAN joint programme of activities for 2021.Available at. https://www.norman-network.net/sites/default/files/files_private/JointProgramme2021/NORMAN%20JPA%202021_Final_Feb2021.pdf Last accessed 28th April 2021.

NORMAN NetworkAalizadeh, Reza, Alygizakis, Nikiforos, Schymanski, Emma, Slobodnik, Jaroslav, Fischer, Stellan, Cirka, Lubos, 2020.. S0 | SUSDAT | Merged NORMAN Suspect List: SusDat. https://zenodo.org/record/3900203.

Ort, C., Bijlsma, L., Castiglioni, S., Covaci, A., de Voogt, P., Emke, E., et al., 2018. Wastewater analysis for community-wide drugs use assessment. Handb. Exp. Pharmacol. 252, 543–560.

Parlapani, E., Holeva, V., Voitsidis, P., Blekas, A., Gliatas, I., Porfyri, G.N., et al., 2020. Psychological and behavioral responses to the COVID-19 pandemic in Greece. Front. Psychiatry 11, 821.

Patricio Silva, A.L., Prata, J.C., Walker, T.R., Duarte, A.C., Ouyang, W., Barcelo, D., et al., 2021. Increased plastic pollution due to COVID-19 pandemic: challenges and recommendations. Chem. Eng. J. 405, 126683.

Reemtsma, T., Quintana, J.B., Rodil, R., Garcí´a-López, M., Rodrí´guez, I., 2008. Organophosphorus flame retardants and plasticizers in water and air I. Occurrence and fate. TrAC Trends Anal. Chem. 27, 727–737.

Reinstadler, V., Ausweger, V., Grabher, A.L., Kreidl, M., Huber, S., Grander, J., et al., 2021. Monitoring drug consumption in Innsbruck during coronavirus disease 2019 (COVID-19) lockdown by wastewater analysis. Sci. Total Environ. 757, 144006.

Rousis, N.I., Zuccato, E., Castiglioni, S., 2017. Wastewater-based epidemiology to assess human exposure to pyrethroid pesticides. Environ. Int. 99, 213–220.

Rousis, N.I., Gracia-Lor, E., Reid, M.J., Baz-Lomba, J.A., Ryu, Y., Zuccato, E., et al., 2020. Assessment of human exposure to selected pesticides in Norway by wastewater analysis. Sci. Total Environ. 723, 138132.

Schulze, B., Jeon, Y., Kaserzon, S., Heffernan, A.L., Dewapriya, P., O'Brien, J., et al., 2020. An assessment of quality assurance/quality control efforts in high resolution mass spectrometry non-target workflows for analysis of environmental samples. TrAC Trends Anal. Chem. 133, 116063.

Senta, I., Rodríguez-Mozaz, S., Corominas, L., Petrovic, M., 2020. Wastewater-based epidemiology to assess human exposure to personal care and household products – a review of biomarkers, analytical methods, and applications. Trends Environ. Anal. Chem. 28.

Singh, S., Roy, D., Sinha, K., Parveen, S., Sharma, G., Joshi, G., 2020. Impact of COVID-19 and lockdown on mental health of children and adolescents: a narrative review with recommendations. Psychiatry Res. 293, 113429.

Stein, S.E., Scott, D.R., 1994. Optimization and testing of mass spectral library search 522 algorithms for compound identification. J. Am. Soc. Mass Spectrom. 5, 859–866.

Tautenhahn, R., Bottcher, C., Neumann, S., 2008. Highly sensitive feature detection for high resolution LC/MS. BMC Bioinformatics 9, 504.

Thomaidis, N.S., Gago-Ferrero, P., Ort, C., Maragou, N.C., Alygizakis, N.A., Borova, V.L., et al., 2016. Reflection of socioeconomic changes in wastewater: licit and illicit drug use patterns. Environ. Sci. Technol. 50, 10065–10072.

Tscharke, B., Chen, C., Gerber, C., White, J.M., 2016. Temporal trends in drug use in Adelaide, South Australia by wastewater analysis. Sci. Total Environ. 565, 384–391.

van Doremalen, N., Morris, D.H., Holbrook, M.G., Gamble, A., Williamson, B.N., Tamin, A., et al., 2020. Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. N. Engl. J. Med. 382, 1564–1567.

Van Hal, G., 2019. Sewage as an information source on (illicit) substance use: a sociological and public health view. Curr. Opin. Environ. Sci. Health 9, 34–39.

van Zyl-Smit, R.N., Richards, G., Leone, F.T., 2020. Tobacco smoking and COVID-19 infection. Lancet Respir. Med. 8, 664–665.

Wang, S., Green, H.C., Wilder, M.L., Du, Q., Kmush, B.L., Collins, M.B., et al., 2020. High-throughput wastewater analysis for substance use assessment in Central New York during the COVID-19 pandemic. Environ. Sci. Process. Impacts 22, 2147–2161.

WHO, 2021. Weekly epidemiological update.Available at. https://www.who.int/publications/m/item/weekly-epidemiological-update—27-january-2021 Last accessed 1st February 2021.

Zaami, S., Marinelli, E., Vari, M.R., 2020. New trends of substance abuse during COVID-19 pandemic: an international perspective. Front. Psychiatry 11, 700.

Zuccato, E., Castiglioni, S, 2011. Assessing Illicit Drug Consumption by Wastewater Analysis: History, Potential, and Limitation of a Novel Approach. Illicit Drugs in the Environment: Occurrence, Analysis, and Fate Using Mass Spectrometry. Wiley-Blackwell, Oxford, pp. 291–304.