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Spatial and temporal assessment of crack cocaine use in 13 European cities through wastewater-based epidemiology

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Abstract:	Already in early 2000s, concerns have been growing in the EU about increasing use of cocaine and it is estimated that below 1% of the population administer the drug by smoking crack cocaine. New available data suggests an increase in the use of crack cocaine and an increase in the number of crack cocaine users entering treatment has been reported in several European countries. Robust estimations of crack cocaine use are however not available yet. The use of crack cocaine has long been associated with severe adverse socio-economic conditions as well as mental health problems, such as suicide ideation and depression. The aim of this study was to assess spatial trends in population-normalized mass loads of crack cocaine biomarkers (i.e., anhydroecgonine and anhydroecgonine methyl ester) in 13 European cities in six countries (the Netherlands, Belgium, Ireland, Portugal, Spain and Italy). Furthermore, temporal trends over a five-year period were evaluated through the analysis of historic samples collected in the Netherlands. Finally, the stability of the crack cocaine biomarkers in wastewater was investigated through batch experiments. The samples were analyzed with a new developed and validated hydrophilic interaction liquid chromatography

	coupled to mass spectrometry method. Targeted crack cocaine biomarkers were found in all cities. Also, crack cocaine biomarker was detected in wastewater from 2017 to 2021 in the Netherlands, but no significance between the years were found. With respect to biomarker in-sample stability, AEME was found to be stable in wastewater. This study assessed crack cocaine use for the first time on a broad scale, both temporal and in cities across Europe, with wastewater-based epidemiology and it shows the importance of wastewater analysis to monitor community loads of crack cocaine use.
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Dear Editor,

Please find enclosed our manuscript entitled "*Spatial and temporal assessment of crack cocaine use in 13 European cities through wastewater-based epidemiology*".

Our paper presents the spatial and temporal assessment of crack cocaine use in 13 European cities by sewage surveillance, also referred to as wastewater-based epidemiology (WBE). This innovative method is increasingly being used to gather information about exposure and emissions of a wide range of compounds at a community level. However, in the particular case of crack cocaine, only few studies have tackled this issue. Here we present a thorough investigation of crack cocaine biomarkers in influent wastewater streams in 13 European cities. The goal of our study was to assess the spatial trend in population-normalized mass loads of crack cocaine across Europe and to evaluate temporal trend in Amsterdam, the Netherlands, over a five-year period.

We were able to develop and validate an analytical method to detect crack cocaine biomarker (AEME) in influent wastewater. The detection frequency of AEME was 100% in all analysed samples. Furthermore, in-sample stability tests confirms that AEME is stable in wastewater and thus a suitable biomarker for the assessment of crack cocaine use. Furthermore, a positive correlation between AEME and BE (cocaine use biomarker) mass loads was observed, but a formal causality link between cocaine usage/availability and crack use cannot be established on this data solely..

We believe that the novel approach presented is highly compelling for future studies and is of particular interest for the readership of the special issue of *Science of the Total Environment.* We hereby attest that the current manuscript has not been previously published and that it is not under consideration by any other journal. Supporting information for publication is also provided.

On behalf of all co-authors,

Ruud Steenbeek, MSc.

Spatial and temporal assessment of crack cocaine use in 13 European cities through wastewater-based epidemiology

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Graphical Abstract

No graphical abstract is used for this manuscript

Highlights

- An analytical method was developed and validation for the measurement of crack cocaine biomarker in influent wastewater
- Anhydroecgonine methyl ester was found to be stable in wastewater after in-sample stability tests.
- AEME was found with a detection frequency of 100% in all samples from 13 European cities and from Amsterdam over a five-year period.
- A positive correlation between AEME and benzoylecgonine (cocaine biomarker) mass loads was observed, but a formal causality link cannot be established based on the data solely.

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Supporting Information

Spatial and temporal assessment of crack cocaine use in 13 European cities through wastewater-based epidemiology

Ruud Steenbeek[1]•, Erik Emke[1], Dennis Vughs[1], João Matias[2], Tim Boogaerts[3], Sara Castiglioni[4], Marina Campos-Mañas[5], Adrian Covaci[3], Pim de Voogt[1], Thomas ter Laak[1,6], Félix Hernández[5], Noelia Salgueiro-González[4], Wim G. Meijer[7], Mario J. Dias[8], Susana Simões[8] Alexander L.N. van Nuijs[3], Lubertus Bijlsma[5], Frederic Béen[1]

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Keywords: Wastewater-based epidemiology, crack cocaine, spatial variability, temporal variability, HILIC

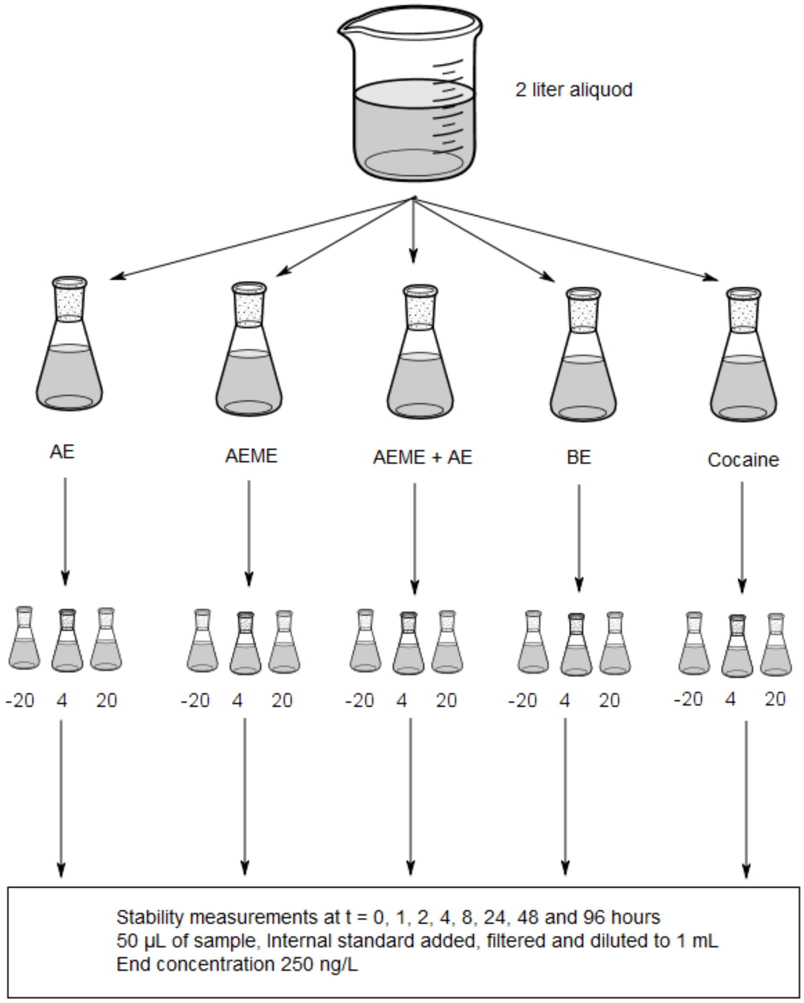

Figure S1: The set-up of the in-sample stability tests of AEME and AE.

Table S1: The absolute concentration, the flow and the load of the 13 European cities between October 2020 and June 2021.

Day	WWTP	Compound	Concentration (ng/L)	Flow (m^3/day)	Load (mg/day/1000 inh)
Wednesday	Eindhoven	AEME	7.17	163805	2.57
Thursday	Eindhoven	AEME	7.88	149869	2.58
Friday	Eindhoven	AEME	7.85	130484	2.24
Saturday	Eindhoven	AEME	9.65	124446	2.63
Sunday	Eindhoven	AEME	14.64	118477	3.79
Monday	Eindhoven	AEME	8.50	123995	2.31
Tuesday	Eindhoven	AEME	10.65	122749	2.86
Wednesday	Amsterdam	AEME	36.58	145000	7.91
Thursday	Amsterdam	AEME	23.41	152000	5.31
Friday	Amsterdam	AEME	28.85	149000	6.41
Saturday	Amsterdam	AEME	29.66	146000	6.46
Sunday	Amsterdam	AEME	34.34	146000	7.48
Monday	Amsterdam	AEME	30.68	142000	6.50
Tuesday	Amsterdam	AEME	32.88	141000	6.92
Wednesday	Utrecht	AEME	10.55	85740	3.38
Thursday	Utrecht	AEME	14.37	51463	2.76
Friday	Utrecht	AEME	16.71	48987	3.06
Saturday	Utrecht	AEME	22.06	48166	3.97
Sunday	Utrecht	AEME	15.76	48500	2.85
Monday	Utrecht	AEME	14.25	48580	2.59
Tuesday	Utrecht	AEME	16.13	48478	2.92
Wednesday	Antwerp	AEME	15.69	44640	5.38
Thursday	Antwerp	AEME	16.88	41808	5.42
Friday	Antwerp	AEME	18.33	49696	7.00
Saturday	Antwerp	AEME	17.82	58800	8.05
Sunday	Antwerp	AEME	21.65	43120	7.17
Monday	Antwerp	AEME	19.22	44944	6.63
Tuesday	Antwerp	AEME	19.05	44832	6.56
Wednesday	Brussels	AEME	12.43	243843	3.18
Thursday	Brussels	AEME	12.69	241247	3.21
Friday	Brussels	AEME	15.53	241758	3.94
Saturday	Brussels	AEME	11.89	241725	3.01
Sunday	Brussels	AEME	13.68	244242	3.50
Monday	Brussels	AEME	16.88	242086	4.28

Tuesday	Brussels	AEME	10.88	241110	2.75
Wednesday	Rome	AEME	2.88	777600	2.03
Thursday	Rome	AEME	3.07	787104	2.20
Friday	Rome	AEME	3.53	779328	2.50
Saturday	Rome	AEME	2.39	786240	1.71
Sunday	Rome	AEME	3.32	796608	2.40
Monday	Rome	AEME	2.66	788832	1.91
Tuesday	Rome	AEME	3.06	778464	2.17
Wednesday	Milan	AEME	4.04	321270	1.24
Thursday	Milan	AEME	3.89	318420	1.18
Friday	Milan	AEME	3.49	313750	1.04
Saturday	Milan	AEME	5.48	310200	1.62
Sunday	Milan	AEME	4.62	306740	1.35
Monday	Milan	AEME	3.66	325485	1.14
Tuesday	Milan	AEME	4.10	325775	1.28
Wednesday	Bologna	AEME	9.23	118400	1.82
Thursday	Bologna	AEME	7.49	118320	1.48
Friday	Bologna	AEME	9.77	118740	1.93
Saturday	Bologna	AEME	10.69	119960	2.14
Sunday	Bologna	AEME	11.98	120340	2.40
Monday	Bologna	AEME	9.49	115968	1.83
Tuesday	Bologna	AEME	8.50	118820	1.68
Wednesday	Bari	AEME	19.48	78759	3.34
Thursday	Bari	AEME	6.85	79150	1.18
Friday	Bari	AEME	10.13	76029	1.68
Saturday	Bari	AEME	4.68	77751	0.79
Sunday	Bari	AEME	15.64	96802	3.29
Monday	Bari	AEME	1.76	84570	0.32
Tuesday	Bari	AEME	10.52	80641	1.85
Wednesday	Lisbon	AEME	8.11	138973	2.64
Thursday	Lisbon	AEME	8.43	133571	2.64
Friday	Lisbon	AEME	9.33	125138	2.73
Saturday	Lisbon	AEME	8.28	113498	2.20
Sunday	Lisbon	AEME	10.32	104641	2.53
Monday	Lisbon	AEME	7.01	120360	1.98
Tuesday	Lisbon	AEME	8.31	140196	2.73
Wednesday	Almada	AEME	5.48	32200	1.27
Thursday	Almada	AEME	6.15	33200	1.47

Friday	Almada	AEME	4.45	32300	1.04
Saturday	Almada	AEME	7.37	34900	1.85
Sunday	Almada	AEME	8.87	32300	2.07
Monday	Almada	AEME	6.48	34000	1.57
Tuesday	Almada	AEME	6.58	36100	1.71
Wednesday	Dublin	AEME	10.01	344923	1.82
Thursday	Dublin	AEME	8.94	339168	1.60
Friday	Dublin	AEME	9.16	337346	1.63
Saturday	Dublin	AEME	8.50	322948	1.45
Sunday	Dublin	AEME	10.87	325695	1.86
Monday	Dublin	AEME	10.74	337731	1.91
Tuesday	Dublin	AEME	10.00	339684	1.79
Wednesday	Castellon	AEME	17.61	39336	3.86
Thursday	Castellon	AEME	16.00	37738	3.36
Friday	Castellon	AEME	14.46	36369	2.93
Saturday	Castellon	AEME	11.55	40550	2.61
Sunday	Castellon	AEME	15.34	35788	3.06
Monday	Castellon	AEME	15.69	38982	3.41
Tuesday	Castellon	AEME	17.61	40473	3.97

Table S2: The absolute concentration, the flow and the load of Amsterdam from 2017 to 2021.

Day	Year	Compound	Concentration (ng/L)	Flow (L/day)	Load (mg/day/1000 inh)
Wednesday	2017	AEME	22.39	1.36E+08	4.71
Thursday	2017	AEME	n.a.	1.36E+08	n.a
Friday	2017	AEME	27.39	1.37E+08	5.80
Saturday	2017	AEME	35.74	1.38E+08	7.60
Sunday	2017	AEME	38.36	1.34E+08	7.94
Monday	2017	AEME	34.63	1.4E+08	7.46
Tuesday	2017	AEME	31.56	1.46E+08	7.12
Wednesday	2018	AEME	21.10	1.43E+08	4.60
Thursday	2018	AEME	24.61	1.42E+08	5.32
Friday	2018	AEME	32.61	1.77E+08	8.79
Saturday	2018	AEME	22.78	1.46E+08	5.06
Sunday	2018	AEME	27.87	1.45E+08	6.17
Monday	2018	AEME	31.72	2.16E+08	10.46
Tuesday	2018	AEME	28.65	3.13E+08	13.70
Wednesday	2019	AEME	25.64	2.05E+08	7.94
Thursday	2019	AEME	23.92	1.51E+08	5.44
Friday	2019	AEME	26.76	1.45E+08	5.87
Saturday	2019	AEME	34.65	1.43E+08	7.47
Sunday	2019	AEME	35.59	1.45E+08	7.81
Monday	2019	AEME	29.84	1.45E+08	6.52
Tuesday	2019	AEME	32.72	1.89E+08	9.36
Wednesday	2020	AEME	26.76	1.46E+08	5.84
Thursday	2020	AEME	25.53	1.42E+08	5.43
Friday	2020	AEME	35.82	1.43E+08	7.64
Saturday	2020	AEME	26.43	1.41E+08	5.58
Sunday	2020	AEME	30.54	1.4E+08	6.37
Monday	2020	AEME	40.62	1.38E+08	8.40
Tuesday	2020	AEME	36.24	1.35E+08	7.32
Wednesday	2021	AEME	36.58	1.52E+08	8.30
Thursday	2021	AEME	23.41	1.49E+08	5.20
Friday	2021	AEME	28.85	1.49E+08	6.41
Saturday	2021	AEME	29.66	1.46E+08	6.46
Sunday	2021	AEME	34.34	1.46E+08	7.48
Monday	2021	AEME	30.68	1.42E+08	6.50
Tuesday	2021	AEME	32.88	1.41E+08	6.92

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Abstract

Already in early 2000s, concerns have been growing in the EU about increasing use of cocaine and it is estimated that below 1% of the population administer the drug by smoking crack cocaine. New available data suggests an increase in the use of crack cocaine and an increase in the number of crack cocaine users entering treatment has been reported in several European countries. Robust estimations of crack cocaine use are however not available yet. The use of crack cocaine has long been associated with severe adverse socio-economic conditions as well as mental health problems, such as suicide ideation and depression. The aim of this study was to assess spatial trends in population-normalized mass loads of crack cocaine biomarkers (i.e., anhydroecgonine and anhydroecgonine methyl ester) in 13 European cities in six countries (the Netherlands, Belgium, Ireland, Portugal, Spain and Italy). Furthermore, temporal trends over a five-year period were evaluated through the analysis of historic samples collected in the Netherlands. Finally, the stability of the crack cocaine biomarkers in wastewater was investigated through batch experiments. The samples were analyzed with a new developed and validated hydrophilic interaction liquid chromatography coupled to mass spectrometry method. Targeted crack cocaine biomarkers were found in all cities. Also, crack cocaine biomarker was detected in wastewater from 2017 to 2021 in the Netherlands, but no significance between the years were found. With respect to biomarker in-sample stability, AEME was found to be stable in wastewater. This study assessed crack cocaine use for the first time on a broad scale, both temporal and in cities across Europe, with wastewater-based epidemiology and it shows the importance of wastewater analysis to monitor community loads of crack cocaine use.

1. Introduction

Already in the early 2000s, concern has been growing in the EU about the increasing use of cocaine (EMCDDA, 2001). Twenty years later, cocaine is the second most commonly used illicit drug in Europe, although prevalence levels and trends differ considerably between countries, with 4.8% of the adult population having used cocaine at least once in their lifetime (EMCDDA, 2021). Cocaine is available in Europe mainly in two forms: cocaine hydrochloride, a salt often referred to as 'cocaine powder' that can be snorted, swallowed or injected, and "crack" cocaine, which has been processed into a freebase form using cocaine hydrochloride as the starting material, that can be smoked, swallowed or injected.

Smoking 'crack cocaine' radically transforms the effects of the drug; the rapidity and intensity of onset lead to a sensation of euphoria ('rush') followed by a sharp drop ("crash") that frequently leads to a craving for another dose (UNODC, 2021). Most treatment entrants citing cocaine as their main problem drug are powder cocaine users: 45 000 users in 2019 in Europe, 14% of all drug clients. With respect to crack-related treatment, around 92% of the 8000 entries in 2019 were reported by 8 EU countries. (EMCDDA, 2021). Cocaine has long been associated with severe adverse socio-economic conditions and serious psychological and physical health outcomes, for example respiratory damage or the transmission of Hepatitis C and other blood-borne diseases (Janssen et al., 2020), higher "binge" use and increased risk of polydrug use (Carvalho et al., 2008; Jeppesen et al., 2015). Epidemiological data indicate that crack cocaine use became increasingly prevalent in the Americas from the 1990s forward (Dunn et al., 1996; Edlin et al., 1992; Fischer and Coghlan 2007; Werb et al., 2010). In France, a 2017 capture-recapture study estimated the prevalence of high-risk crack cocaine use at 0.07% of the population. In the three largest Dutch cities (Amsterdam, Rotterdam and The Hague) 0.5% of the population is addicted to crack (van Miltenburg et al., 2020). The remaining crack users are reported mainly by Belgium, Spain and France (EMCDDA, 2020).

New available data suggest an increase in the number of crack cocaine users entering treatment in Belgium, Ireland, Italy, Portugal, United Kingdom (EMCDDA, 2019) and France (Janssen et al., 2020). A possible worrying is the observation that some countries may be seeing an increase in crack cocaine availability and use (European Drug Report, 2021). Unfortunately, population surveys, which are mostly performed by known drug users, do not easily reach those who use 'crack cocaine' or do not even ask separately about the patterns of 'crack cocaine' use and evaluation based upon observational studies or self-reports for the use of illicit drugs may be inaccurate (Lu et al., 2001).

For research and monitoring purposes, people who use cocaine may be categorised in different ways, according to the setting, the product used or the motivation for use. Among regular consumers, a broad distinction can be made between typically more socially integrated users, who sniff powder cocaine, and marginalised users, who inject cocaine or smoke crack cocaine, sometimes alongside the use of opioids. In many datasets, it is not possible to distinguish between the two forms of cocaine (cocaine powder or crack) and the term cocaine use covers both (EMCDDA, 2019). Furthermore, assessment of the prevalence of crack cocaine smoking cannot be based upon seized amounts as users often prepare crack cocaine from cocaine hydrochloride by 'freebasing' techniques described online (Jeppesen et al. 2015). Therefore, robust population estimates of crack cocaine use do not exist (Butler et al., 2017).

When smoking crack, anhydroecgonine methyl ester (AEME or methylecgonidine) is formed as a result of the elimination of benzoic acid from cocaine at high temperature and the methyl ester can be hydrolyzed to anhydroecgonine (AE or ecgonidine) in human plasma due to butyryl cholinesterase and nonenzymatic processes (Fandino et al., 2002). AEME and AE have been identified in the urine of crack smokers (Zhang and Foltz, 1990; Paul et al., 1999; Kintz et al., 1995; Shimomura et al., 2001) and in influent wastewater (Castiglioni et al., 2011; Bisceglia et al., 2010; Gonzalez-Marino et al., 2019). To quantify these pyrolysis products in wastewater, hydrophilic interaction liquid chromatography (HILIC) can be applied to improve the separation of small and polar analytes that are poorly retained by traditional reversed-phase chromatographic columns (RPLC) (Castiglioni et al 2011; Gheorghe et al. 2008). Furthermore, mixed-mode chromatography has recently been used to determine pyrolytic products of cocaine in wastewater (Gonzalez-Mariño et al., 2018).

Through the analysis of illicit drug residues in wastewater, wastewater-based epidemiology (WBE) provides a quantitative measure of the mass loads of a substance released in a specific sewer catchment. Mass loads are then normalized by the population size to provide the daily load released per 1000 people. (Gonzalez-Mariño et al., 2020). Estimations of cocaine use has been made through the determination of its main urinary metabolite benzoylecgonine (BE) in wastewater for more than a decade. However, a distinction between crack cocaine and powder cocaine use in WBE is less common (González-Mariño et al, 2019; Castiglioni et al. 2011), while this has been more commonly done in urine (Jeppesen et al., 2015).

The aim of the present study was to assess spatial trends in population-normalized mass loads of crack cocaine biomarkers (i.e., AE and AEME) in influent wastewater from 13 European cities from six countries (the Netherlands, Belgium, Ireland, Portugal, Spain and Italy) collected in 2020 and 2021 to obtain complementary information about population-wide crack cocaine use. These countries were chosen because of the increase in the number of crack cocaine users entering treatment since 2014 (EMCDDA, 2019). Furthermore, temporal trends in Amsterdam, the Netherlands, over a five-year period (2017-2021) were evaluated through the analysis of historic samples . This study is, to our knowledge, the first published approach to assess crack cocaine use in several European countries by WBE.

2. Materials and methods

2.1 Materials and reagents

Reference standards of anhydroecgonine and anhydroecgonine methyl ester and their deuterated standards were purchased from Lipomed AG (Lipomed, Arlesheim, Switserland). Acetonitrile, ammonium hydroxide and methanol (ultra-gradient HPLC grade) were obtained from Boom B.V. (Meppel, the Netherlands). Formic acid and hydrochloric acid were purchased from Sigma-Aldrich (Steinheim, Germany). Stock solutions of the reference standards, including internal standards, were prepared at a concentration of 3.5 mg/L in acetonitrile. Individual stock solutions were stored at -20 °C. Working solutions containing all individual standards were freshly prepared in acetonitrile with 5% ultrapure water (18.2 MΩ/cm, ELGA LabWater, Lane End, UK) (35 μg/L) each time a new set of samples was processed and analyzed.

2.2 Wastewater sampling

Influent wastewater samples were collected between October 2020 and April 2021 at the entrance of the wastewater treatment plants (WWTPs) of the 13 cities mentioned in Table 1. The influent wastewater samples from Amsterdam were historic samples collected from previous sampling campaigns from 2017 to 2021. At the time of sampling, different COVID-19 related restrictions were in places in those cities with different Government Response Stringency Index (GRSI) (University of Oxford, 2020). All samples were 24-hour composite samples, collected following the protocols established in the yearly monitoring campaigns coordinated by the Sewage Analysis Core Group Europe (SCORE) (González- Mariño et al., 2020; Castiglioni et al., 2013; SCORE, 2020). Additional data, such as population and wastewater flows, were provided by the WWTPs personnel and were used to compute population normalized daily mass loads (expressed in milligram per day per 1000 inhabitants [mg/day.1000 inhabitants]).

2.3 Sample preparation

For solid-phase extraction, 50 mL of each sample was transferred in a precleaned HDPE bottle. Internal standard work solution was added to each sample to reach a concentration of 100 ng/L in wastewater and the sample was adjusted to pH = 2.0 with HCl. Samples were horizontally shaken for 5 min at 120 rpm and filtered through a 0.20 µm filter. Samples were then extracted with Oasis MCX cartridges (3 mL, 60 mg, Waters, USA). Cartridges were washed 6 mL of methanol, followed by 3 mL of ultrapure water and 3 mL of acidified ultrapure water (pH = 2.0). Samples were then gently loaded onto the cartridges. Subsequently, the cartridges were washed with 6 mL of acidified ultrapure water (pH = 2.0) and dried under vacuum for 1 h. Thereafter, the cartridges were eluted with 6 mL of MeOH with 2% ammonium hydroxide. Eluates were collected in glass tubes and evaporated to dryness under a gentle stream of nitrogen at 40 °C. Eluates were then reconstituted in 500 μL in acetonitrile with 5% ultrapure water and vortexed for 5 seconds. The extract was filtered through a 0.45 μm filter and transferred in 1.8 mL vials with inserts for analysis.

2.4 Method development

A Tribrid Orbitrap Fusion mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) equipped with an electrospray ionization (ESI) source was interfaced to a Vanquish HPLC system (Thermo Fisher Scientific, Bremen, Germany). Every batch run mass calibration was performed using a Pierce ESI positive ion calibration solution. The ion transfer tube temperature and the vaporizer temperature were set to 300 °C and 350 °C respectively. The sheath, auxiliary and sweep gas were maintained at arbitrary units of 45, 5 and 5 respectively. The source voltage was set to 3000 V in positive mode. The RF lens was set to 60% and the scan range was set in the range of 100-400 *m/z*. The Orbitrap resolution was set to 120,000 FWHM and the quadruple isolation was used for acquisition with a 5 ppm mass window. Data-dependent acquisition was performed with a High Collision Dissociation (HCD) of 30%.

For the chromatographic separation an Agilent Zorbax HILIC plus (150 mm x 2.1 mm, 1.8 μm) connected to a krudkatcher ULTRA HPLC In-line Filter, 0.5 μm was used. The column temperature was maintained at 25 °C. Mobile phase A consisted of 95% ultrapure water and 5% acetonitrile (v/v) with 5 mM ammonium formate at a pH = 3. Mobile phase B consisted of 95% acetonitrile and 5% ultrapure water (v/v) with 5 mM ammonium formate at a pH = 3. A linear gradient from 100% B to 20% B in 15 min was used. Next, B was held at 20% for 5 min. Then

%B was increased to 100% in 1 min and after this the column was equilibrated at 100% B for 6 min which results in a total run time of 27 min. The flow rate was 0.300 mL/min and 50 µL of sample was injected onto the LC column.

2.5 Method validation

The validation was based on the guidelines developed by Peters et al. (2007) and the guidelines for bioanalytical method validation by the European Medicines Agency (EMA) (van Amsterdam et al., 2013). During the validation, performance parameters such as precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), linearity, matrix effects, recovery, selectivity and carry-over were evaluated. The method validation was performed in tap water and industrial wastewater (free of any targeted compounds and mimicking the composition of urban wastewater) spiked at 0, 1, 5 and 100 ng/L. This was done four times per matrix on two separate days (eight measurements in total per matrix and concentration). The LOD in industrial wastewater is defined as three times the standard deviation of the repeatability for the lowest concentration that was spiked (1 ng/L), taking into account a confidence interval of 99% with one-side probability. The LOQ was determined by multiplying the LOD by 3. Matrix effect was investigated at 100 ng/L, where the ratio between the concentration in the tap water and the concentration in wastewater multiplied by 100 is computed as the matrix effect (in %). Repeatability and accuracy were determined at 1, 5 and 100 ng/L on two separate days and recovery was determined at a spiked concentration of 100 ng/L. Calibration curves ($R^2>0.99$) were based on seven concentration levels ranging from 0-500 ng/L. Calibration curves (quadratic, weighted 1/x) were constructed by plotting the ratio of the peak area against the peak area of the corresponding deuterated internal standard. Carry-over was determined by analyzing a procedural blank after the highest concentration of the calibration curve.

2.6 In-sample stability of crack cocaine biomarkers

To assess stability of AEME and AE in different conditions, an experimental set-up based on McCall et al. (2016) was used. In brief, a large wastewater pool of 2 L was divided in aliquots of 50 mL three days before the start of the analysis to form biofilm in the bottles. Idem, 2 L of drinking water was divided in 50 mL HPDE bottles for the blank controls. After this, aliquots were spiked at 5 µg/L with the following compound combinations: i) AE + AEME, ii) AE, iii) AEME, iv) benzoylecgonine (metabolite of cocaine) and v) cocaine. Benzoylecgonine and cocaine were analyzed to see if crack cocaine biomarkers were formed during the experiment. The aliquots were placed at 20 °C, 4 °C , and -20 °C and every condition of the experiment was tested in triplicate. The spiking of the aliquots was considered as time point 0 hours. At 0, 2, 4, 8, 24, 48, 72 and 96 h, 50 µL was taken out of the aliquot and 28 µL of the internal standard was added (final concentration of 50 ng/L). At every time step and temperature, a non-spiked wastewater sample was taken to correct for background concentrations. 50 µL of ultrapure water was added and after this the sample was diluted with acetonitrile to 1 mL and filtered with a 0.45 µm filter into a vial. The final concentration of the compounds in the vial is approximately 250 ng/L. The samples were stored at -20 °C until analysis. A graphical illustration of the experimental set-up of the in-sample stability tests can be found in Figure S1.

2.7 Data analysis

All statistical tests were performed using R (R Core Team, 2021) and p-values < 0.05 were considered significant. Differences in population-normalized loads of AEME between cities were evaluated using the nonparametric Wilcoxon rank-sum test because in most cases data were not normally distributed. Differences in mass loads between days of the week were evaluated using a non-parametric Kruskal-Wallis test, where the data were normalized to the weekly average of the particular city or year. Also, a nonparametric Wilcoxon test was used to compare the pooled normalized weekdays data with the pooled weekend data to investigate differences in use between weekdays (Tuesday, Wednesday, Thursday, Friday) and weekends (Saturday, Sunday, Monday). Pairwise Wilcoxon tests were used to compare mass loads of the different cities. Temporal trends were evaluated by fitting a linear regression to the mass loads of the crack biomarkers and evaluating the significance of the slope of the regression line. The WWTP of the city of Amsterdam covers 77% of its population (personal communication, WWTP Amsterdam West). The number of registered inhabitants for each of the considered years was hence multiplied by 0.77 to avoid using a static figure which does not account for the increasing population. For the comparison between benzoylecgonine and AEME, a linear regression model was computed to determine if there was a relationship between benzoylecgonine and AEME loads and a Spearman Rank Sum test was conducted to find a correlation between those two biomarkers.

3 Results

3.1 Method validation

For AEME, the selectivity was confirmed by the analysis of three blank samples, all of which showed no interference. No carry-over was found at the blank samples after the highest level of the calibration level (500 ng/L). The linearity of the calibration curve was $R^2 = 0.9955$ (quadratic, weighted 1/x). The lowest limit of quantification (LLOQ) of 1 ng/L was found for the MS2 fragment of AEME. Quality controls of 1, 5, and 100 ng/L were used for the within-run and between-run accuracy and precision because it was expected that AEME would be found at low concentrations (< 10 ng/L). Within-run (86.6 -105.2%) and between-run accuracy (95.6 – 110.4%) and precision (1.32 -7.95%) were within the range of 15% bias. Matrix effect (n=8) was 79% and recovery (n=8) was 91.7%. These results are also summarized in Table 2. For AE, the performance criteria for method validation provided by the EMA were not met because of accuracy, precision and sensitivity. Therefore, AE could not be used to evaluate its suitability as biomarker for crack cocaine use.

3.2 In-sample stability tests

In-sample stability of the analysed biomarkers was evaluated to determine whether these could be formed in wastewater and hence bias obtained results (See Figure 1). Based on the rating of the stability classes proposed by McCall et al. (2016), AEME was highly stable (0-20% transformation) in wastewater after 96 h, except for -20 °C, probably due to the eight freeze and thaw cycles during the experiment. Furthermore, no AEME was formed within 96 h when BE or cocaine was added to the samples. This suggests that there are no other apparent sources of AEME other than crack cocaine consumption. Formation of AEME from cocaine residues due to analytical conditions, as is the case for gas chromatography (Toennes et al., 2003; Cone et al., 1995; Gonzalez et al., 1995), can be excluded here as analyses were performed with LC. Results found here are in line with a previous study which showed that AEME was found to be stable in urine for up to 30 days in samples stored at 4 °C and -20 °C and at pH to 6.0 (Carvalho et al., 2008). Unfortunately, in the present study AE was not stable in wastewater with an increase up to 140% at 4 °C. When AEME or BE was added to the solution, an increase in AE was observed. Based on obtained results, it appears that AEME was stable in wastewater and can hence be used as a biomarker to monitor crack cocaine use through WBE.

3.3 Spatial patterns

Because AE was not stable in wastewater and did not meet the criteria for the method validation, only AEME was further used as a biomarker for crack cocaine use in wastewater. Concentrations of AEME found in wastewater were between 1.8 and 36.6 ng/L. All individual concentrations can be found in Table S1.

Figure 2 shows the AEME population normalized mass loads in all locations in the 13 European cities included in this study. The detection frequency of AEME was 100%. The population-normalized mass loads ranged from 0.3 to 8.0 mg/day/1000 inhabitants. Highest average population-normalized mass loads were found in Antwerp and Amsterdam (6.6 and 6.7 mg/day/1000 inhabitants respectively), while in the other cities AEME concentrations were in the 1.2-3.4 mg/day/1000 inhabitants range. Overall, no significant differences between AEME loads in the 13 European cities were found (Kruskal-Wallis test, p-value > 0.05).

In fact, AEME could be measured even in the four Italian cities analysed. This is in contrast with earlier findings by Castiglioni et al. (2011), where AEME was not detected in influent wastewater collected from various Italian cities higher than the LOQ of 7.5 ng/L. However, it should be noted that only Milan was measured in both this study and the one conducted in 2011 by Castiglioni et al. From previous research in Santiago de Compostela, AEME was not found in concentrations higher than the LOD (i.e. 3 ng/L) (Gonzalez-Mariño et al., 2020). With respect to weekly trends, no significant difference between sampling days was found (Kruskal-Wallis test, p-value > 0.05), as shown in Figure 3. This is in line with expectations that crack cocaine is used regularly and does not exhibit an increased use during weekends unlike other substances e.g. MDMA or snorted cocaine. This non-significant difference between weekdays and weekend days was found in this present study (nonparametric Wilcoxon test, p > 0.05). Similar results were obtained in a study conducted in Brasilia, where mass loads of AEME (4.1-7.2 ng/L) and AE (6.6-8.5 ng/L were found to be stable over four days (Gonzalez-Mariño et al., 2020).

3.4 Temporal trends in Amsterdam

Figure 4 shows the AEME population-normalized mass loads in Amsterdam from 2017 to 2021. Over the considered period, the population of Amsterdam increased (CBS,2022), which was taken into account as detailed previously (see section 2.7). AEME was detected in all samples collected, except for one sample from 2017 (Thursday, April 20 2017), but this was due to insufficient sample volume. Mass loads measured in the five-year period ranged from 4.6 to 13.7 mg/day/1000 inhabitants. No significant difference in AEME mass loads could be found between years (Kruskal-Wallis test and pair-wise Wilcoxon test, p-value > 0.05). In agreement with findings from the analysis of samples collected across European cities, no significant difference could be found between weekdays (Kruskal-Wallis test, p-value > 0.05).

This was a different outcome compared to results from online survey data based on mixed methods and expert perception, which suggested a possible increase in crack cocaine availability and use associated with the COVID-19 pandemic in Europe (EMCDDA, 2021a). Another development observed by experts in several countries (Belgium, Ireland, Spain, France and Portugal) is that the use and availability of crack is increasing largely related to more paraphernalia that is being distributed for crack use by harm reduction services during 2020 (EMCDDA, 2021a). In addition, an increase in the number of crack cocaine users entering treatment has been reported in Belgium, Ireland, France, Italy, Portugal, United Kingdom (EMCDDA, 2020) and France (Janssen et al., 2020).

In this study, only historic wastewater data for the city of Amsterdam was available, hence it is not possible to corroborate whether increased consumption of crack cocaine is taking place in the mentioned countries. Nevertheless, at least in Amsterdam, wastewater data seems to suggest that this is not the case. It would however be highly advisable to extend the monitoring of AEME levels over time to determine if changes are taking place or not. As a first step in data triangulation, a Dutch study by Nabben and Benschop (2021) estimated crack cocaine use in Amsterdam and this was compared with the results of this study. According to studies conducted by these two institutes, there are an estimated 2500 crack cocaine users in Amsterdam (Pérez et al., 2013), consuming on average € 135 worth of crack per week. The street price of cocaine is on average € 50 per gram and purity is approximately 70% (Nabben and Benschop., 2021). Based on this information, the amount of excreted AEME is 3.4 mg/day/1000 inhabitants (assuming that 0.19% of cocaine base will be excreted as AEME after smoking (Baker et al., 2014)), which is in the same order of magnitude as AEME loads found in Amsterdam (6.7 mg/day/1000 inhabitants).

3.5 Crack *versus* cocaine biomarkers

Figure 5 shows the relationship between benzoylecgonine (BE), used as a biomarker to monitor overall cocaine use, and AEME mass loads in the 12 European cities (except for Dublin for which

BE mass loads were not available). For Amsterdam, data from 2017-2021 was included. For the 12 European cities a significant positive correlation of $\rho = 0.78$ (Spearman's Rank correlation test, $p < 0.05$) is observed and for the data from Amsterdam also a significant positive correlation ($\rho = 0.79$) is found ($p < 0.05$). Although local/cultural specificities, which might drive crack cocaine use, cannot be excluded, these findings suggest that there is indeed a positive correlation between general cocaine use (and availability) and crack cocaine use. Nevertheless, a formal causality link between cocaine usage/availability and crack use cannot be established based on these data solely. In surveys the distinction between crack and powder cocaine use is not made often. In fact, in a study about cocaine treatments retrieved from observational studies, no distinction was made for some countries (Germany, Luxemburg), while for others (the Netherlands, Belgium, Ireland) only partially (Antoine et al., 2021). The proportion of crack use among cocaine users as primary substance treatment entrance also varied a lot: from the analysed countries in the current study, Italy had the lowest share of crack (<10%), followed by Spain and Ireland (10-20%) and the Netherlands and Belgium above 30% (Portugal not mentioned). A correlation between crack and powder cocaine use in this study was found, but unfortunately no clear explanation is provided by the authors why this is occurring. Further research is needed to better understand this correlation and its determinants.

4. Conclusions

An analytical method was developed and validated for the measurement of crack cocaine biomarker AEME in influent wastewater. AEME was found stable in wastewater and the concomitant presence of cocaine or BE in a sample does not result in formation of additional AEME. The method was applied to evaluate crack cocaine use in 13 European cities between

October 2020 and June 2021 and in Amsterdam from 2017 to 2021. This is, to the author's knowledge, the first study which covers a broad range of European cities to investigate crack cocaine use. In all cities AEME was found and Amsterdam and Antwerp exhibited the highest population-normalized mass loads of AEME. Our results showed no trends in AEME mass loads in Amsterdam from 2017 to 2021, where there are signals of a possible increase in crack cocaine use and availability. Calculations based on the number of users, street price and amount of crack use per week in Amsterdam yield, results similar to those that are based on the mass loads observed in influent wastewater. Also a positive correlation between AEME and BE mass loads was observed, but a formal causality link between cocaine usage/availability and crack use cannot be established based on this data solely This study highlights the importance of wastewater analysis to monitor community-wide loads of crack cocaine use. More routinely monitoring of AEME and the comparison between WBE data and surveys focused on crack use versus powder cocaine use needs to be done to get more insight in crack cocaine consumption.

Credit author statement

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Declaration of Interest Statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Tables and Figures

Table 1: Sample locations

Country	Location	Sampling period
the Netherlands	Amsterdam	18/03/2021 – 24/03/2021
	Utrecht	17/03/2021 – 23/03/2021
	Eindhoven	17/03/2021 – 23/03/2021
Italy	Rome	19/10/2020 – 25/10/2020
	Milan	02/11/2020 – 08/11/2020
	Bologna	19/10/2020 – 25/10/2020
	Bari	19/10/2020 – 25/10/2020
Belgium	Brussels	13/04/2021 – 19/04/2021
	Antwerp	23/03/2021 – 29/03/2021
Portugal	Lisbon	27/04/2021 – 03/05/2021
	Almada	21/04/2021 – 27/04/2021
Spain	Castellon	07/04/2021 – 13/04/2021
Ireland	Dublin	13/06/2021 – 19/06/2021

Table 2: Validation parameter

Compound	IS	Linearity (R^2)	Inter-day precision (%RSD, n = 8)			Intra-day precision (%RSD, n = 8)			Matrix effect (%)	Recovery (%)
			1 ng/L	5 ng/L	100 ng/L	1 ng/L	5 ng/L	100 ng/L		
AEME	AEME-d3	0.9955	7.19	6.08	1.32	9.38	2.07	0.16	78.85	91.70

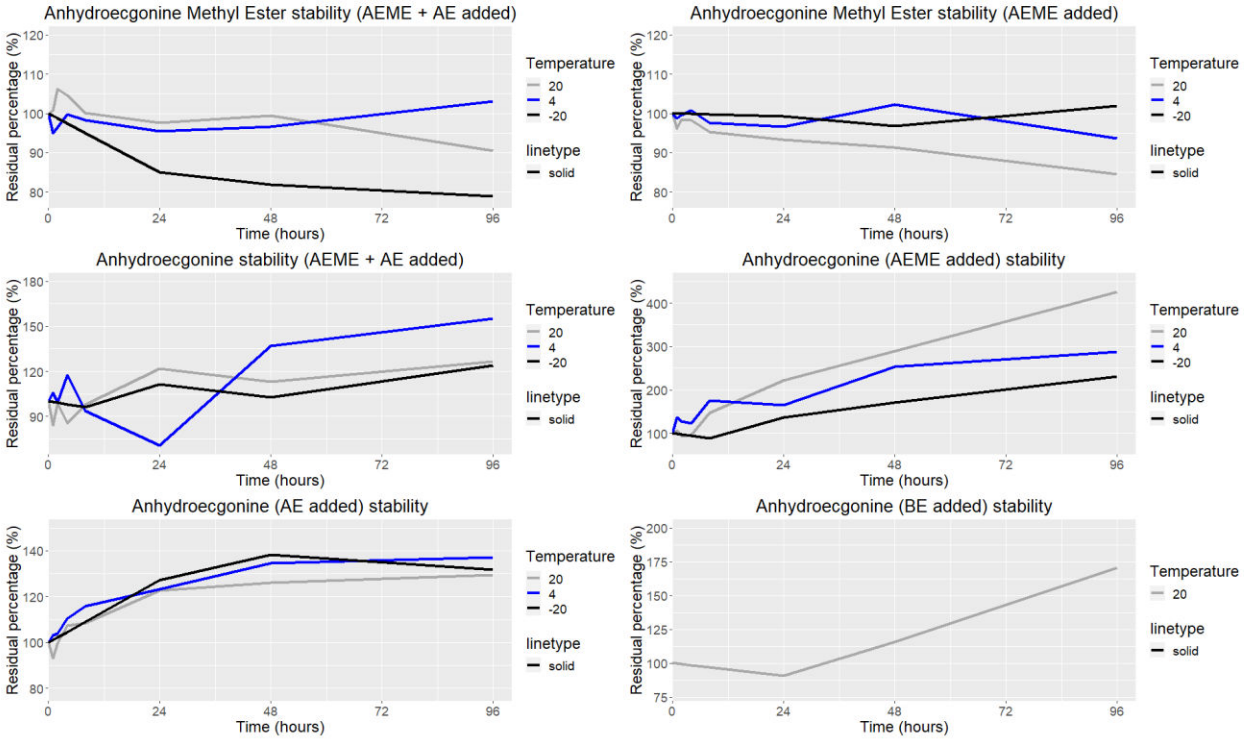

Figure 1: The residual percentages of the six stability tests for the stability of AE and AEME after 96 h.

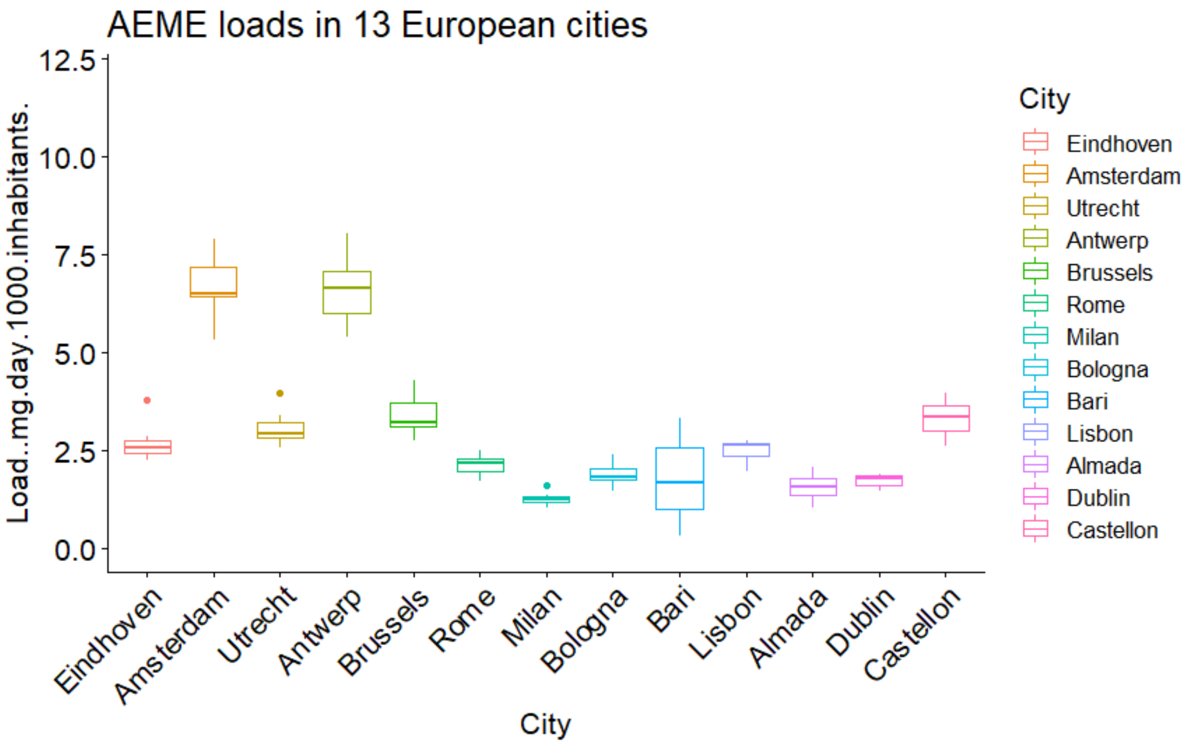

Figure 2: AEME loads in 13 European cities. The dots represent outlier in the data and the error bars represent the range of the mass loads.

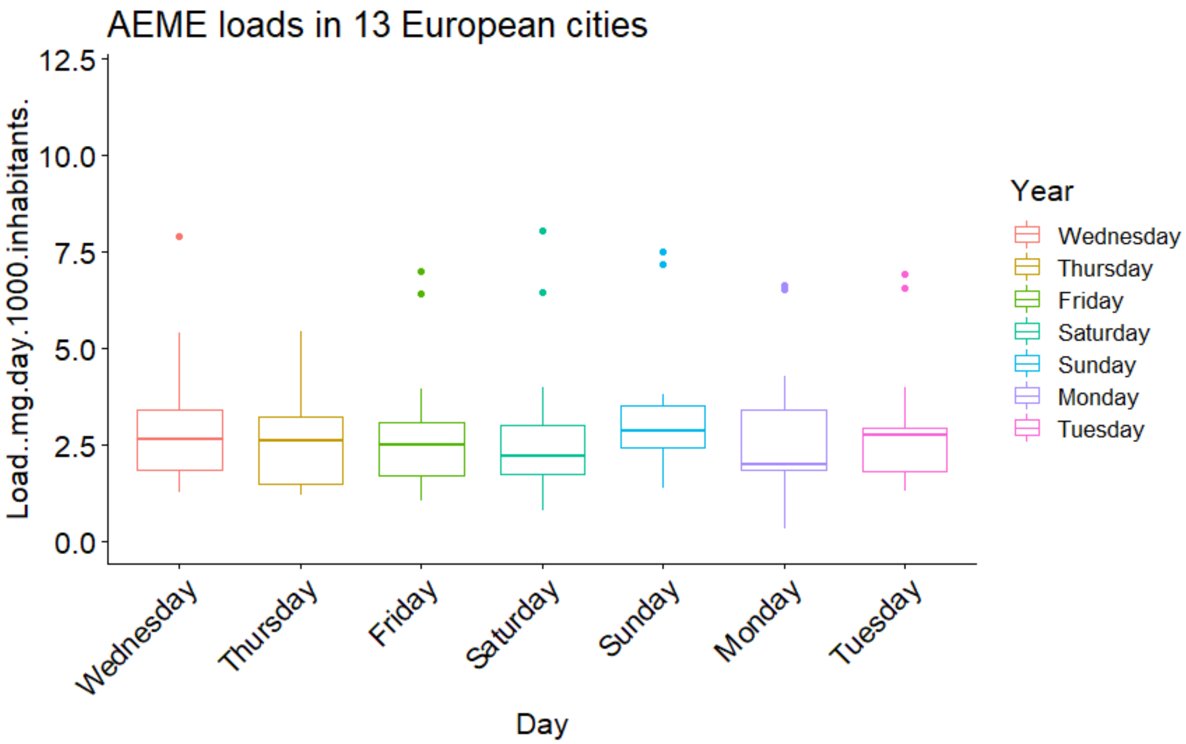

Figure 3: AEME load in 13 European cities per day of the week

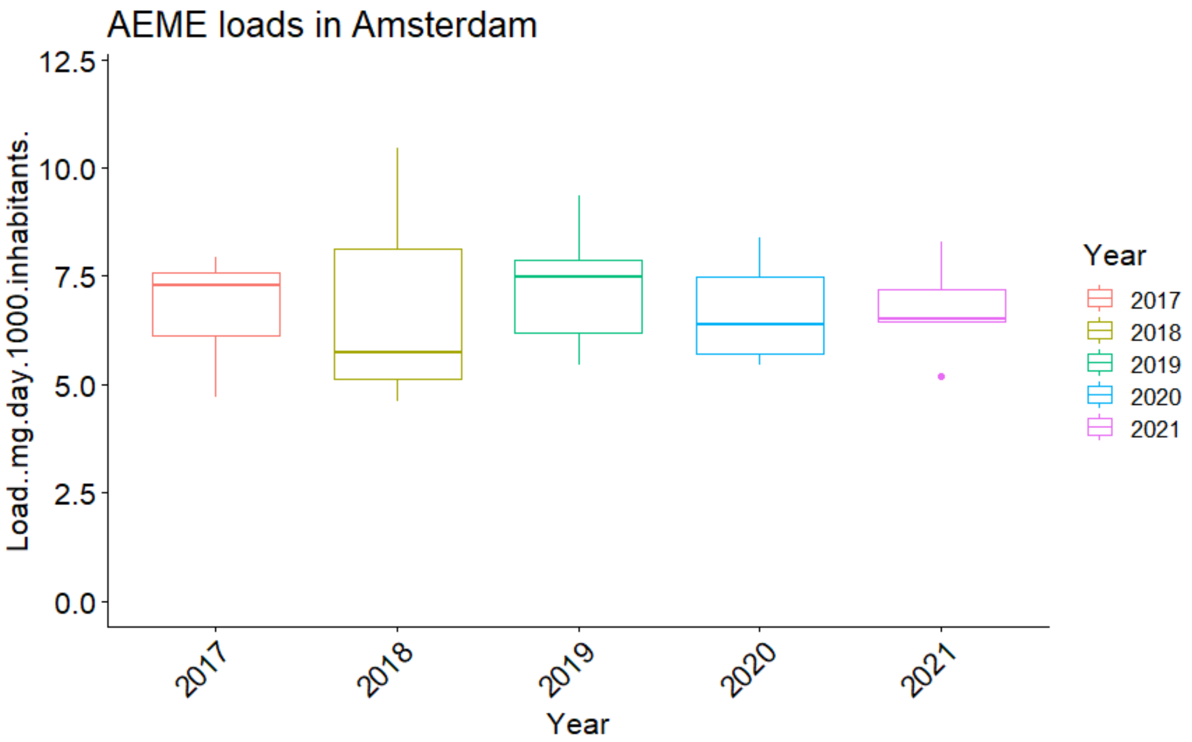

Figure 4: AEME loads in Amsterdam from 2017 to 2021.

Declaration of Interest Statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Credit author statement

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