Human population as a key driver of biochemical burden in an inter-city system: implications for One Health concept

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Abstract:

This paper tests the hypothesis that human population and city function are key drivers of biochemical burden in an inter-city system, which can be used to inform One Health actions as it enables a holistic understanding of city's metabolism encompassing all of the activities of a city in a single model: from lifestyle choices, through to health status and exposure to harmful chemicals as well as effectiveness of implemented management strategies. Chemical mining of wastewater for biochemical indicators (BCIs) was undertaken to understand speciation of BCIs in the context of geographical as well as community-wide socioeconomic factors. Spatiotemporal variabilities in chemical and biological target groups in the studied inter-city system were observed. A linear relationship (R^2 >0.99) and a strong positive correlation between most BCIs and population size (r >0.998, p <0.001) were observed which provides a strong evidence for the population size as a driver of BCI burden. BCI groups that are strongly correlated with population size and are intrinsic to humans' function include mostly high usage pharmaceuticals that are linked with long term non-communicable conditions (NSAIDs, analgesics, cardiovascular, mental health and antiepileptics) and lifestyle chemicals. These BCIs can be used as population size markers. BCIs groups that are produced as a result of a specific city's function (e.g. industry presence and occupational exposure or agriculture) and as such are not correlated with population size include: pesticides, PCPs and industrial chemicals. These BCIs can be used to assess city's function, such as occupational exposure, environmental or food exposure, and as a proxy of community-wide health. This study confirmed a strong positive correlation between antibiotics (ABs), population size and antibiotic resistance genes (ARGs). This confirms the population size and AB usage as the main driver of AB and ARG levels and provides an opportunity for interventions aimed at the reduction of AB usage to reduce AMR. Holistic evaluation of biophysicochemical fingerprints (BCI burden) of the environment and data triangulation with socioeconomic fingerprints (indices) of tested communities are required to fully embrace One Health concept.

Key words: water fingerprinting, wastewater-based epidemiology, AMR, pharmaceuticals, illicit drugs, pesticides, lifestyle, exposure

1. Introduction

One Health assumes that the health of people is closely linked with the health of animals and surrounding environment. It is a cross sectoral and multidisciplinary effort aimed at holistic understanding and management of public and environmental health. One Health has been widely adopted in the antimicrobial resistance (AMR) challenge as it is multifaceted with human and animal health impacts, as well as food security and safety. One Health model incorporates a dynamic set of biophysicochemical (e.g. multichemical complex mixtures impacting environmental and public health via variable exposure status) and socioeconomic/health indicators (e.g. level of industrial/agricultural activity, deprivation index, disease prevalence) that are difficult to unravel. Here we present an approach that enables research within the One Health domain – wastewater fingerprinting or wastewater-based epidemiology (WBE).

Wastewater represents a fingerprint of a city's production, metabolism and disposal. It is a complex mixture of substances of biological and chemical origin including city stressors (e.g. toxicants and infectious agents) and urban physiological processes (e.g. specific disease-linked proteins, genes and stressor metabolites). The quantitative measurement of these substances continuously pooled by the sewerage system can provide evidence of a city's exposure to stressors (Kasprzyk-Hordern 2019). Wastewater can also provide data on the biochemical burden released by a city (Figure 1). Several papers focussed on quantification of various

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chemicals and biological agents in wastewater but none, to the authors knowledge, attempted to correlate biochemical fingerprint with city's size and its function.

In order to understand biochemical burden produced by a city, WBE pipelines developed in Bath (Kasprzyk-Hordern, Proctor et al. 2021) were used in this manuscript. WBE focusses on wastewater as a medium for epidemiological information about a community contributing to the wastewater (usually a city or town). WBE currently informs illicit drug use trends (Ort, van Nuijs et al. 2014) (Thomas, Bijlsma et al. 2012) (González-Mariño, Baz-Lomba et al. 2020) and other lifestyle chemical use: e.g., alcohol (Reid, Langford et al. 2011, Baz-Lomba, Salvatore et al. 2016), tobacco (Castiglioni, Senta et al. 2014), counterfeit medicines (Venhuis, de Voogt et al. 2014) (Causanilles, Cantillano et al. 2018, Causanilles, Nordmann et al. 2018), antibiotics and corresponding resistance genes (Castrignano, Yang et al. 2020) as well as levels of stress biomarkers such as isoprostanes (Ryu, Gracia-Lor et al. 2016, O'Brien, Choi et al. 2019). WBE has revolutionised population health studies, especially in the context of the COVID pandemics (Ahmed, Angel et al. 2020, Bivins, North et al. 2020, Medema, Been et al. 2020, Sodre, Brandao et al. 2020). WBE has also focussed on public exposure to chemicals: pesticides (Rousis, Gracia-Lor et al. 2017) (O'Brien, Choi et al. 2019, Rousis, Gracia-Lor et al. 2020) and industrial chemicals (Lopardo, Petrie et al. 2019) (Been, Bastiaensen et al. 2018).

This paper tests the hypothesis that human population and city function are key drivers of biochemical burden in an inter-city system, which can be used to inform One Health actions. Several groups of chemical and biological agents (biochemical indicators, BCIs) were subject of investigation: water quality indicators (COD, BOD, N, P), industrial chemicals, personal care products, pesticides, illicit drugs, lifestyle chemicals, prescription pharmaceuticals, as well as genetic targets, such as antibiotic resistance genes (ARGs). We have selected five contrasting town/cities served by five major wastewater treatment plants (WWTPs) contributing to one river catchment in the South-West UK and covering an area of approximately 2,000 km^2 and a population of ~1.5 million (this constitutes >75% of the overall population in the catchment). Chemical mining of wastewater for BCIs was undertaken to understand spatiotemporal speciation of BCIs in the context of geographical as well as community-wide socioeconomic factors. The five cities and towns tested have different characteristics: (1) they are different in size, as well as (2) in industry presence and socioeconomic status. We applied WBE pipelines to:

(1) Understand spatiotemporal variabilities in chemical and biological target groups in the studied inter-city system.

(2) Identify target groups that are strongly correlated with population size and are intrinsic to human function.

(3) Identify target groups that are produced as a result of a specific city's function (e.g. industrial presence or agriculture) and as such are not correlated with population size.

(4) Select markers that can inform the size of population served by WWTPs.

(5) Test which BCIs can be used as proxies for city health and AMR prevalence, including potential for at source interventions.

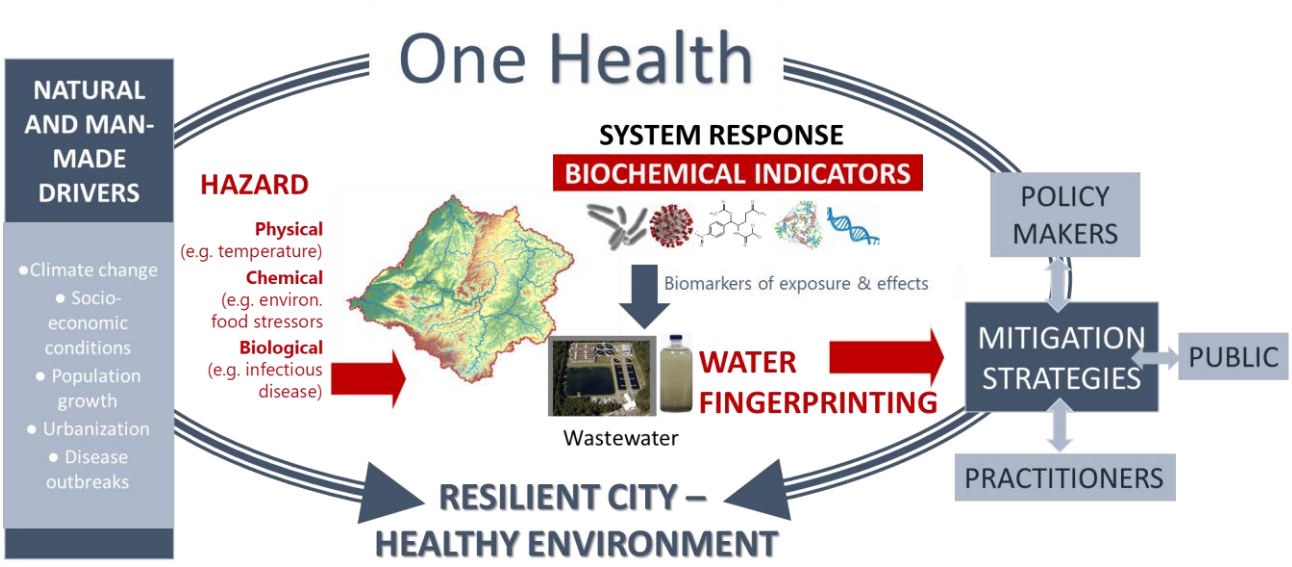


Figure 1. Water Fingerprinting and One Health

2. Materials and Methods

2.1. Reagents and analytical standards

Several BCI groups were studied (Table 1). These include pharmaceuticals, chemicals in personal care products, pesticides, industrial chemicals, illicit drugs and other lifestyle chemicals as well as genetic material (ARGs) and water quality indicators. The internal standards (IS) used in chemical analysis are discussed in (Proctor, Petrie et al. 2021) and are also gathered in Table S1, S2. Water was purified using a Milli-Q purification system from Millipore (Nottingham, UK). All solvents used were of HPLC grade or higher. MeOH, HCOOH, HCl, NaOH, NH_4OH, NH_4F and 2-propanol were purchased from Sigma (UK) and Fisher (UK). All glassware was deactivated using a 5% (v/v) dimethyldichlorosilane (DMDCS) in toluene (Sigma, UK) to prevent loses from analyte sorption according to the procedure described in (Proctor, Petrie et al. 2021).

Table 1. Classes of BCIs.

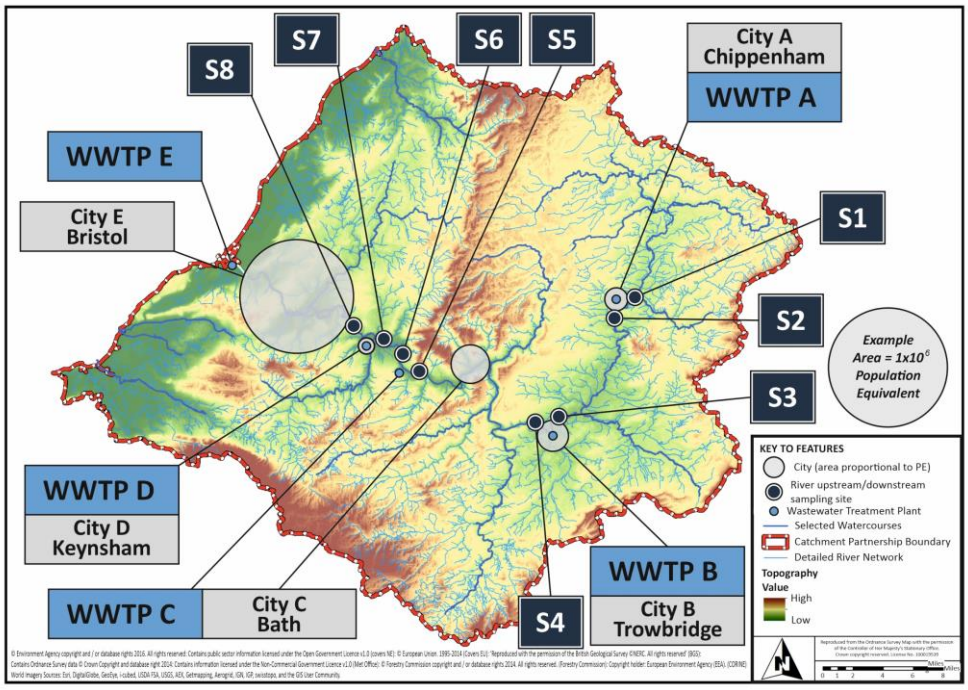
Class	Compound	Class	Compound
UV Filter	Benzophenone-1	Anaesthetic and metabolite	Ketamine
	Benzophenone-2		Norketamine
	Benzophenone-3	Anti-depressants	Venlafaxine
	Benzophenone-4		Desvenlafaxine
Parabens	Methylparaben		Fluoxetine
	Ethylparaben		Norfluoxetine
	Propylparaben		Sertraline
	Butylparaben		Mirtazapine
Plasticizer	Bisphenol-A		Citalopram
	Bisphenol A sulphate		Desmethylcitalopram
Steroid Estrogens	E1		Paroxetine
	E2		Duloxetine
	EE2		Amitriptyline
Antibiotics and Antibacterial	Sulfasalazine		Nortriptyline
	Clarithromycin		Norsertraline
	Azithromycin		
	Trimethoprim	Anti-epileptic	Carbamazepine
	Sulfamethoxazole		Carbamazepine10,11-epoxide
	Triclosan		10,11-Dihydro-10-hydroxycarbamazepine
	Triclosan sulphate		
	Amoxicillin	Calcium-channel blocker	Diltiazem
	Metronidazole		Verapamil
	Sulfadiazine	Hypnotic	Temazepam
	Cefalexin		Oxazepam
	Ofloxacin		Diazepam
	Ciprofloxacin	Anti-psychotic	Quetiapine
	Tetracycline		Risperidone
	Danofloxacin	Dementia	Donepezil
	Oxytetracycline		Memantine
	Chloramphenicol	Creatinine	Creatinine
	Penicillin G	Lifestyle Chemicals	Nicotine
	Penicillin V		Caffeine
	Erythromycin		Cotinine
	Prulifloxacin		1,7 dimethylxantine
	Norfloxacin	Analgesics and Metabolites	Morphine
	Nalidixic acid		
Antifungal	Griseofulvin		Dihydromorphine
	Ketoconazole		Normorphine
Hypertension	Valsartan		Methadone
	Irbesartan		EDDP
	Lisinopril		Codeine
NSAIDs	Ketoprofen		Norcodeine
	Ibuprofen		Dihydrocodeine
	Naproxen		Tramadol
	Diclofenac		N-desmethyltramadol
	Acetaminophen		O-desmethyltramadol
Lipid regulator	Bezafibrate	Stimulants and metabolites	Amphetamine
	Atorvastatin		Methamphetamine
Anti-hyperlipidemic	Gemfibrozil		MDMA
Anti-hyperintensive	Candesartan Cilexetil		MDA
Antihistamine	Fexofenadine		Cocaine
	Cetirizine		Benzoylecgonine
GUD/ED	Sildenafil		Anhydroecgonine methylester
Diabetes	Metformin		Cocaethylene
	Gliclazide		Mephedrone
	Sitagliptin		MDPV
Cough suppressant	Pholcodine	Opioid and metabolite	Heroin
Beta-blocker	Atenolol		6-acetylmorphine
	Metoprolol		Thiamethoxam

	Propranolol	Pesticides, fungicides and herbicides	Imidacloprid
	Bisoprolol		Clothianidin
H2 receptor agonist	Ranitidine		Metazachlor
	Cimetidine		Terbuthylazine
X-ray contrast media	Iopromide		Methiocarb
Various	Buprenorphine		Dichlofluanid
Drug precursor	Ephedrine/pseudoephedrine		Flufenacet
	Norephedrine		Oxadiazon
Anti-cancer	Azathioprine		Chlorpyrifos
	Methotrexate		Triallate
			3PBA (3-phenoxybenzoic acid)
	Ifosfamide	Veterinary Pharma	Tylosin
	Tamoxifen		Sulfapyridine
	Imatinib		Sarafloxacin
	Capecitabine		Ceftiofur
	Bicalutamide		Diazinon
Genes/ARGs	16SRNA	WQIs	Ammonia N
	ermB		COD
	qnrS		N total
	sulf		Nitrite as N
	catA		Nitrate as N
			Orthophos
			Chloride

2.2. Sample collection

Untreated wastewater samples (1L) were collected (between coarse screening and primary sedimentation) for 7 consecutive days from Wednesday to Tuesday between June and October 2015 from five major WWTPs (Figure 2, sites A-E) serving 5 cities and towns: Chippenham (town), Trowbridge (town), Bath (city), Keynsham (town) and Bristol (city). These WWTPs contribute to >75% of the overall population in studied Avon River Catchment (an area of approximately 2,000 km^2 and the population of ~1.5 million).

Untreated wastewater samples were collected as volume proportional 24 h composites with average sub-sample collection frequencies of approximately 15 minutes using an ISCO 3700 autosampler packed with ice to maintain 4^{o}C during collection to limit biological activity (Petrie *et al.*, 2017). River water samples were collected as grab samples on the same days as wastewater samples (see S1-S8 in Figure 2). All samples were transported on ice to the laboratory, spiked with the internal standards and stored at -18°C until sample preparation and analysis.



Site	Population	Industrial Contribution	Sewer Residence (h)	Wastewater Treatment Process*	Solid Retention Time (d)	Hydraulic Retention Time (h)	River sampling distance to discharge point (km)	
							Up stream	Down Stream
WWTP A (serving Chippenham)	37,714	0.4%	<0.5 -4	AS	19	46.2	0.5	0.5

WWTP B (serving Trowbridge)	68,453	30.0%	<0.5 -4	TF	n.a	24.5	0.5	0.5
WWTP C (serving Bath)	109,543	1.2%	<0.5 -9	TF	n.a	13.9	2	2
WWTP D (serving Keynsham)	18,274	0.1%	<0.5 -2	TF	n.a	17.6	1	1
WWTP E (serving Bristol)	867,244	23.9%	<1 -24	90% SBR 10% AS	4 8	10.9 25.8	-	-

* AS – activated sludge, TF – trickling filter, SBR – sequencing batch reactor.

Figure 2 Site information of studied WWTPs and corresponding river locations (note: Towns A, B and D are called City A, B and C in the text for simplicity reasons).

2.3. Sample preparation and analysis

2.3.1. SPE/MAE-UHPLC-QqQ - targeted analysis of chemical BCIs

Methodology used in this paper was as published by Proctor et al. (Proctor et al. 2019). Briefly, liquid samples (50 mL, at pH 7.5 -8.5) were filtered with GF/F glass microfibre 0.7 µm filter (Whatman, UK), and spiked with 50 ng of internal standard (IS) mix (50 µL of a 1 µg mL^{-1} methanolic IS solution). Solid phase extraction (SPE) with 60 mg Oasis HLB sorbents (Waters, UK), pre-conditioned using 2 mL MeOH and 2 mL H_2O at 1 mL min^{-1}, was used to extract and concentrate BCIs from the matrix. 50 mL of wastewater samples were then loaded at 5 mL min^{-1} and dried under vacuum. BCIs were then eluted using 4 mL MeOH at a rate of 1 mL min^{-1}. Methanolic extracts were dried under nitrogen using a TurboVap evaporator (Caliper, UK, 40 ºC, N^2, <5 psi). Dried extracts were reconstituted in 500 µL 80:20 H_2O:MeOH and analysed with UHPLC-QqQ.

Suspended particulate matter (SPM) obtained from GF/F filters was freeze-dried and 0.25 g samples were spiked with 50 ng of IS mix (50 µL of a 1 µg mL^{-1} methanolic IS solution). Microwave assisted extraction (MAE) was used to extract BCIs from SPM. Briefly, samples were mixed with 25 mL of 50:50 MeOH:H_2O (pH 2), heated at 110 ºC using a 800 W MARS 6 microwave (CEM, UK) and methanolic extracts adjusted to <5 % of MeOH using H_2O (pH 2). The extracts were then passed through pre-conditioned Oasis MCX SPE cartridges (Waters, UK) and eluted with 2 mL 0.6 % HCOOH in MeOH (acidic compounds) followed by 3mL 7% ammonium hydroxide in MeOH (basic compounds). After drying, the extracts were reconstituted in 500 µL 80:20 H_2O:MeOH, filtered using pre-LCMS 0.2 µm PTFE filters (Whatman, Puradisc) and analysed with the method described below.

Extracted BCIs were analysed using a Waters Acquity UPLC system (Waters, Manchester, UK) equipped with BEH C18 column (150 x 1.0 mm, 1.7 µm particle size) (Waters, Manchester, UK) and coupled with Xevo TQD Triple Quadrupole Mass Spectrometer (Waters, Manchester, UK) equipped with an electrospray ionisation source. Analysis was performed in both ESI+ and ESI- with a capillary voltage of 3.20 kV, the desolvation temperature of 400 °C and the source temperature of 150 °C. Nitrogen was used as the nebulising and desolvation gas, and argon as the collision gas. The cone gas flow was 100 L $h^{-1}$and the desolvation gas flow was 550 L h^{-1}. Further details regarding method's conditions and performance can be found in Proctor et al. (Proctor et al. 2019) and in Tab S1 and Fig S1.

2.3.2. SPE-UHPLC-QTOF – retrospective analysis of chemical BCIs

Methodology used in this paper was as published by (Lopardo, Rydevik, and Kasprzyk-Hordern 2018). 100 mL of unfiltered wastewater samples were spiked with IS mix (25 µL of a methanolic solution, 1 µg mL^{-1}), filtered using GF/F glass microfibre filter (Whatman, UK) and passed through Oasis HLB. BCIs were then eluted using 4 mL MeOH at a rate of 1 mL min^{-1}. Methanolic extracts were dried under nitrogen using a TurboVap evaporator (Caliper, UK, 40 ºC, N^2, <5 psi). Dried extracts were reconstituted in 500 µL 80:20 H_2O:MeOH and analysed with UHPLC-QTOF. Extracted BCIs were analysed using Dionex Ultimate 3000 HPLC equipped with a BEH C18 column (50 x 2.1 mm, 1.7 µM, Waters UK) coupled with a Bruker Maxis HD Q-TOF equipped with an ESI source, which was operated in both positive and negative ionisation mode. The source settings were as follows: capillary voltage was set at 4.5 kV, the end plate offset was set to 500 V, a pressure of 3 Bar was used for the nebulizer gas, the drying gas (nitrogen) flow was 11 L min^{-1} and the drying temperature was set at 220°C. Analysis was run in both full scan mode (MS) and broadband collision induced dissociation (bbCID) mode. Calibrant solution was injected before each run. Further details regarding method's conditions and performance can be found in (Lopardo, Rydevik, and Kasprzyk-Hordern 2019) and in Table S2 and Fig S2.

2.3.3. Gene analysis

Unfiltered influent wastewater (1 mL) was centrifuged (3000 g, 5 min) and the pellet formed was re-suspended in 200 µL of phosphate buffered saline (PBS) to which 5 µL of lysozyme was added followed by incubation at 37 °C for 15 minutes. 200 µL of binding buffer and 40 µL of proteinase K were then added and samples incubated for 10 minutes at 70 °C. DNA was then extracted using the High Pure PCR Template preparation Kit (Roche, Germany) following manufacturer's instructions. After extraction, the DNA was quantified using a Thermofisher Nanodrop instrument and stored a -80 °C before genetic BCIs' quantification using the QuantStudio 3D Digital PCR system and the QuantaStudio 3D PCR V2 Kit (Life Technologies, Thermo Fisher Scientific). PCR reaction consisted of 7.3 µL Master Mix V2, 0.7 µL ARG specific TaqMan assay (20 X primer/probe mix), 1.5 µL DNase free water and 6.0 µL of DNA sample. 14.5 µL of PCR mix was then loaded onto the high density nanofluidic PCR chip. Amplification was carried out using a GeneAmp PCR 9700 system. The reaction was initiated through heating to 95 °C and held for 10 minutes with thermocycling carried out for 40 cycles; 2 minutes at 60 °C followed by 98 °C for 30 seconds. Each chip was processed using the QuantStudio 3D Digital PCR system and Thermo Scientific AnalysisSuite™ software was used to analyse results. Further details regarding the method are presented in Castrignano et al. and Elder et al. (Castrignanò *et al.*, 2020)(Elder, Proctor et al. 2021).

2.3.4. Water quality indicators

Water quality indicators (WQIs) were analysed at Wessex Water Scientific Centre. The Aquakem (Thermo Scientific) analyser was used for the quantitative measurement of water quality indicators except for COD. The following parameters were studied: Ammonia N, N total (NON), Nitrite, Nitrate, Orthophosphate, Chloride, COD. Detailed methodology is included in the Supplementary Section.

2.4. Calculations

Daily mass loads of BCIs (mg day^{-1}) were calculated by multiplying total BCI concentrations (mg L^{-1}) in a 24 h composite raw wastewater sample by daily wastewater flow rates (L day^{-1}). Total BCI concentrations in raw wastewater were calculated after accounting for both liquid and SPM fractions using the following formula:

$$BCI_{load}[mg\ day^{-1}] = C_{BCI}\ x\ V$$

where: C_{BCI} is the total concentration of *BCI* (mg L^{-1}) in influent wastewater (both liquid and SPE phase), *V* is the volume of wastewater received by the WWTP per day (L day^{-1}).

Population normalised daily mass loads (mg day^{-1} $1000inh^{-1}$) were calculated using the following formula:

$$BCI_{PNDL}[mg\ day^{-1}\ 1000inh^{-1}] = \frac{BCI_{load}}{PE_{WW\ or\ NHS}}\ x\ 1000$$

where: BCI_{load} is the daily mass load of *BCI* (mg day^{-1}) in influent wastewater, PE_{WW} is the water utility estimate and PE_{NHS} is population size of patients registered in primary care (see Figure 3).

Statistical analysis was undertaken using Excel and Regression Analysis. ANOVA was used to calculate p value. PCA analysis was undertaken using Analyse-it. Seven sampling days, each analysed in duplicate, in five different cities were investigated. Constant values for population equivalents were applied for system calibration. Two population size estimates were used (Figure 3): PE-WW and PE-NHS. PE-WW was calculated based on water utility estimates as presented in Figure 3. Resident population estimate was calculated by multiplying number of properties by occupancy rate, adjusted for care homes, residential schools etc. The occupancy rate is set at district level.

Resident population included care homes, schools, universities, prisons and military bases. Tourism was counted under the non-resident population. Day trippers were not counted. Commercial waste was calculated based on supply flow to commercial properties and estimate of 60 g BOD per capita per day. Tankered waste imports were calculated based on COD strength. As the volume of waste was known, therefore a load could be calculated and converted into a PE (using the assumption of 120 g COD per capita per day). However, tankered waste could not be associated only with 'septic' waste as a proportion of the waste was of industrial origin.

PE-NHS (population size by GP surgeries) was calculated based on the number of people registered in the GP surgeries located in the WWTPs catchment zone. GP surgeries information, such as, postcode and number of

people registered were obtained from NHS Digital (https://digital.nhs.uk/). Briefly, we have used PrAna (Jagadeesan et al., manuscript in submission, http://pranaviz.bath.ac.uk:3838/pranaviz/) tool to identify the GP surgeries present in each WWTPs catchment zone. Briefly, the WWTPs catchment maps were used to identify and collect GP surgeries information inside each catchment region, including number of patients registered using R, an open source software for statistical computing and graphics.

PE-WW: directly linked with sewage catchment but might lead to PE overestimation in the presence of industrial effluent

PE-NHS: accounts for patients registered in the catchment with likely PE underestimation due to not accounting for commuters/visitors

	PE-WW (2015)				
Wessex Water Population Data	WWTP A	WWYP B	WWTP C	WWTP D	WWTP E
City/Town served	Chippenham	Trowbridge	Bath	Keynsham	Bristol
Domestic-Billed Properties	15,472	20,537	43,807	8,074	288,702
Average Household Size	2	2	2	2	2
Resident Population Estimate	35,121	44,771	93,747	17,278	612,048
Non-Resident Population	504	329	8,350	123	18,671
Commercial PE	1,491	2,448	6,182	531	29,090
Trade Effluent PE	149	18,209	1,264	27	41,640
Tankered Waste PE	0	2,328	0	0	165,795
Total PE served by WWTP	**37,714**	**68,453**	**109,543**	**18,274**	**867,244**
	PE-NHS				
Patients registered	**40,184**	**47,834**	**113,128**	**23,493**	**732,173**
% CV	3.2	17.7	1.6	12.5	8.4

Figure 3. Populations equivalents used in the study (2015)

As seen from Figure 3 both PE-WW and PE-NHS provide comparable PE estimates, especially in Cities A and C. The highest % CV are observed for City B and E, likely due to industry presence, and in City D, likely due to small population size.

3. Results and discussion

3.1. Spatiotemporal patterns of BCIs in the inter-city system

Most pharmaceutical targets are used to treat chronic conditions. These are cardiovascular, diabetes, and mental health pharmaceuticals. Due to their long-term usage daily loads showed low temporal variability (as seen in Figure S3) both in terms of lack of weekly trends (no 'weekend' effect that is characteristic for illicit drugs) and inter-city variability. Most cities had similar population normalised drug loads (PNDLs) (Figure S4), also discussed in our previous paper ((Kasprzyk-Hordern, Proctor et al. 2021), with some inter-city variabilities that are discussed in section 3.4.1. for example, city D showed slightly higher presence of cardiovascular drugs and city C, as opposed to city E, showed lower prevalence of antidiabetics, but higher prevalence of cardiovascular drugs. As opposed to pharmaceuticals a clear 'weekend' trend of increased PNDLs was observed in the case of illicit drugs (cocaine and MDMA). Interestingly, the largest studied city (E) had the highest illicit drug share, more than double, when compared to city A (Figure S5). Population normalised daily loads of caffeine and nicotine stayed relatively constant across the week in all studied cities, with city C and D showing relatively higher PNDLs when compared to cities B and E. IPCPs (industrial and personal care products) were city-function dependent with the highest pesticide PNDLs (for imidacloprid and diazinon) recorded in cities A, B and E (Figure S6). Industrial chemical PNDLs of IPCPs (BPA, benzophenones and parabens) were much higher in cities B and E due to a much more substantial industrial presence including food manufacture, toiletry manufacture, paint stripping commercial laundrette, vehicle washing, packaging industry, food warehousing and distribution (Figure S6). Further discussion on using pharmaceuticals as a proxy for public health can be found in section 3.4.1.

3.2. BCIs' intercity daily loads as a function of city's population size

Linear regression was applied to describe statistical relationship between daily BCI loads and population size with R^2 in most cases >0.99 showing very good fit of the model. Pearson's r being on average >0.998, indicated a very strong positive linear correlation between cumulative weekly and daily average (from 7 days) BCI loads and PE. The *p*-value obtained for all but a few BCIs was <0.001 proving further evidence of a significant correlation between BCIs loads and PEs described by the model (Table 2).

The results clearly indicate that there is a strong positive correlation between BCIs and population size with a very few BCIs showing weaker correlations. BCIs were divided in three main groups (Table 2):

Group 1: BCIs with the strongest correlations (R^2>0.998, r>0.999, p<0.001), with usage independent of city functions. These are mostly, as expected, (non-communicable disease) NCD pharmaceuticals with multi-spectrum applications focussed on chronic disease and high prescription patterns: analgesics (e.g. tramadol and its metabolites), antidepressants (e.g. citalopram and its metabolites), antidiabetics (e.g. glicazide), antiepileptics (e.g. carbamazepine and its metabolites), NSAIDs (e.g. naproxen), and most importantly lifestyle chemicals (e.g. nicotine, caffeine and their metabolites) as well as some cardiovascular drugs (e.g. irbesartan or propranolol).

Group 2: BCIs with medium-high correlation (0.990<R^2>0.998 and 0.999 <r>0.990, 0.05>p>0.001) and with usage of seasonal nature. These are mostly cardiovascular pharmaceuticals, antibiotics and WQIs.

Group 3: BCIs with lower correlations (R^2 <0.990, r<0.999 and p>0.05) with usage dependent on city function. These are mostly individual pharmaceuticals with low usage, seasonal/short-term prescription patterns and specific application e.g. anticancer drugs, antihistamines, as well as some individual pesticides, personal care products and ARGs.

Table 2. Biochemical indicator daily loads in wastewater influent vs population size (calculated using PE-WW)

	Group	Compound	R^2	r	p-value
Group I	Analgesics and metabolites	Tramadol	0.9993	0.9997	0.000007
		N-demethyltramadol	0.9961	0.9980	0.000104
		O-demethyltramadol	0.9972	0.9986	0.000064
		Morphine	0.998	0.9990	0.000037
		Dihydromorphine	0.9985	0.9992	0.000025
		Normorphine	0.9986	0.9993	0.000021
		Codeine	0.9987	0.9993	0.000020
		Norcodeine	0.9991	0.9996	0.000011
		Dihydrocodeine	0.9986	0.9993	0.000022
	Antidepressants	Amitriptyline	0.9996	0.9890	0.001376
		Nortriptyline	0.9994	0.9997	0.000007
		Sertraline	0.9994	0.9990	0.000036
		Norsertraline	0.998	0.9990	0.000035
		Fluoxetine	0.995	0.9975	0.000149
		Norfluoxetine	0.9934	0.9967	0.000229
		Citalopram	0.9998	0.9999	0.000001
		Desmethylcitalopram	0.9996	0.9998	0.000004
		Venlafaxine	0.9995	0.9998	0.000004
		Desmethylvenlafaxine	0.9998	0.9999	0.000001
		Mirtazapine	0.9981	0.9991	0.000034
	Antidiabetics	Metformin	0.9973	0.9986	0.000060
		Glicazide	0.9992	0.9996	0.000010
		Sitagliptin	0.9983	0.9991	0.000030
	Anti-epileptic	Carbamazepine	0.9965	0.9982	0.000089
		Carb.-10,11-epoxide	0.992	0.9960	0.000302
		10,11-dihydro-10-hydroxycarb.	0.9988	0.9994	0.000018
	Lifestyle	Caffeine	0.9923	0.9962	0.000285
		1,7-dimethylxantine	0.9979	0.9990	0.000040
		Nicotine	0.999	0.9995	0.000014
		Cotinine	0.9993	0.9996	0.000008
		Cocaine	0.998	0.9990	0.000038
		Benzoylecgonine	0.9976	0.9988	0.000050
		Amphetamine	0.9989	0.9994	0.000016
		Methamphetamine	0.9977	0.9989	0.000046
		MDMA	0.9986	0.9993	0.000022
		MDA	0.9965	0.9983	0.000087
		Methadone	0.9992	0.9996	0.000010
		EDDP	0.9991	0.9996	0.000011
		Mephedrone	0.9917	0.9959	0.000319
	NSAIDs	Ibuprofen	0.9998	0.9999	0.000001
		Naproxen	0.9999	1.0000	0.000000
		Paracetamol	0.9996	0.9998	0.000003
		Diclofenac	0.9996	0.9998	0.000004
Group II	Anaesthetics	Ketamine	0.9934	0.9981	0.000097
		Norketamine	0.9926	0.9963	0.000270
	Antibiotics	Sulfamethoxazole	0.9974	0.9987	0.000056
		Chloramphenicol	0.9909	0.9955	0.000366
		Trimethoprim	0.9951	0.9976	0.000144
		Sulfapyridine	0.999	0.9995	0.000013
		Sulfasalazine	0.9955	0.9978	0.000127
		Clarithromycin	0.9997	0.9998	0.000002
		Azithromycin	0.9874	0.9937	0.000600
		Sulfamethoxazole	0.9974	0.9987	0.000056
		Metronidazole	0.967	0.9834	0.002567
		Cefalexin	0.9275	0.9631	0.008476
		Ciprofloxacin	0.9944	0.9972	0.000177
		Ofloxacin	0.9959	0.9979	0.000113
		Norfloxacin	0.9896	0.9948	0.000452
		Nalidixic acid	0.9936	0.9968	0.000219
	Cardiovascular	Propranolol	0.9985	0.9992	0.000025
		Atenolol	0.9983	0.9991	0.000030
		Metoprolol	0.9322	0.9655	0.007649
		Bisoprolol	0.9905	0.9953	0.000392
		Diltiazem	0.9973	0.9987	0.000059
		Verapamil	0.9582	0.9789	0.003680
		Valsartan	0.9905	0.9952	0.000394
		Irbesartan	0.9993	0.9996	0.000009
		Lisinopril	0.9939	0.9970	0.000201
		Bezafibrate	0.9611	0.9804	0.003291
		Atorvastatin	0.9893	0.9947	0.000468
	Various	Sildenafil	0.9977	0.9988	0.000047
		E1	0.9992	0.9996	0.000009
		Buprenorphine	0.9896	0.9948	0.000454
		(Pseudo)ephedrine	0.9994	0.9997	0.000007
		Quetiapine	0.9968	0.9984	0.000076
	WQI	Ammonia N	0.9942	0.9971	0.000187
		COD	0.9985	0.9993	0.000024
		N total	0.1762	0.4197	0.481737
		Nitrite as N	0.9945	0.9973	0.000172
		Nitrate as N	0.0002	0.0124	0.984196
		Ortophos	0.9898	0.9949	0.000440
		Chloride	0.9953	0.9976	0.000137
Group III	Anti-cancer	Capecitabine	0.9891	0.9945	0.000485
		Imanitib	0.9585	0.9790	0.003630
		Bicalutamide	0.9299	0.9643	0.008043
	Antifungals	Ketoconazole	0.9752	0.9875	0.001675
	Antihistamines	Cetirizine	0.8996	0.9485	0.013939
		Fexofenadine	0.9968	0.9984	0.000077
		Ranitidine	0.9844	0.9922	0.000829
		Cimetidine	0.9783	0.9891	0.001370
	Pesticides	Diazinon	0.997	0.9985	0.000071
		Ceftiofur	0.0545	0.2336	-
		Oxadiazon	0.9936	0.9968	0.000217
		Flufenacet	0.9944	0.9972	0.000176
		Methicarb	0.9466	0.9730	0.005318
		Clothiniadin	0.991	0.9955	0.000362
		Imidacropid	0.986	0.9930	0.000705
		Thiamethoxam	0.0966	0.3108	-
		3PBA	0.9975	0.9988	0.000053
	PCPS	BP-1	0.9708	0.9853	0.002132
		BP-2	0.991	0.1195	0.848225
		BP-3	0.9931	0.9966	0.000242
		BP-4	0.991	0.9769	0.004194
		EP	0.9904	0.9952	0.000398
		MP	0.8133	0.9018	0.036379
		PP	0.9734	0.9866	0.001851
		BPA	0.9915	0.9958	0.000332
		BPA sulphate	0.9908	0.9954	0.000377
		Triclosan	0.9927	0.9963	0.000266
		Triclosan suphate	0.9756	0.9877	0.001627
	Urinary marker	Creatinine	0.9399	0.9695	0.006374
	Gene/ARG	16SRNA	0.8786	0.9373	0.018657
		ermB	0.9939	0.9969	0.000204
		qnrS	0.9613	0.9804	0.003272
		sulf	0.5292	0.7275	0.163623
		catA	0.9701	0.9849	0.002219
	Hypnotic	Temazepam	0.9753	0.9876	0.001656
		Oxazepam	0.9906	0.9953	0.000391
		Diazepam	0.9871	0.9935	0.000627

R^2	r	p-value
1	1	<0.005
0.99	0.99	≥0.005
0.8	0.8	

3.3. Intercity WBE as a support tool in One Heath strategy for Planetary Health

3.3.1. Pharma usage as a proxy for population health

As discussed in section 3.2, PNDLs can provide invaluable information on community-wide pharmaceutical consumption, which can then be used as a proxy of the prevalence of certain diseases. Figure 4 shows cumulative PNDLs calculated using PE-WW (PNDLs calculated using both PE-NHS and PE-WW are shown in Fig S9).

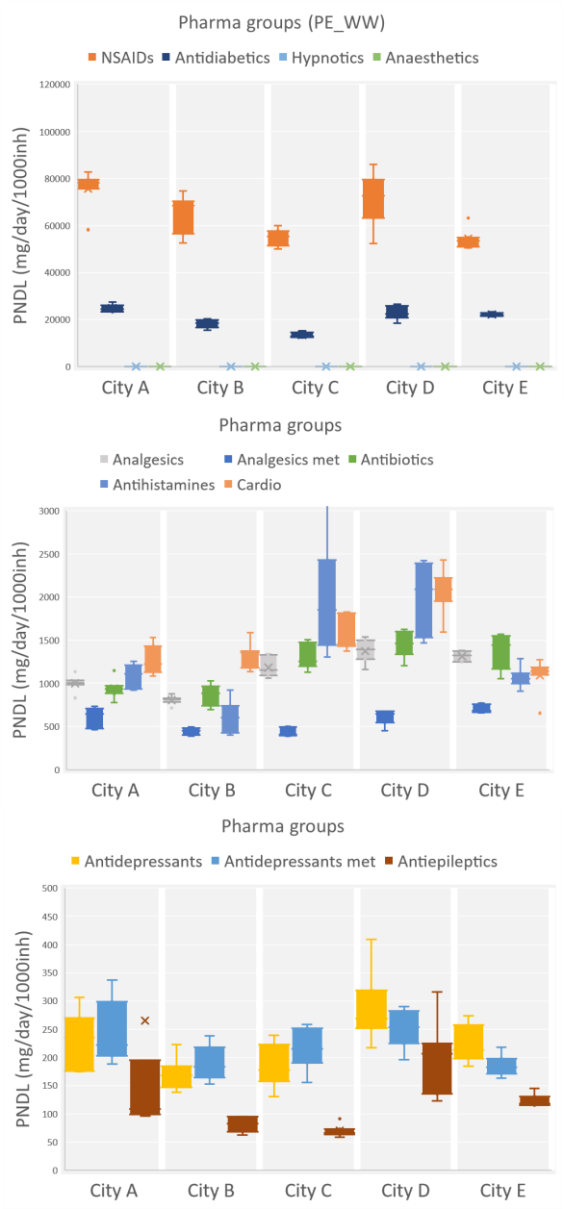


Figure 4. Population normalised daily loads of pharmaceuticals (calculated using PE-WW) in the intercity system

While prescription data can provide information on prescription patterns, only WBE can inform actual use at a community level. This is of particular importance in the case of pharmaceuticals that can be sourced over the counter, such as those used for pain treatment. It is important to note that PNDLs do not allow for a differentiation of pharmaceuticals' consumption vs direct disposal. In order to estimate consumption(intake), human metabolic transformation by-products need to be used as BCIs (see (Kasprzyk-Hordern, Proctor et al. 2021)).

The UK ONS Index of Multiple Deprivation (IMD) 2015 is presented in Table 3 for the two largest cities: C and E. IMD is a measure of multiple deprivation based on combining seven distinct domains of deprivation: Income Deprivation, Employment Deprivation, Education, Skills and Training Deprivation, Health Deprivation and Disability, Crime, Barriers to Housing and Services, and Living Environment Deprivation. It is interesting to note that the comparison of two largest cities: City C and city E with different IMDs, clearly shows that usage of pharmaceuticals increases with higher IMD as well as with population demographics. It is for example notable that antidiabetics usage is lower in city C with lower IMD despite older population. This is clear in the case of both PE-NHS and PE-WW normalised PNDLs. On the other hand, city C's geographical location makes it prone to lower air quality which is manifested in higher antihistamines intake. Interestingly, some high usage pharmaceuticals (e.g. analgesics, NSAIDs) do not show high inter-city variability. These pharmaceuticals could be used as population equivalent indicators as discussed in section 3.4.5.

Table 3. The UK ONS Index of Multiple Deprivation (IMD) 2015 (https://www.ons.gov.uk/peoplepopulationandcommunity/housing/datasets/townsandcitiesanalysis)

City			City C	City E
Census	Age	Population aged 0-15	15.2	18.5
		Population aged 16-64	68.2	67.5
		Population aged 65+	16.6	14.0
		Population aged 85+	2.8	2.2
	% health	Population "limited a lot" by a health problem or disability, aged 16-64	3.9	5.1
		Population "limited a little" by a health problem or disability, aged 16-64	6.2	6.9
		Population "not limited" by a health problem or disability, aged 16-64	90.0	88.0
	Students %	Proportion of Full Time Students, aged 16-74	21.4	12.1
	Qualifications	Proportion of resident population with no qualifications, aged 16+	14.0	20.3
IMD		Number of LSOAs	61.0	333.0
		IMD rank*	88.0	62.0
		IMD: Proportion of LSOAs in most deprived 20%	8.2	23.4
		Income Deprivation Rank	92.0	59.0
		Income Deprivation: Proportion of LSOAs in most deprived 20%	8.2	21.9
		Employment Deprivation Rank	87.0	60.0
		Employment Deprivation: Proportion of LSOAs in most deprived 20%	8.2	23.4
		Health Deprivation and Disability Rank	83.0	62.0
		Health Deprivation and Disability: Proportion of LSOAs in most deprived 20%	11.5	24.0
		Education, Skills and Training Deprivation Rank	94.0	61.0
		Education, Skills and Training: Proportion of LSOAs most deprived	11.5	27.6
		Crime Rank	98.0	39.0
		Crime: Proportion of LSOAs in most deprived 20%	3.3	31.2
		Barriers to Housing and Services Rank	68.0	40.0
		Barriers to Housing and Services: Proportion of LSOAs most deprived	3.3	9.0
		Living Environment Deprivation Rank	68.0	29.0
		Living Environment Deprivation: Proportion of LSOAs most deprived	11.5	29.7
** A rank of one indicates the most deprived town or city and a rank of 109 the least.*				

3.3.2. BCI burden and city function

Multi-biomarker suite analysis describing city metabolism can also provide a holistic understanding to encompass all of the activities of a city in a single model: from lifestyle choices (caffeine intake, nicotine) through to health status (pharmaceuticals) and exposure to harmful chemicals due to environmental and industrial exposures (e.g. pesticide intake and industrial exposure). Figure 5 (Fig S10) shows that city C has the lowest pharmaceutical PNDLs. Higher exposure to industrial chemicals in city B and E indicates industry presence and is linked with occupational exposure, especially pronounced in higher levels of bisphenol A and its metabolites PNDLs during working days vs weekends (see (Kasprzyk-Hordern, Proctor et al. 2021) for further discussion). Higher usage of illicit drugs in city E than city C might be linked with higher IMD as well as it being a larger urban area (González-Mariño, Baz-Lomba et al. 2020). Larger cities (city C and E) have also higher nicotine intake.

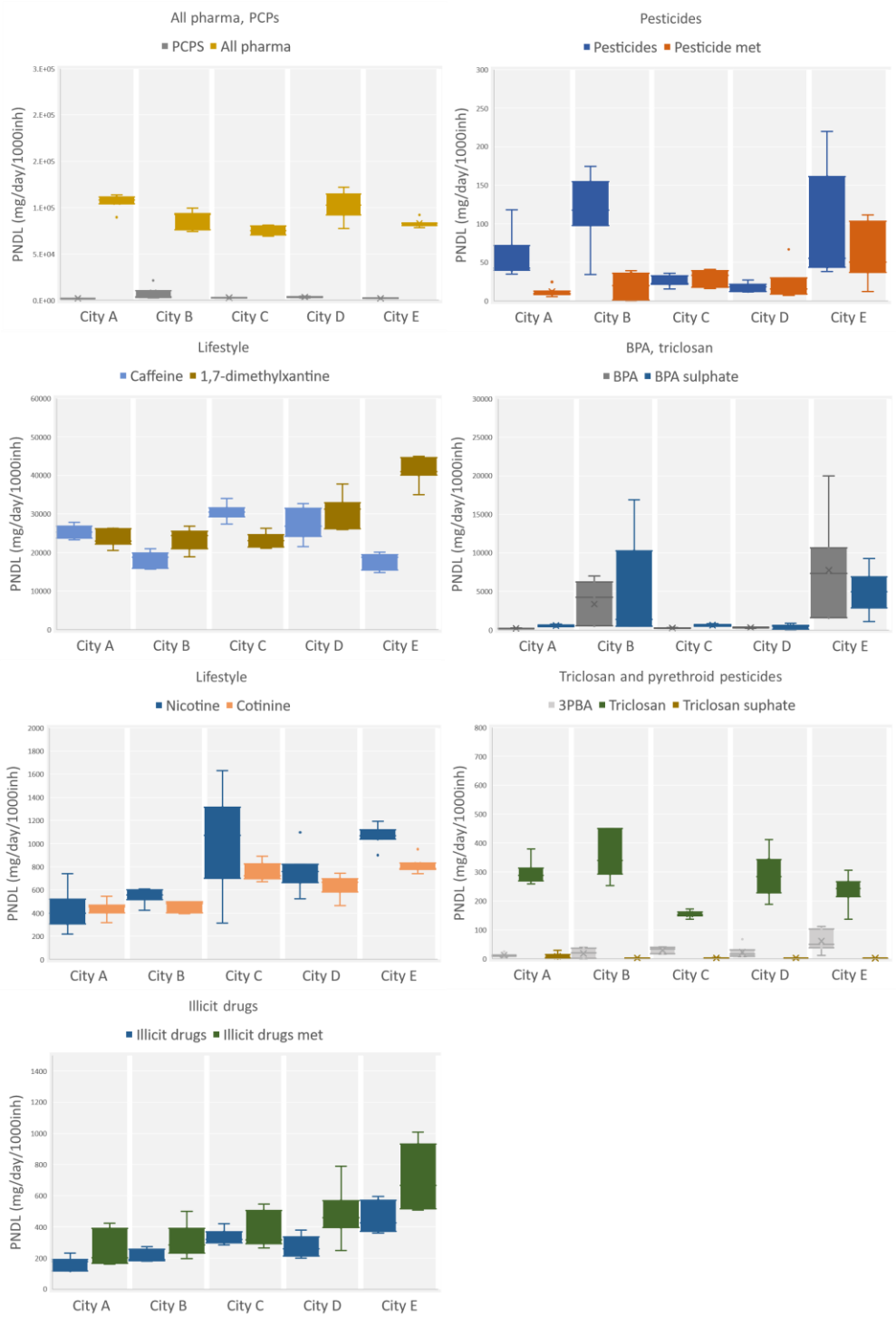
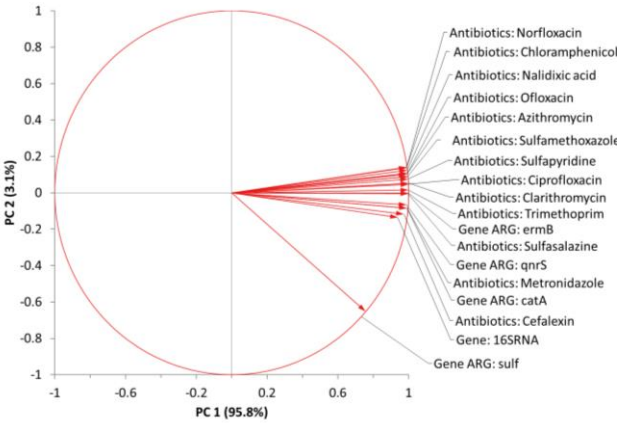


Figure 5: Population normalised daily loads of BCIs driven by city function

3.3.3. Population as a driver of AMR

AMR requires urgent action and due to its multifaceted nature, it is in need of holistic solutions. In this project we have confirmed that there is a strong positive correlation between ABs and PE as well as ARGs and PE with *p* values in most cases <0.001 as well as Pearson coefficient >0.99 (Table 2). There is also a strong positive correlation between ABs and ARGs (Figure 6). The results indicate that WBE can prove very useful in understanding antibiotic and resistance genes' (ARGs) fluxes in a community/at sewerage/river catchment and an intercity level. The strong positive correlation between three variables is also apparent form multivariate regression with *p* value for all AB groups and relevant ARGs denoting <0.005.



Pearson coefficient		
	PE	Sulfamethoxazole
Sulfamethoxazole	0.998704	1
Sul1	0.727473	0.694801
	PE	Chloramphenicol
Chloramphenicol	0.995464	1
catA	0.984915	0.967056
	PE	Macrolides
Macrolides	0.999576	1
ermB	0.996935	0.994663
	PE	Fluoroquinolones
Fluoroquinolones	0.998584	1
qnrS	0.980448	0.976258

Figure 6. Correlation between AB daily loads, ARG daily loads and PE in five cities: (a) PCA correlation monoplot (98.9%) for PEs calculated using ABs and ARGs and (b) Pearson coefficients.

PNDLs of individual ABs and corresponding ARGs can be seen in Fig 7 (Fig S11). It is apparent that the place of residence seems not to matter as PNDLs for individual ABs and ARGs are relatively comparable across all sites; the number of people contributing to the catchment does though.

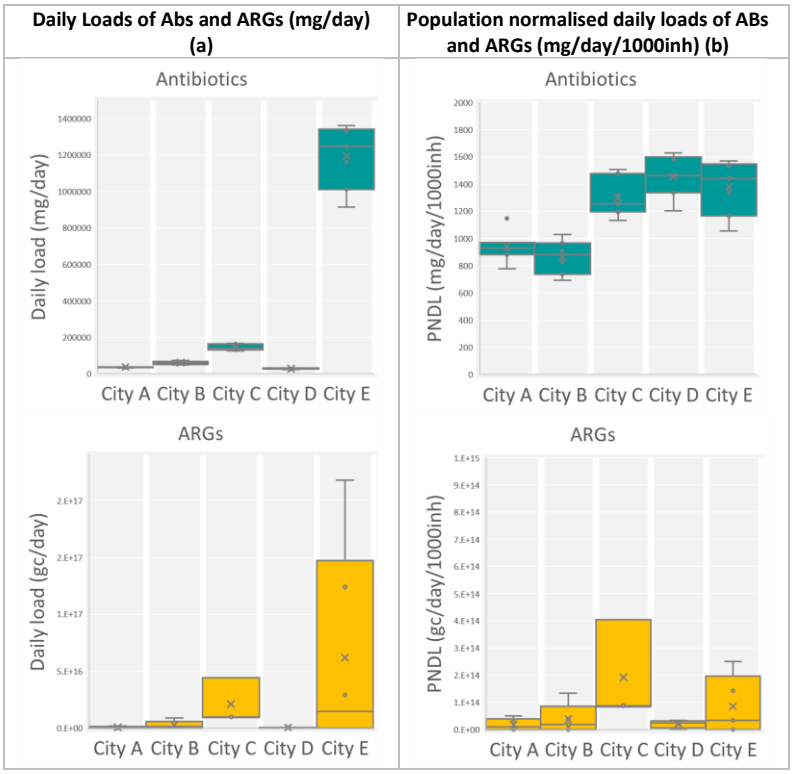


Figure 7. ABs and ARGs: (a) daily loads and (b) population normalised daily loads (chloramphenicol was excluded in total AB calculation).

This is of particular importance in the context of One Health, especially if testing the hypothesis that human population, and its antibiotics' usage, is the key driver of AMR. Human gut is considered as one of environmental reservoirs of AMR (Penders, Stobberingh et al. 2013, Singh, Verma et al. 2019). Human activities including consumption of antibiotics are responsible for the accumulation of AMR genes in the human gut, and there is a strong link between these and environmental AMR gene carriage (Singh, Verma et al. 2019). Excessive antibiotics' use and lack of patient AB prescription compliance (e.g. not finishing prescribed dose or using leftover antibiotics for self-diagnosis and administration) could be curbed with certain simple intervention strategies, not only in hospital settings but also in the wider community. Careful management of AB usage should therefore help with the reduction of AMR prevalence. Possible interventions aimed at management of AB usage might include educational campaigns and reductions in healthcare usage.

WBE's role could be to identify hotspots as well as monitor effectiveness of interventions. WBEs could be for example used to monitor resistance genes within a population (as well as the prevalence of certain microorganisms), and in conjunction with resistance data from national health service, it could be used to inform antibiotic stewardship within a catchment or intercity as well as national levels.

3.3.4. Public health interventions and One Heath

Fluxes of biomarker groups in an inter-city system are critical in understanding a city's function and have strong potential to enable city-system focussed interventions. An understanding of population as a driver of environmental burden of BCIs provides an opportunity to introduce interventions at source. These could include either social, technology or policy focussed interventions aimed at reduction in usage of BCIs or change of practice. Environment/water fingerprinting is best placed to provide a comprehensive evaluation of such interventions, as it is multifaceted, comprehensive and relatively low cost. There are very few examples of WBE applications where quantitative evidence gathering tools have been used. The 2016 policy intervention to limit NPS (new psychoactive substances) usage (UK NPS bill) has been described by Rice et al. (Rice, Kannan et al. 2020). The potential for wide-ranging applications is apparent and results from proof-of-concept studies are very encouraging. Future work should focus on the holistic management of industrial and communal inputs into river catchment systems based on evidence driven WBE to truly embrace the One Heath philosophy.

3.3.5. Population size estimation using BCIs

Lack of robust and dynamic population size estimation tools in WBE is the key obstacle in quantitative measurements of per capita exposure or disease status. Current approaches focus mainly on PE estimates provided by water utilities that, although might be accurate, cannot show inter-day changes in population size resulting from, for example, commuting or tourism. Chemical analysis of certain BCI groups, especially metabolites of high-usage pharmaceuticals (e.g. desmethylvenlafaxine or desmethylcitalopram) with well-defined consumption patterns, can provide important insides into diurnal changes in population size contributing to wastewater. As there is a strong positive correlation between averaged daily loads of NCD pharma (and their metabolites) and PE-WW and PE-NHS we have considered a catchment calibration approach using a linear regression model to calculate PE-REG. Our modelling indicated that city B and E might have population overestimated by <30% due to industry inputs, if using PE-WW, or underestimated by <30% if PE-NHS is used due to not accounting for commuters and visitors. Therefore, the intercity catchment was calibrated using both PE-WW and PE-NHS. Most BCIs show strong positive correlation with PE in the given catchment (Figure 9). However, the choice of best BCIs for PE calculation should account for: inter-day variabilities (weekday vs weekend, which excludes illicit drugs as markers), seasonal variabilities in usage (which excludes e.g. antibiotics) and variable usage dependent on city's socioeconomic status (e.g. antidiabetics pharma). Population equivalents calculated using selected Group 1 BCIs with the strongest positive correlation are shown in Figure 8. As expected, metabolites show the lowest spatiotemporal variability in the studied intercity catchment (e.g. <12% for desmethylcitalopram) than their respective parent pharma (that might be subject to direct disposal), which indicates their best suitability as population markers. Analysis of interday patterns indicates that there is little PE variability between weekday and weekend days, which shows that any population change in this intercity catchment is within method uncertainties (<30%). Indeed, according to ONS (https://www.ons.gov.uk/peoplepopulationandcommunity/housing/datasets/townsandcitiesanalysis) there was estimated 16,602 net in commuting (aged 16-74) in City C and 9092 net in commuting in City E in 2015. Interestingly, there is a slight increase in PE numbers in City C during weekend, which might be linked with influx of day visitors (tourism and shopping) as City C is the largest city with established weekend shopping/leisure destination in the region and a UNESCO site.

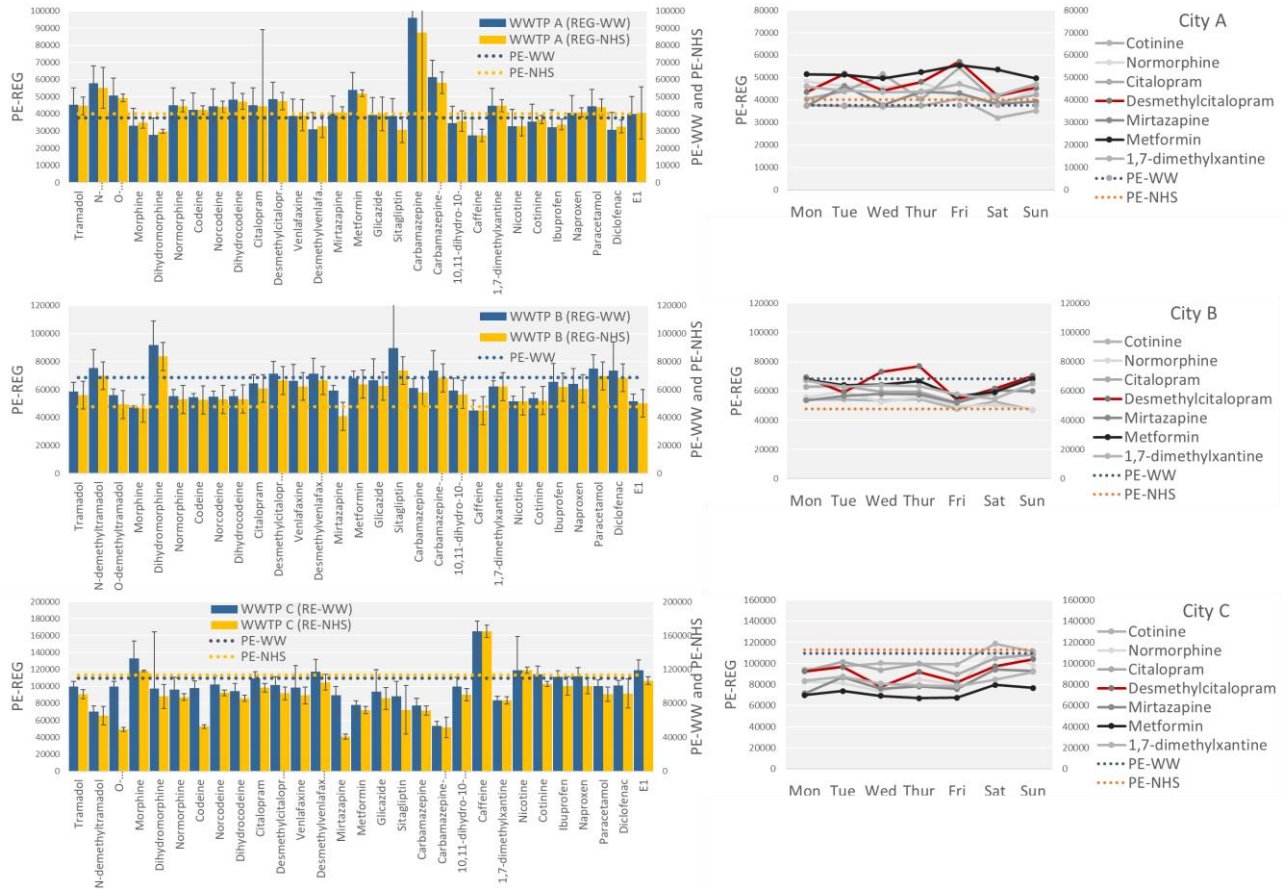


Figure 8. PEs calculated using example Group 1 BCIs.

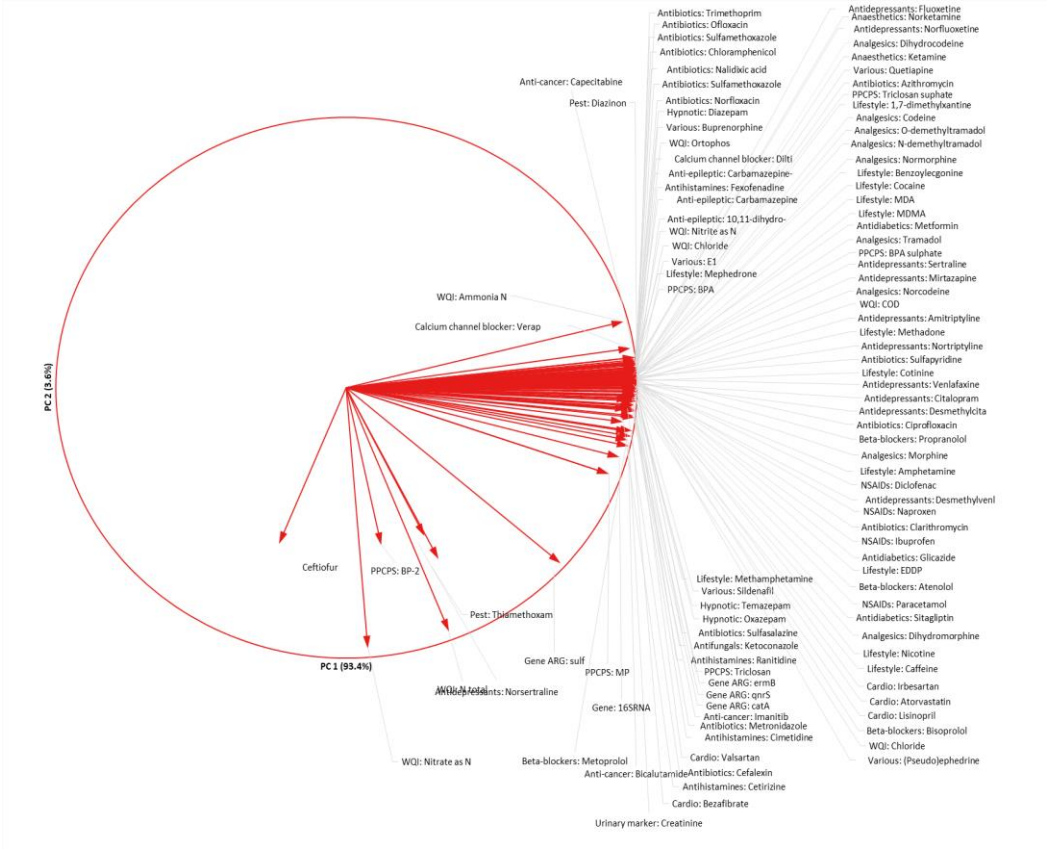


Figure 9. PCA correlation monoplot (97%) for PEs calculated using BCIs.

Desmethylcitalopram is shown as a promising example. There is a significant positive relationship between desmethylcitalopram daily loads and population size served by respective wastewater treatment plants (Pearson coefficient, r= 0.9998, p<0.00001). Population equivalents were calculated using linear regression and inter-city calibration using wastewater measured daily desmethylcitalopram loads and PE-WW (coefficient of determination, R^2 = 0.9998) as seen in Figure 8. Measurements were undertaken over 7 consecutive days in 5 towns/cities. Daily PE-REG variability in the studied catchment was <12%. It is important to mention that there are limitations to this study: 5 cities in one geographic location as well as 1 week not accounting for seasonal changes in chemicals. Hence, only NCD chemicals and lifestyle chemicals are recommended as PEs. Key points in biomarker selection should consider as follows: (1) Appreciation of temporal changes both weekday-weekend and seasonal; (2) Taking advantage of prescription datasets; (3) Using spectrum of biomarkers to get more comprehensive assessment of PE changes in studied communities.

3.3.6. BCIs burden and environmental health – closing the loop in One Health

A holistic understanding of the sources, fate and behaviour of BCIs at a catchment level is also important from environmental health perspective and particularly important in One Health concept. Knowledge of BCI levels in wastewater influent is critical in understanding community stressors as well as resulting public health effects. Daily monitoring of wastewater influent can enable evaluation of public health interventions aimed at increased public health. An extension of WBE into longitudinal spatio-temporal monitoring of BCI levels reaching receiving environment (in this case, the River Avon) can provide invaluable information on the impact of communities on the receiving environment, which in turn can trigger carefully designed, evidence driven interventions aimed at environmental and public health protection. Detailed discussion on chemicals of emerging concern can be found in a paper by Proctor et al. ((Proctor, Petrie et al. 2021)). Fig 9 (Fig S12) shows an increase in BCI daily loads with an increase in population size contributing to the receiving river. Several factors contribute to BCI levels in the receiving environment. These include, efficiency of treatment, rainfall and runoff, climate and weather (e.g. sunlight and temperature), BCI load resulting from other geographic areas arriving with river tributaries to the catchment. It is however apparent that daily loads of those BCIs that are strongly positively correlated with PE (see Tab 3) are directly linked with population contributing to environmental burden (e.g. lifestyle chemicals, NCD pharmaceuticals). BCIs that are city function driven, will be manifested with more variable daily loads linked with their usage which is independent of PE and rather linked with e.g. industrial activities or agriculture (e.g. BPA or pesticides).

BCI presence, which is directly proportional to the size of the population producing these BCIs, is directly linked with environmental risks. Indeed, in the studied catchment, several antibiotics (ciprofloxacin, clarithromycin, azithromycin, and erythromycin) were regularly found exceeding $PNEC_{enviro}$ and $PNEC_{MIC}$ in wastewater influent and effluent and at very few occasions in receiving waters (see (Elder, Proctor et al. 2021)) for further discussion. In another study in the same catchment, pharmaceuticals such as the painkillers ibuprofen and acetaminophen, have been shown to pose low chronic risk throughout the catchment (concentrations 1-10% of the PNEC). Other pharmaceuticals, such as carbamazepine and diazepam, show sporadic increases in concentration up to and over 50% of the PNEC (carbamazepine up to 3.2x PNEC, however the sampling location (R2) at this point was not well mixed). The concentrations of the lifestyle chemicals caffeine and nicotine also indicated they might pose a risk to the environment with concentration of caffeine reaching >13% of its PNEC, and nicotine exceeding the PNEC at several instances across the catchment (Proctor and Kasprzyk-Hordern 2021). Overall, individual pharmaceuticals have been shown to be low risk to this catchment, however their combined risk, especially for BCIs, may lead to a combined risk greater than the individual compounds, as shown in the paper by Fraker and Smith which showed increased behavioural effects in tadpoles when exposed to caffeine and acetaminophen than when exposed to the compounds individually (Fraker and Smith 2004) .

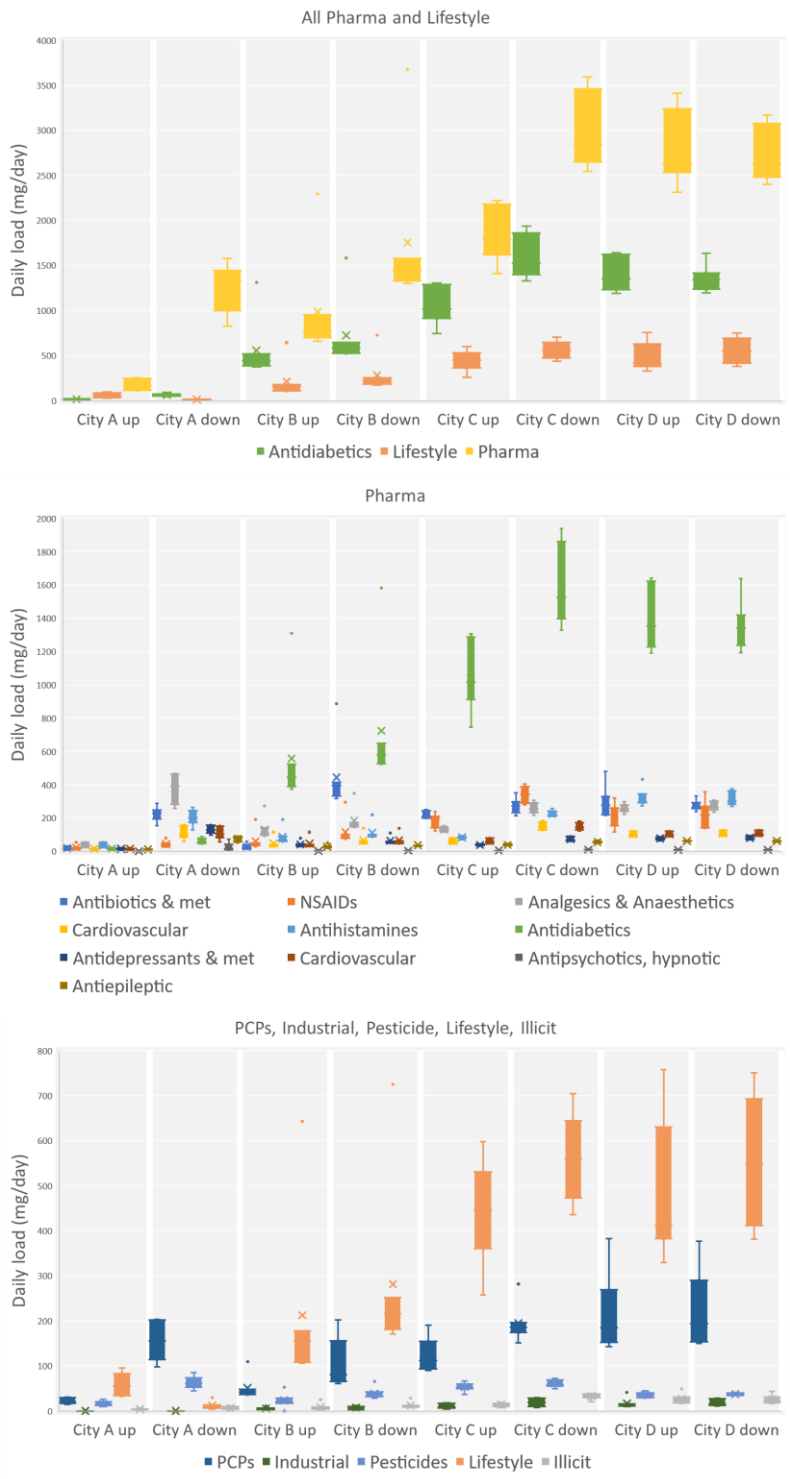


Figure 10. Daily loads of BCIs in receiving waters.

Conclusions

This paper tested the hypothesis that a biochemical burden in a given catchment (derived from wastewater from this catchment and measured with WBE tools) is driven by population contributing to this catchment and city's function, which could enable management strategies aimed at increased environmental and public health in this catchment. Such an approach is particularly promising in the context of One Health as it enables a holistic understanding of city's metabolism encompassing all of the activities of a city in a single model: from lifestyle choices, through to health status and exposure to harmful chemicals as well as effectiveness of implemented management strategies.

Several groups of BCIs were the subject of investigation (water quality indicators, industrial chemicals, personal care products, pesticides, illicit drugs, lifestyle chemicals, prescription pharmaceuticals, as well as genetic targets, such as antibiotic resistance genes) in an intercity system including five cities/towns located in

one river catchment. Chemical mining of wastewater for BCIs was undertaken to understand spatiotemporal speciation of BCIs in the context of geographical as well as community-wide socioeconomic factors.

The main conclusions enabling One Heath are as follows:

1. There are spatiotemporal variabilities in chemical and biological target groups in the studied inter-city system. There is a linear relationship (R^2 >0.99) and a strong positive correlation between most BCIs and population size (r >0.998, p <0.001) which provides a strong evidence for the population size as a driver of BCI burden. BCI groups that are strongly correlated with population size and are intrinsic to humans' function include mostly high usage pharmaceuticals that are linked with long term non-communicable conditions (NSAIDs, analgesics, cardiovascular, mental health and antiepileptics) and lifestyle chemicals. These BCIs can be used as population size markers.
2. BCIs groups that are produced as a result of a specific city's function (e.g. industry presence and occupational exposure or agriculture) and as such are not correlated with population size include: pesticides, PCPs and industrial chemicals, as well as pharmaceuticals that are used to treat less common disease over shorter periods of time. These BCIs can be used to assess city's function, such as occupational exposure, environmental or food exposure. Measurement of pharma daily loads in wastewater can be also used as a proxy of community-wide health.
3. There is a strong positive correlation between ABs and PE as well as ARGs and PE with *p* values in most cases <0.001 as well as Pearson coefficient >0.99. There is also a strong positive correlation between ABs and ARGs. This confirms the population size and AB usage as the main driver of AB and ARG levels and provides an opportunity for interventions aimed at the reduction of AB usage.
4. Holistic evaluation of biophysicochemical fingerprints (BCI burden) of the environment and data triangulation with socioeconomic fingerprints (indices) of tested communities are required to fully embrace One Health concept.

Acknowledgments

The support of Wessex Water Services Ltd and EPSRC Impact Acceleration Account (Project number: EP/K503897/1 and EP/R51164X/1, ENTRUST IAA) is greatly appreciated. The support of the Leverhulme Trust (Project No RPG-2013-297) and NERC NWESP project (Project No NE/V010441/1) is also greatly appreciated. All data supporting this study are provided as supporting information accompanying this paper, as well as in Proctor et al. SI (Proctor, Petrie et al. 2021) and Elder et al. SI (Elder, Proctor et al. 2021).

CRediT authorship contribution statement

Barbara Kasprzyk-Hordern: Conceptualization, Methodology, Formal analysis, Writing-original draft, Writing – review-editing, Data curation, Visualization, Project administration, Funding acquisition, Resources. Kathryn Proctor: Writing - review & editing, Data curation, Methodology. Kishore Jagadeesan: Writing - review & editing, Data curation, Methodology. Felicity Edler: Writing - review & editing, Methodology. Richard Standerwick: Writing - review & editing, Project administration, Resources, Methodology. Ruth Barden: Funding acquisition, resources.

Supplementary Information

Table S1 SPE/MAE-UHPLC-QqQ - method performance.

Table S2 SPE-UHPLC-QTOF – method performance.

Figure S1. SPE/MAE-UHPLC-QqQ – schematic overview.

Figure S2 SPE-UHPLC-QTOF – schematic overview.

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Supplementary Material

Human population as a key driver of biochemical burden in an inter-city system: implications for One Health concept

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Table of contents:

S2.3.4. Water Quality Indicators analysis

Ammonia N: utilises ammonia reaction with sodium salicylate and hypochlorite ions, which are generated in situ by the alkaline hydrolysis of sodium dichloroisocyanurate. The absorbance of a blue product formed at pH 12.6 in the presence of sodium nitroprusside is measured spectrophotometrically at 660 nm and related to the ammonia concentration in the sample by means of a calibration curve (LOQ, 0.02mg/l, range: 0.02-100mg/l)

N total (TON): Nitrate is reduced to nitrite by hydrazine under alkaline conditions, using copper (II) ions as a catalyst. The total nitrite is then treated with sulphanilamide and N-1-naphthylethylenediamine dihydrochloride under acidic conditions (in the presence of orthophosphoric acid). The absorbance of a characteristic pink azo – dye is measured spectrophotometrically at 540 nm and related to the total oxidised nitrogen concentration in the sample by means of a calibration curve. (LOQ, 0.3 mg/l, range: 0.3-50mg/l)

Nitrite: The diazotisation of sulphanilamide by nitrite in the presence of orthophosphoric acid, at pH 1.9, leads to the formation of an azo-dye with N-1-napthylethylenediamine. Its absorbance is then measured at 540 nm and is related to the nitrite concentration by means of a calibration curve. (LOQ, 0.03mg/L, range: 0.03-10mg/l)

Nitrate: Nitrate is calculated using TON minus Nitrite. The calculation takes place after the samples have been analysed for both chemistries.

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Ortophosphate: Orthophosphate ions react with a solution containing molybdic acid, ascorbic acid and antimony (II) ions in the presence of acid, to form a 12-molybdophosphoric acid. This is reduced in situ to a blue heteropoly compound (phosphomolybdenum blue) in which antimony is incorporated. The absorbance of the compound is measured spectrophotometrically at 880 nm and related to the orthophosphate concentration in the sample by means of a calibration curve. Soluble reactive phosphorus uses the same method as above, but the sample is filtered through a 0.45µm filter prior to analysing (LOQ, 0.06 mg/l, range 0.6-20 mg/l).

Chloride: Chloride ions were mixed with acid chloride colour reagent containing mercury (II) thiocyanate. The released thiocyanate ions then react in acid solution with iron (III) nitrate to give a reddish-brown coloured iron (III) thiocyanate complex. The resulting intensity of the stable colour produced is measured at a wavelength of 480 nm and is related to the chloride concentration by means of a calibration curve. (LOQ 1mg/l, range 1-1000 mg/l)

COD: COD was analysed spectrophotometrically. Briefly, samples, either shaken or settled, were oxidised in tubes with chromic acid, a mixture of potassium dichromate, sulphuric acid in the presence of silver sulphate as a catalyst and mercuric ions to counteract interference from chloride. The sealed tubes are heated to 150 °C for three hours, cooled and the degree of oxidation determined by spectrophotometry. The procedure is calibrated by processing a solution of potassium hydrogen phthalate as a standard material.

Table S1a: Instrumental performance data for ECs of interest in mobile phase (ordered by class) (Proctor et al. 2019)

Class of Analyte	**Analyte**	RT	RRT	Linearity		Intra-day instrumental performance		Inter-day instrumental performance		$IDL_{S/N}$ (ug L^{-1})	$IQL_{S/N}$ (ug L^{-1})
				Range (ug L^{-1})	r^2	Precision (Deviation) (%)	Accuracy (%)	Precision (%)	Accuracy (%)		
UV Filter	Benzophenone-1	9.6	0.9	0.06 – 684.0	0.996	2.3	106.8	3.3	106.7	0.01	0.06
	Benzophenone-2	7.7	1.0	0.05 – 583.8	0.997	1.1	99.6	4.2	97.6	0.01	0.05
	Benzophenone-3	21.2	1.2	0.05 – 404.0	0.995	3.2	84.9	4.5	86.8	0.01	0.05
	Benzophenone-4	6.9	0.9	1.01 – 502.5	0.997	2.3	103.0	3.8	105.1	0.31	1.01
Parabens	Methylparaben	7.5	1.0	0.06 – 1122	0.998	1.1	93.3	6.0	97.4	0.01	0.06
	Ethylparaben	8.3	1.0	0.11 – 663.6	0.997	2.6	112.3	2.1	113.1	0.03	0.11
	Propylparaben	9.2	1.0	0.12 – 462.0	0.997	5.7	96.4	4.3	98.4	0.04	0.12
	Butylparaben	10.1	1.0	0.06 – 696.6	0.997	5.0	97.1	3.6	100.3	0.01	0.06
Plasticizer	Bisphenol A	9.0	1.1	0.10 – 626.4	0.997	2.4	103.6	1.3	104.6	0.03	0.10
Steroid estrogens	E1	9.8	1.0	0.49 – 989.0	0.998	1.8	96.9	2.1	98.6	0.10	0.49
	E2	9.8	1.0	0.47 – 949.0	0.997	3.1	96.6	2.6	96.3	0.09	0.47
	EE2	9.7	1.0	0.48 – 950.0	0.997	2.6	94.6	3.3	93.2	0.10	0.48
Antibiotics and Antibacterial	Sulfasalazine	7.1	0.8	0.90 – 904.0	0.999	3.9	105.2	2.4	104.7	0.27	0.90
	Clarithromycin	18.9	1.1	0.06 – 561.0	0.999	2.6	99.8	2.4	101.8	0.01	0.06
	Azithromycin	14.0	0.9	0.001 – 1000	0.998	4.5	108.9	1.5	102.0	0.01	0.05
	Trimethoprim	8.4	1.0	0.10 – 500.0	0.998	3.0	96.9	2.2	99.5	0.03	0.10
	Sulfamethoxazole	9.6	1.0	0.10 – 1000	0.999	3.5	95.1	2.4	96.0	0.03	0.10
	Triclosan[a]	12.3	1.2	1.13 – 225.6 112.8 – 1128	0.997 / 0.998	9.4	69.1	6.5	71.4	0.34	1.13
	Amoxicillin	3.1	0.2	0.06 – 439.5	0.995	5.3	105.7	6.7	94.4	0.02	0.06
	Metronidazole	5.3	1.0	1.00 – 1000	0.999	2.5	105.0	1.2	102.9	0.06	0.21
	Sulfadiazine	4.8	0.9	0.05 – 795.2	0.999	2.8	105.3	1.5	104.4	0.01	0.03
	Cefalexin[b]	9.2	0.3	15.9 – 200	0.995	9.5	111.3	12.3	102.9	4.78	15.94
	Ofloxacin	9.6	1.0	0.23 – 986.0	0.998	4.2	97.4	2.8	95.9	0.07	0.23
	Ciprofloxacin	9.9	1.0	1.18 – 902	0.999	8.7	89.0	5.5	90.2	0.35	1.18
	Tetracycline	10.0	1.0	0.06 – 864.0	0.999	6.8	115.1	8.5	113.1	0.02	0.06
	Danofloxacin	10.2	1.0	1.05 – 1000	0.998	7.3	106.0	6.0	99.2	0.32	1.05
	Oxytetracycline	10.4	1.1	2.36 – 800.8	0.997	4.6	93.5	3.0	88.9	0.71	2.36
	Chloramphenicol	12.6	0.6	1.74 – 400	0.999	3.8	103.5	3.0	100.8	0.52	1.74
	Penicillin G	13.1	0.5	4.68 – 93.6	0.994	10.3	115.5	4.4	111.7	0.02	0.07
	Penicillin V	14.5	0.8	5.00 – 200	0.993	4.4	88.5	15.0	96.8	0.15	0.49
	Erythromycin	17.2	1.0	204.4 – 1022	0.999	2.3	94.4	2.9	95.2	0.20	0.65
	Prulifloxacin	18.0	1.9	100 – 1000	0.997	4.4	98.7	8.9	86.4	2.44	8.13
	Norfloxacin	9.7	1.0	0.01 – 1000	0.996	4.1	85.5	4.4	85.1	0.002	0.01

Class of Analyte	**Analyte**	RT	RRT	Linearity		Intra-day instrumental performance		Inter-day instrumental performance		$IDL_{S/N}$ (ug L^{-1})	$IQL_{S/N}$ (ug L^{-1})
				Range (ug L^{-1})	r^2	Precision (Deviation) (%)	Accuracy (%)	Precision (%)	Accuracy (%)		
Antifungal	Griseofluvin	17.2	0.9	0.26 – 205.2	0.999	1.6	89.2	3.0	91.6	0.08	0.26
	Ketoconazole	21.7	1.2	0.02 – 800.0	0.999	3.8	94.8	2.5	91.7	0.01	0.02
Hypertension	Valsartan	7.6	0.9	1.12 – 1122	0.998	1.9	115.8	3.5	118.6	0.34	1.12
	Irbesartan	8.6	1.0	0.50 – 603.6	0.998	2.6	96.9	4.1	98.3	0.10	0.50
	Lisinopril	7.1	0.9	0.93 – 372.5	0.995	2.2	97.2	7.2	95.2	0.09	0.93
NSAIDs	Ketoprofen[b]	7.9	0.9	0.54 – 1085	0.998	2.2	99.9	2.6	99.4	0.11	0.54
	Ibuprofen[b]	9.8	1.0	0.05 – 1071	0.998	2.4	93.7	2.3	94.2	0.01	0.05
	Naproxen	8.1	1.0	0.49 – 989.0	0.998	1.5	97.7	2.5	98.3	0.10	0.49
	Diclofenac[b]	9.0	1.0	0.10 – 619.2	0.997	7.9	89.6	4.5	91.8	0.03	0.10
	Acetaminophen	5.1	1.0	0.54 – 1070	0.998	1.6	97.4	2.6	99.0	0.11	0.54
Lipid regulator	Bezafibrate	7.9	1.0	0.10 – 976.0	0.998	2.3	97.8	2.8	97.9	0.03	0.10
	Atorvastatin	9.3	1.1	0.05 – 500.0	0.997	2.6	98.0	3.5	100.9	0.01	0.05
Anti-hyperlipidemic	Gemfibrozil	23.3	1.2	1.01 – 100.5	0.994	7.8	118.5	6.9	121.1	0.11	0.35
Anti-hyperintensive	Candesartan Cilexetil	23.0	0.9	226.8 – 680.4	0.995	5.2	100.5	0.9	106.9	1.58	5.28
Antihistamine	Fexofenadine	8.4	1.0	0.09 – 937.5	0.998	2.1	106.3	6.5	104.6	0.03	0.09
	Cetirizine	18.7	1.0	0.08 – 417.7	0.999	1.3	100.5	1.3	100.8	0.02	0.08
GUD/ED	Sildenafil	18.3	1.0	0.01 – 1000	1.000	3.5	99.5	3.0	99.1	0.002	0.01
Diabetes	Metformin	2.8	1.0	0.43 – 862.5	0.998	1.5	96.3	1.3	97.0	0.09	0.43
	Gliclazide	17.8	1.0	0.05 – 508.0	0.997	2.1	93.2	2.8	95.3	0.01	0.05
	Sitagliptin	11.8	0.7	0.08 – 646.4	0.998	3.2	111.7	3.0	110.3	0.01	0.02
Cough suppressant	Pholcodine	3.7	0.9	1.14 – 570.0	0.999	4.7	99.5	3.3	99.2	0.35	1.14
Beta-blocker	Atenolol	4.3	1.0	0.10 – 502.5	0.999	2.1	95.3	2.3	96.8	0.03	0.10
	Metoprolol	11.2	1.0	0.05 – 507.5	0.999	1.3	96.8	2.0	96.1	0.01	0.05
	Propranolol	15.1	1.0	0.09 – 434.9	0.999	2.0	105.4	1.0	106.2	0.03	0.09
	Bisoprolol	13.7	0.8	0.10 – 1004	0.999	4.8	100.4	2.0	96.0	0.0004	0.0012
H2 receptor agonist	Ranitidine	4.6	1.1	5.17 – 517.0	0.998	2.5	100.1	9.7	97.4	1.03	5.17
	Cimetidine	5.3	1.0	0.52 – 1043	0.999	4.2	104.1	9.0	99.3	0.10	0.52
X-ray contrast media	Iopromide	4.9	0.9	5.79 – 1158	0.997	5.0	101.2	12.0	105.4	1.16	5.79
Various	Buprenorphine	21.8	1.2	0.08 – 100	0.996	8.9	94.5	11.5	88.2	0.02	0.08
Drug precursor	Ephedrine/pseudoephedrine	7.2	1.0	0.10 – 500.0	0.997	4.1	94.0	3.4	97.3	0.03	0.10
	Norephedrine	6.3	0.9	0.50 – 1000	0.999	4.3	96.3	5.1	95.2	0.01	0.50
Anti-cancer	Azathioprine	7.8	0.9	0.10 – 490.0	0.999	7.6	97.5	13.9	97.4	0.03	0.10
	Methotrexate	7.9	1.0	0.92 – 458.0	0.997	8.7	108.0	4.1	112.2	0.28	0.92
	Ifosfamide	12.7	1.1	0.05 – 509.0	0.999	2.4	93.6	2.7	95.3	0.01	0.05
	Tamoxifen	22.4	1.0	0.03 – 668.4	0.998	4.0	96.0	2.4	96.8	0.01	0.03
	Imatinib	15.4	0.8	0.88 – 88.4	0.994	2.5	103.8	1.5	101.3	0.08	0.28
	Capecitabine	16.1	0.9	0.01 – 594.6	0.999	2.3	89.2	2.8	89.7	0.001	0.004
	Bicalutamide	18.2	0.9	0.10 – 784.0	0.995	2.7	90.1	2.9	92.0	0.03	0.10

Class of Analyte	**Analyte**	RT	RRT	Linearity		Intra-day instrumental performance		Inter-day instrumental performance		$IDL_{S/N}$ (ug L^{-1})	$IQL_{S/N}$ (ug L^{-1})
				Range (ug L^{-1})	r^2	Precision (Deviation) (%)	Accuracy (%)	Precision (%)	Accuracy (%)		
Anaesthetic and metabolite	Ketamine	10.6	1.0	0.05 – 500.0	0.998	1.8	92.5	1.3	93.6	0.01	0.05
	Norketamine	11.1	1.0	0.10 – 500.0	0.999	1.8	94.1	3.2	94.0	0.03	0.10
	Venlafaxine	14.1	1.3	0.04 – 434.8	0.998	2.5	91.2	1.7	90.5	0.01	0.04
	Desmethylvenlafaxine	10.8	1.0	0.10 – 500.0	0.998	2.8	101.3	2.1	102.3	0.03	0.10
	Fluoxetine	18.4	1.0	0.05 – 1000	0.999	1.7	96.8	1.8	98.3	0.01	0.05
	Norfluoxetine[b]	18.4	1.0	0.05 – 500.0	0.998	1.5	102.7	3.1	103.1	0.01	0.05
	Sertraline	19.2	1.0	0.05 – 500.0	1.000	1.6	95.3	1.7	95.7	0.01	0.05
	Mirtazapine[a]	13.5	1.0	0.05 – 100.0, 50.0 – 500.0	0.999/ 0.997	3.4	94.8	2.7	97.6	0.01	0.05
	Citalopram	15.1	1.0	0.50 – 1000	0.999	0.7	101.2	2.6	101.8	0.05	0.50
	Desmethylcitalopram	15.2	1.0	0.05 – 500.0	0.998	1.8	103.0	3.0	103.4	0.01	0.05
	Paroxetine	17.3	0.9	5.00 – 600	0.998	3.2	103.4	1.3	102.1	0.01	0.03
	Duloxetine	17.8	1.0	1.00 – 1000	0.997	3.0	91.2	13.6	78.3	0.003	0.01
	Amitriptyline	18.2	1.0	0.11 – 885.0	1.000	4.5	99.6	2.4	96.8	0.03	0.11
	Nortriptyline	18.4	1.0	0.22 – 800	0.999	4.0	95.5	3.1	92.9	0.07	0.22
	Norsertraline	19.8	1.0	0.23 – 100	0.999	8.7	99.0	11.0	91.8	0.07	0.23
Anti-epileptic	Carbamazepine	16.2	1.0	0.05 – 514.0	1.000	2.0	91.7	1.6	92.7	0.01	0.05
	Carbamazepine 10,11-epoxide	13.5	0.8	0.10 – 1000	0.997	1.6	88.9	2.1	89.9	0.03	0.10
	10,11-Dihydro -10-hydroxycarbamazepine	13.5	0.8	0.50 – 100.0	0.997	2.8	92.2	5.6	93.8	0.05	0.50
Calcium-channel blocker	Diltiazem	16.7	1.0	0.10 – 486.2	0.996	2.3	92.7	2.3	93.6	0.01	0.10
	Verapamil	16.2	1.0	0.01 – 600	0.998	2.9	103.1	2.4	101.9	0.001	0.004
Hypnotic	Temazepam	18.2	1.0	0.05 – 500.0	0.998	1.0	97.0	1.6	97.9	0.01	0.05
	Oxazepam	17.8	1.0	0.10 – 800	0.999	3.3	94.8	3.4	94.3	0.02	0.08
	Diazepam	19.5	1.0	0.01 – 1000	1.000	1.6	100.7	4.5	99.6	0.003	0.01
Anti-psychotic	Quetiapine	17.9	1.0	0.05 – 1000	0.997	1.4	95.3	1.2	96.4	0.01	0.05
	Risperidone	13.7	0.8	0.01 – 200	0.997	3.2	101.6	1.2	96.8	0.002	0.01
Dementia	Donepezil	13.9	0.9	0.01 – 1000	0.998	2.6	110.8	1.3	107.7	0.17	0.58
	Memantine	15.7	1.0	0.05 – 506.4	0.998	3.5	106.3	0.9	104.3	0.02	0.05
Human Indicators	Creatinine	2.7	1.0	1.00 – 1000	0.999	1.4	100.5	2.8	100.1	0.30	1.00
	Nicotine	3.3	0.8	1.00 – 500.0	0.998	1.2	98.3	2.4	98.4	0.30	1.00
	Caffeine	8.3	1.2	0.50 – 500.0	0.999	1.7	99.6	2.8	100.4	0.10	0.50
	Cotinine	7.2	1.0	0.05 – 1000	0.999	1.5	98.4	1.5	98.8	0.01	0.05
	1,7-dimethylxanthine[b]	6.8	0.9	1.00 – 500.0	0.999	6.0	94.3	9.9	94.9	0.30	1.00
Analgaesics and Metabolites	Morphine	3.5	1.0	1.00 – 500.0	0.998	2.9	99.1	2.5	97.5	0.30	1.00
	Dihydromorphine	3.3	1.0	0.05 – 500.0	0.997	4.4	106.0	2.7	108.5	0.01	0.05
	Normorphine	3.4	1.0	1.00 – 500.0	0.999	1.5	100.9	2.2	99.8	0.30	1.00
	Methadone	17.6	1.0	0.05 – 400.0	0.998	1.5	98.7	1.4	100.2	0.01	0.05

Class of Analyte	**Analyte**	RT	RRT	Linearity		Intra-day instrumental performance		Inter-day instrumental performance		$IDL_{S/N}$ (ug L^{-1})	$IQL_{S/N}$ (ug L^{-1})
				Range (ug L^{-1})	r^2	Precision (Deviation) (%)	Accuracy (%)	Precision (%)	Accuracy (%)		
	EDDP	14.8	1.0	0.05 – 500.0	0.999	1.2	96.5	1.1	96.4	0.01	0.05
	Codeine	6.1	1.0	0.50 – 500.0	0.997	2.0	93.5	4.0	95.1	0.10	0.50
	Norcodeine	6.5	1.1	1.00 – 500.0	0.998	2.8	98.5	4.8	98.6	0.30	1.00
	Dihydrocodeine	5.5	0.9	0.10 – 500.0	0.999	1.6	94.2	2.1	94.6	0.03	0.10
	Tramadol	11.0	1.0	1.00 – 500.0	0.999	1.6	100.1	1.9	98.4	0.01	1.00
	N-desmethyltramadol	11.9	1.1	0.50 – 500.0	0.998	2.5	92.5	2.2	94.4	0.01	0.50
	O-desmethyltramadol	8.3	1.2	1.00 – 400.0	0.997	3.3	95.3	4.9	98.5	0.01	1.00
Stimulants and metabolites	Amphetamine	8.4	1.0	0.10 – 500.0	0.999	4.4	100.8	1.6	100.7	0.03	0.10
	Methamphetamine	8.5	1.0	0.10 – 500.0	0.999	2.2	101.0	1.3	101.1	0.03	0.10
	MDMA	8.6	1.0	0.05 – 1000	0.999	1.3	99.2	1.7	99.8	0.01	0.05
	MDA	8.6	1.0	0.10 – 1000	0.998	1.1	98.4	0.7	100.0	0.03	0.10
	Cocaine	11.3	1.0	0.05 – 500.0	0.999	2.2	97.2	1.5	99.0	0.01	0.05
	Benzoylecgonine[a]	9.7	1.0	0.05 – 100.0 , 50.0 – 500.0	0.998/ 0.999	2.4	103.4	0.9	103.2	0.01	0.05
	Anhydroecgoninemethylester	3.5	1.3	0.50 – 500.0	0.999	2.3	101.1	2.4	98.7	0.10	0.50
	Cocaethylene	12.9	1.0	0.05 – 500.0	0.999	2.8	95.1	1.7	94.7	0.01	0.05
	Mephedrone	9.8	1.0	0.05 – 500.0	0.998	1.8	87.1	2.9	85.7	0.01	0.05
	MDPV	12.1	0.9	0.05 – 500.0	0.999	2.2	99.6	0.7	101.4	0.01	0.05
Opiod and metabolite	Heroin	10.9	1.0	0.50 – 500.0	0.999	1.9	98.2	1.8	99.3	0.10	0.50
	6-acetylmorphine	7.7	1.1	0.10 – 500.0	0.997	6.1	95.3	5.1	100.1	0.03	0.10
Pesticides, fungicides and herbicides	Thiamethoxam	8.3	0.4	1.00 – 100	0.994	4.7	93.8	5.4	96.9	0.02	0.06
	Imidacloprid	10.1	0.6	0.10 – 595.2	0.996	2.8	100.5	5.5	103.5	0.01	0.04
	Clothiniadin	10.4	0.5	1.00 – 800	0.999	3.2	97.9	3.3	98.6	0.01	0.04
	Metazachlor	17.1	1.0	0.05 – 1011	0.999	2.5	106.0	2.6	104.7	0.004	0.01
	Terbuthylazine	19.3	1.0	0.05 – 519	1.000	2.4	99.8	3.3	97.5	0.01	0.02
	Methiocarb	19.4	1.0	0.08 – 1007	0.999	1.9	101.8	1.8	100.6	0.02	0.08
	Dichlofluanid	20.4	1.1	6.83 – 1092	0.994	3.8	94.9	4.4	90.9	1.29	4.30
	Flufenacet	20.5	1.2	0.01 – 986.0	0.997	2.0	104.2	2.9	106.2	0.002	0.01
	Oxadiazon	24.2	1.2	1.00 – 99.6	0.996	4.0	95.5	2.8	97.1	0.02	0.08
	Chlorpyrifos[c]	24.8	1.5	1.87 – 98.5	0.985	11.8	80.7	7.8	83.3	0.56	1.87
	Triallate	24.9	1.3	0.03 – 79.0	0.992	7.6	81.3	13.2	70.6	0.01	0.03
Veterinary Pharma	Tylosin	17.3	1.0	0.56 – 560.0	0.999	2.2	99.5	4.0	100.2	0.11	0.56
	Sulfapyridine	6.4	1.2	0.05 – 800	0.999	2.6	110.7	1.1	109.5	0.01	0.03
	Sarafloxacin	10.9	0.7	0.88 – 442	0.995	5.2	112.1	2.3	107.1	0.22	0.75
	Ceftiofur	12.1	1.3	0.28 – 800.0	0.993	3.6	89.5	2.0	86.4	0.08	0.28
	Diazinon	21.9	1.2	0.11 – 2100	0.998	2.7	98.9	4.1	96.0	0.01	0.02

Key: IDL, instrumental detection limit; IQL, instrumental quantification limit.

[a] Linear-range was split into two-overlapping ranges to ensure $r^2 \geq 0.997$.
[b] Semi-quantitative, due to only one MRM transition
[c] Semi-quantitiave, due to poor r^2 value
Where possible instrumental performance was determined at concentrations of 10, 100 and 500 ug L^{-1} i.e. those analytes where these concentrations were outside the range of linearity or results were <LOQ were not included.

Table S1b: Method performance data for ECs of interest (ordered by class) (Proctor et al. 2019)

		Surface water (ng L^{-1})		*Effluent (ng L^{-1})*		*Influent (ng L^{-1})*		*Solid particulate matter (ng g^{-1})*		*Digested solids (ng g^{-1})*	
Class of Analyte	**Analyte**	***MDL***	***MQL***	***MDL***	***MQL***	***MDL***	***MQL***	***MDL***	***MQL***	***MDL***	***MQL***
UV Filter	Benzophenone-1	0.07	0.35	0.14	0.71	0.23	1.15	0.004	0.02	0.14	0.70
	Benzophenone-2	0.16	0.79	0.34	1.68	0.36	1.82	0.004	0.02	0.09	0.44
	Benzophenone-3	0.15	0.77	0.19	0.97	0.37	1.87	-	-	-	-
	Benzophenone-4	2.09	6.90	5.78	19.1	7.83	25.8	0.21	0.70	4.01	13.2
Parabens	Methylparaben	0.08	0.40	0.19	0.94	0.28	1.41	0.003	0.02	0.06	0.31
	Ethylparaben	0.24	0.79	0.46	1.52	0.49	1.61	0.01	0.05	0.17	0.57
	Propylparaben	0.25	0.83	0.47	1.54	0.63	2.08	0.01	0.03	0.22	0.72
	Butylparaben	0.08	0.38	0.14	0.71	0.24	1.21	0.002	0.01	0.10	0.52
Plasticizer	Bisphenol A	0.26	0.86	0.56	1.84	0.85	2.79	0.03	0.09	0.27	0.88
Steroid estrogens	E1	0.78	3.92	0.15	7.69	1.96	9.78	0.04	0.21	1.68	8.38
	E2	0.90	4.48	1.41	7.03	1.84	9.22	0.04	0.21	1.48	7.41
	EE2	0.98	4.91	1.46	7.32	1.83	9.15	-	-	-	-
Antibiotics and Antibacterial	Sulfasalazine	4.31	14.2	9.66	31.9	12.6	41.4	-	-	-	-
	Clarithromycin	0.18	0.90	0.28	1.40	0.34	1.69	-	-	-	-
	Azithromycin	0.08	0.26	0.21	0.68	0.14	0.45	0.03	0.10	0.01	0.04
	Trimethoprim	0.26	0.85	0.51	1.67	0.73	2.41	0.01	0.03	0.07	0.22
	Sulfamethoxazole	0.19	0.63	0.47	1.56	0.72	2.38	0.02	0.08	0.12	0.41
	Triclosan	2.93	9.68	4.55	15.0	4.93	16.3	-	-	-	-
	Amoxicillin	-	-	0.26	0.86	-	-	-	-		
	Metronidazole	0.29	0.98	0.68	2.27	0.57	1.90	0.03	0.09	0.03	0.10
	Sulfadiazine	0.05	0.18	0.18	0.59	0.18	0.62	0.003	0.01	0.003	0.01
	Cefalexin	35.6	118.7	10.2	33.9	18.9	63.1	-	-	-	-
	Ofloxacin	0.35	1.17	0.72	2.40	0.58	1.93	-	-	-	-
	Ciprofloxacin	1.85	6.17	5.10	17.0	3.48	11.6	-	-	-	-
	Tetracycline	0.15	0.50	0.30	1.01	0.18	0.59	-	-	-	-
	Danofloxacin	1.58	5.28	4.45	14.85	3.62	12.08	-	-	2.84	9.45

		Surface water ($ng\ L^{-1}$)		*Effluent ($ng\ L^{-1}$)*		*Influent ($ng\ L^{-1}$)*		*Solid particulate matter ($ng\ g^{-1}$)*		*Digested solids ($ng\ g^{-1}$)*	
Class of Analyte	**Analyte**	***MDL***	***MQL***	***MDL***	***MQL***	***MDL***	***MQL***	***MDL***	***MQL***	***MDL***	***MQL***
	Oxytetracycline	6.04	20.1	10.1	33.6	8.26	27.5	-	-	-	-
	Chloramphenicol	3.18	10.6	6.52	21.7	4.21	14.0	0.21	0.69	0.15	0.48
	Penicillin G	0.89	2.98	-	-	-	-	-	-	-	-
	Penicillin V	0.56	1.86	0.92	3.06	2.40	8.00	0.84	2.80	-	-
	Erythromycin	1.15	3.83	2.35	7.85	2.22	7.41	-	-	-	-
	Prulifloxacin	-	-	51.3	171.0	35.3	117.6	-	-	-	-
	Norfloxacin	0.01	0.04	0.02	0.06	0.02	0.07	-	-	-	-
Antifungal	Griseofluvin	0.32	1.06	0.52	1.74	0.59	1.98	0.05	0.16	0.06	0.21
	Ketoconazole	0.06	0.21	0.03	0.10	0.04	0.12	0.02	0.07	0.00	0.01
Hypertension	Valsartan	2.81	9.26	6.40	21.1	7.24	23.9	-	-	-	-
	Irbesartan	0.89	4.47	1.88	9.38	2.50	12.5	-	-	-	-
	Lisinopril	2.17	21.7	4.25	42.5	3.25	32.5	0.04	0.43	0.25	2.47
NSAIDs	Ketoprofen	0.74	3.72	1.60	8.00	2.38	11.9	0.06	0.28	0.47	2.35
	Ibuprofen	0.06	0.31	0.08	0.42	0.19	0.93	0.005	0.02	0.07	0.36
	Naproxen	0.61	3.07	1.17	5.85	6.29	31.5	0.05	0.25	0.60	3.02
	Diclofenac	0.22	0.73	0.44	1.44	0.67	2.22	0.02	0.06	0.75	2.46
	Acetaminophen	1.20	6.02	2.39	12.0	138.0*	1017*	0.04	0.21	2.74	13.7
Lipid regulator	Bezafibrate	0.22	0.66	0.38	1.25	0.64	2.11	0.02	0.05	0.18	0.60
	Atorvastatin	0.14	0.70	0.17	0.84	0.17	0.85	-	-	-	-
Anti-hyperlipidemic	Gemfibrozil	0.30	1.00	0.63	2.11	1.12	3.75	-	-	0.20	0.67
Anti-hyperintensive	Candesartan Cilexetil	6.89	23.0	-	-	-	-	-	-	-	-
Antihistamine	Fexofenadine	0.21	0.69	0.40	1.32	0.56	1.85	-	-	-	-
	Cetirizine	0.26	0.87	0.32	1.06	0.52	1.72	-	-	-	-
GUD/ED	Sildenafil	0.01	0.03	0.02	0.05	0.01	0.05	0.001	0.003	0.001	0.003
Diabetes	Metformin	156.0*	515.0*	163.0*	460.0*	457.0*	1509*	-	-	-	-
	Gliclazide	0.15	0.77	0.16	0.82	0.22	1.09	-	-	-	-
	Sitagliptin	0.03	0.09	0.08	0.27	0.06	0.22	0.004	0.01	0.003	0.01

		Surface water (ng L^{-1})		*Effluent (ng L^{-1})*		*Influent (ng L^{-1})*		*Solid particulate matter (ng g^{-1})*		*Digested solids (ng g^{-1})*	
Class of Analyte	**Analyte**	***MDL***	***MQL***	***MDL***	***MQL***	***MDL***	***MQL***	***MDL***	***MQL***	***MDL***	***MQL***
Cough suppressant	Pholcodine	2.25	7.42	8.02	26.5	25.3	83.3	0.28	0.92	1.52	5.00
Beta-blocker	Atenolol	0.20	0.66	0.56	1.84	0.71	2.35	0.01	0.05	0.10	0.33
	Metoprolol	0.07	0.35	0.19	0.96	0.28	1.40	0.01	0.03	0.03	0.14
	Propranolol	0.29	0.96	0.73	2.41	0.68	2.25	0.01	0.04	0.13	0.42
	Bisoprolol	0.001	0.004	0.004	0.01	0.003	0.01	0.0001	0.0005	0.0001	0.0005
H2 receptor agonist	Ranitidine	7.96	39.8	22.3	111.4	14.8	73.8	0.44	2.19	4.81	24.1
	Cimetidine	1.60	7.98	3.12	15.6	5.06	25.3	-	-	-	-
X-ray contrast media	Iopromide	5.97	29.9	14.1	70.6	24.5	123.0	-	-	-	-
Various	Buprenorphine	0.06	0.20	0.11	0.36	0.18	0.61	0.02	0.07	0.01	0.05
Drug precursor	Ephedrine/pseudoephedrine	0.60	1.97	1.62	5.36	1.32	4.36	0.02	0.07	0.11	0.35
	Norephedrine	0.18	8.82	0.35	17.3	0.37	18.6	0.01	0.39	0.04	1.85
Anti-cancer	Azathioprine	0.17	0.55	0.36	1.20	0.41	1.36	-	-	-	-
	Methotrexate	6.13	20.2	9.04	29.8	7.11	23.5	0.16	0.53	1.64	5.42
	Ifosfamide	0.08	0.40	0.24	1.22	0.31	1.53	-	-	-	-
	Tamoxifen	14.5	72.6	0.76	3.82	0.70	3.50	0.004	0.01	2.23	11.14
	Imatinib	0.88	2.93	1.13	3.76	1.78	5.95	0.10	0.35	0.06	0.21
	Capecitabine	0.01	0.02	0.01	0.03	0.01	0.03	0.002	0.01	0.001	0.003
	Bicalutamide	0.22	0.72	0.31	1.02	0.32	1.07	0.02	0.06	0.01	0.03
Anaesthetic and metabolite	Ketamine	0.07	0.37	0.19	0.93	0.24	1.20	0.005	0.02	0.03	0.17
	Norketamine	0.23	0.76	0.56	1.86	0.72	2.37	0.02	0.05	0.10	0.33
	Venlafaxine	0.07	0.37	0.24	1.20	0.37	1.83	0.01	0.03	0.08	0.38
	Desmethylvenlafaxine	0.24	0.80	0.66	2.18	0.85	2.79	0.01	0.05	0.09	0.29
	Fluoxetine	1.14	5.71	1.42	7.08	0.50	2.52	0.005	0.02	0.11	0.53
	Norfluoxetine	1.64	8.21	1.27	6.35	0.42	2.12	0.004	0.02	0.14	0.68
	Sertraline	1.61	8.07	1.21	6.05	0.74	3.72	0.002	0.01	0.17	0.86
	Mirtazapine	0.09	0.44	0.25	1.25	0.39	1.94	0.01	0.03	0.05	0.27
	Citalopram	0.61	6.08	1.41	14.1	1.24	12.4	0.02	0.24	0.16	1.64

		Surface water (ng L^{-1})		*Effluent (ng L^{-1})*		*Influent (ng L^{-1})*		*Solid particulate matter (ng g^{-1})*		*Digested solids (ng g^{-1})*	
Class of Analyte	**Analyte**	***MDL***	***MQL***	***MDL***	***MQL***	***MDL***	***MQL***	***MDL***	***MQL***	***MDL***	***MQL***
	Desmethylcitalopram	0.14	0.69	0.36	1.82	0.31	1.54	0.01	0.03	0.05	0.24
	Paroxetine	0.18	0.59	0.21	0.69	0.13	0.45	0.01	0.02	0.005	0.02
	Duloxetine	0.04	0.13	0.05	0.18	0.04	0.12	0.003	0.01	0.002	0.01
	Amitriptyline	0.16	0.55	0.33	1.09	0.30	1.02	0.02	0.07	0.01	0.03
	Nortriptyline	0.33	1.11	0.63	2.11	0.61	2.03	0.03	0.10	0.02	0.06
	Norsertraline	-	-	-	-	1.07	3.58	0.09	0.28	-	-
Anti-epileptic	Carbamazepine	0.08	0.38	0.19	0.93	0.27	1.37	0.01	0.03	0.10	0.48
	Carbamazepine 10,11-epoxide	0.16	0.53	0.55	1.82	0.53	1.76	-	-	-	-
	10,11-Dihydro -10-hydroxycarbamazepine	0.34	3.37	0.84	8.41	0.99	9.94	0.02	0.25	0.43	4.35
Calcium-channel blocker	Diltiazem	0.11	1.11	0.32	3.23	0.27	2.68	-	-	-	-
	Verapamil	0.01	0.02	0.01	0.04	0.01	0.03	0.001	0.002	0.0004	0.001
Hypnotic	Temazepam	0.08	0.38	0.14	0.69	0.18	0.92	0.01	0.04	0.16	0.82
	Oxazepam	0.11	0.36	0.22	0.72	0.20	0.66	-	-	0.01	0.03
	Diazepam	0.02	0.06	0.04	0.13	0.04	0.12	0.002	0.01	0.002	0.01
Anti-psychotic	Quetiapine	0.10	0.48	0.21	1.07	0.26	1.32	0.004	0.02	0.05	0.26
	Risperidone	0.01	0.02	0.02	0.06	0.02	0.06	0.001	0.004	0.002	0.01
Dementia	Donepezil	0.55	1.83	1.54	5.12	1.48	4.93	0.09	0.30	0.09	0.29
	Memantine	0.04	0.14	0.11	0.36	0.12	0.39	0.02	0.07	0.01	0.04
Human Indicators	Creatinine	511*	1686*	771*	2544*	945*	3118*	-	-	-	-
	Nicotine	3.34	11.0	5.44	18.0	508*	2296*	0.16	-	0.66	2.19
	Caffeine	0.37	1.83	1.11	5.57	121*	581*	-	-	-	-
	Cotinine	0.07	0.35	0.21	1.06	0.27	1.34	0.005	0.02	0.24	1.22
	1,7-dimethylxanthine	3.19	10.5	11.4	37.6	560*	2165*	-	-	-	-
Analgaesics and Metabolites	Morphine	2.65	8.75	6.34	20.9	8.85	29.2	0.11	0.37	1.92	6.33
	Dihydromorphine	0.11	0.55	0.32	1.59	0.05	2.51	0.01	0.04	0.09	0.45
	Normorphine	3.54	11.7	7.84	25.9	9.99	33.0	0.12	0.39	1.74	5.75
	Methadone	0.11	0.54	0.21	1.04	0.20	1.01	0.01	0.03	0.03	0.17

		Surface water (ng L^{-1})		*Effluent (ng L^{-1})*		*Influent (ng L^{-1})*		*Solid particulate matter (ng g^{-1})*		*Digested solids (ng g^{-1})*	
Class of Analyte	**Analyte**	***MDL***	***MQL***	***MDL***	***MQL***	***MDL***	***MQL***	***MDL***	***MQL***	***MDL***	***MQL***
	EDDP	0.21	1.05	0.29	1.47	0.23	1.13	0.01	0.03	0.04	0.20
	Codeine	0.74	3.71	1.46	7.31	2.56	12.8	0.04	0.21	0.33	1.66
	Norcodeine	2.88	9.52	8.32	27.4	8.53	28.2	0.19	0.64	1.26	4.17
	Dihydrocodeine	0.23	0.75	0.55	1.83	0.88	2.89	0.02	0.05	0.11	0.36
	Tramadol	0.08	8.20	0.21	21.3	0.30	30.0	0.01	0.62	0.03	3.26
	N-desmethyltramadol	0.12	5.92	0.30	15.0	0.56	27.9	0.01	0.30	0.04	2.02
	O-desmethyltramadol	0.09	8.53	0.28	27.8	0.31	31.4	-	-	-	-
Stimulants and metabolites	Amphetamine	0.68	2.23	1.11	3.65	1.23	4.07	0.01	0.05	0.09	0.29
	Methamphetamine	0.32	1.05	0.71	2.35	0.95	3.13	0.01	0.04	0.09	0.30
	MDMA	0.10	0.50	0.27	1.35	0.34	1.70	0.01	0.03	0.04	0.18
	MDA	0.53	1.74	1.00	3.30	0.99	3.26	-	-	-	-
	Cocaine	0.07	0.35	0.22	1.11	0.46	2.31	0.01	0.03	0.03	0.15
	Benzoylecgonine	0.07	0.34	0.18	0.91	0.21	1.07	0.005	0.02	0.03	0.14
	Anhydroecgoninemethylester	0.93	4.67	1.99	9.96	2.95	14.8	-	-	-	-
	Cocaethylene	0.07	0.35	0.21	1.04	1.31	6.54	0.01	0.03	0.03	0.17
	Mephedrone	0.22	1.09	0.44	2.19	0.55	2.75	0.01	0.04	0.06	0.31
	MDPV	0.04	0.22	0.12	0.59	0.48	2.41	0.01	0.03	0.04	0.20
Opiod and metabolite	Heroin	0.92	4.62	3.44	17.2	4.18	20.9	0.05	0.25	0.56	2.79
	6-acetylmorphine	0.28	0.94	0.76	2.50	0.89	2.95	-	-	-	-
Pesticides, fungicides and herbicides	Thiamethoxam	0.13	0.42	0.44	1.46	0.53	1.76	0.01	0.03	0.01	0.02
	Imidacloprid	0.04	0.15	0.10	0.33	0.10	0.33	0.01	0.02	-	-
	Clothiniadin	0.06	0.19	0.14	0.47	0.15	0.50	0.004	0.01	-	-
	Metazachlor	0.02	0.06	0.04	0.14	0.04	0.13	0.002	0.01	0.002	0.01
	Terbuthylazine	0.03	0.11	0.07	0.22	0.07	0.23	0.01	0.02	0.01	0.02
	Methiocarb	0.13	0.43	0.27	0.91	0.26	0.86	0.01	0.04	0.01	0.04
	Dichlofluanid	-	-	-	-	25.2	83.8	-	-	-	-
	Flufenacet	0.01	0.04	0.02	0.07	0.02	0.07	0.002	0.01	0.002	0.01
	Oxadiazon	0.15	0.49	0.26	0.85	0.30	0.98	0.08	0.26	0.05	0.16

Class of Analyte	Analyte	*Surface water (ng L^{-1})*		*Effluent (ng L^{-1})*		*Influent (ng L^{-1})*		*Solid particulate matter (ng g^{-1})*		*Digested solids (ng g^{-1})*	
		MDL	***MQL***	***MDL***	***MQL***	***MDL***	***MQL***	***MDL***	***MQL***	***MDL***	***MQL***
Veterinary Pharmaceuticals	Chlorpyrifos	12.9	42.9	8.54	28.5	-	-	-	-	0.33	1.09
	Triallate	0.11	0.37	0.20	0.68	0.09	0.31	-	-	-	-
	Tylosin	1.28	6.39	2.23	11.1	3.27	16.3	-	-	-	-
	Sulfapyridine	0.04	0.14	0.11	0.37	0.10	0.33	-	-	-	-
	Sarafloxacin	0.83	2.78	2.66	8.86	2.01	6.72	-	-	-	-
	Ceftiofur	2.17	7.23	1.32	4.41	1.02	3.39	-	-	-	-
	Diazinon	0.03	0.11	0.07	0.23	0.06	0.21	0.00	0.01	0.003	0.01

* Calculated for direct injection

Table S2. SPE/MAE-UHPLC-QTOF- method performance

Analyte	IS	Linearity Range [µg L^{-1}]	R^2	Accuracy* [%]	Precision* [%]	IDL [µg L^{-1}]	IQL [µg L^{-1}]
Bisphenol A Sulphate	4-chloro-3-methylphenol-D2	1.4 - 103.4	0.997	98.3	2.1	0.41	1.39
3-PBA	4-chloro-3-methylphenol-D2	0.03-100	0.994	90.1	2.5	0.01	0.03
Triclosan sulphate	4-chloro-3-methylphenol-D2	1.59 - 100	0.999	101.3	0.3	0.51	1.59

**concentration levels: 0.1, 5 and 100 ng/mL used for inter-day precision and accuracy*

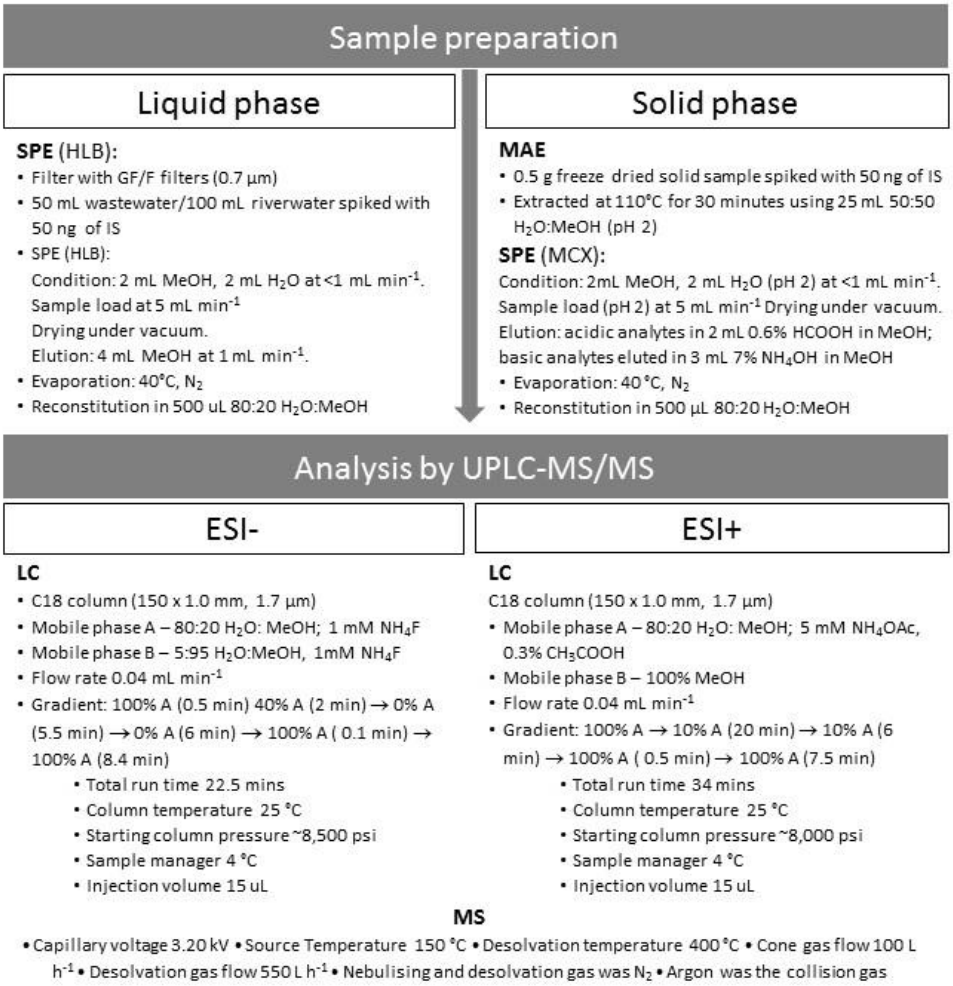

Figure S1. SPE/MAE-UHPLC-QqQ – schematic overview.

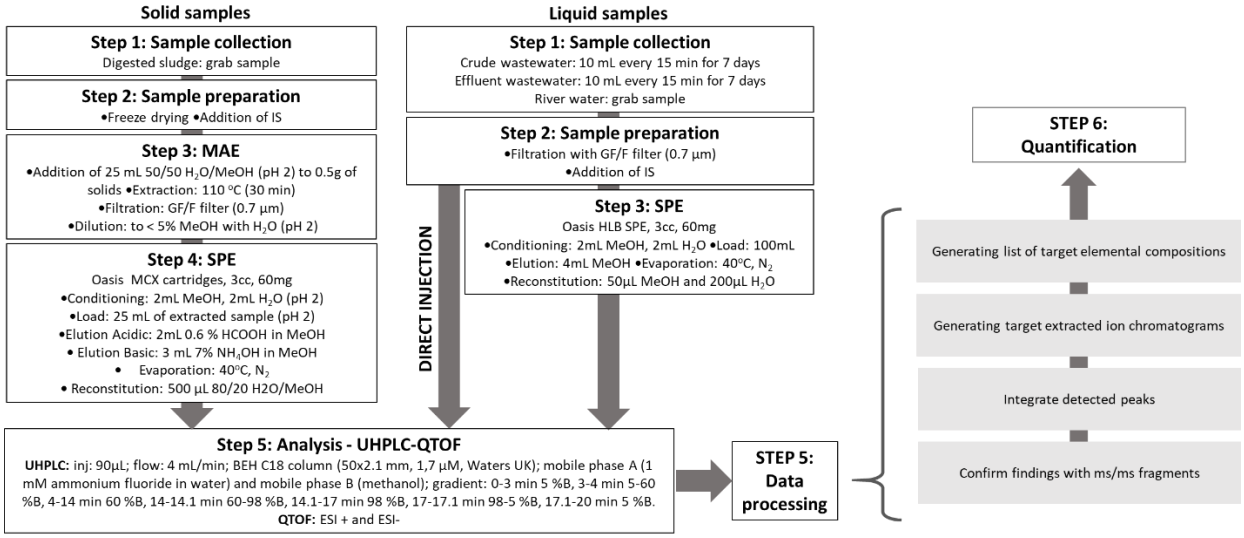

Figure S2 SPE-UHPLC-QTOF – schematic overview.

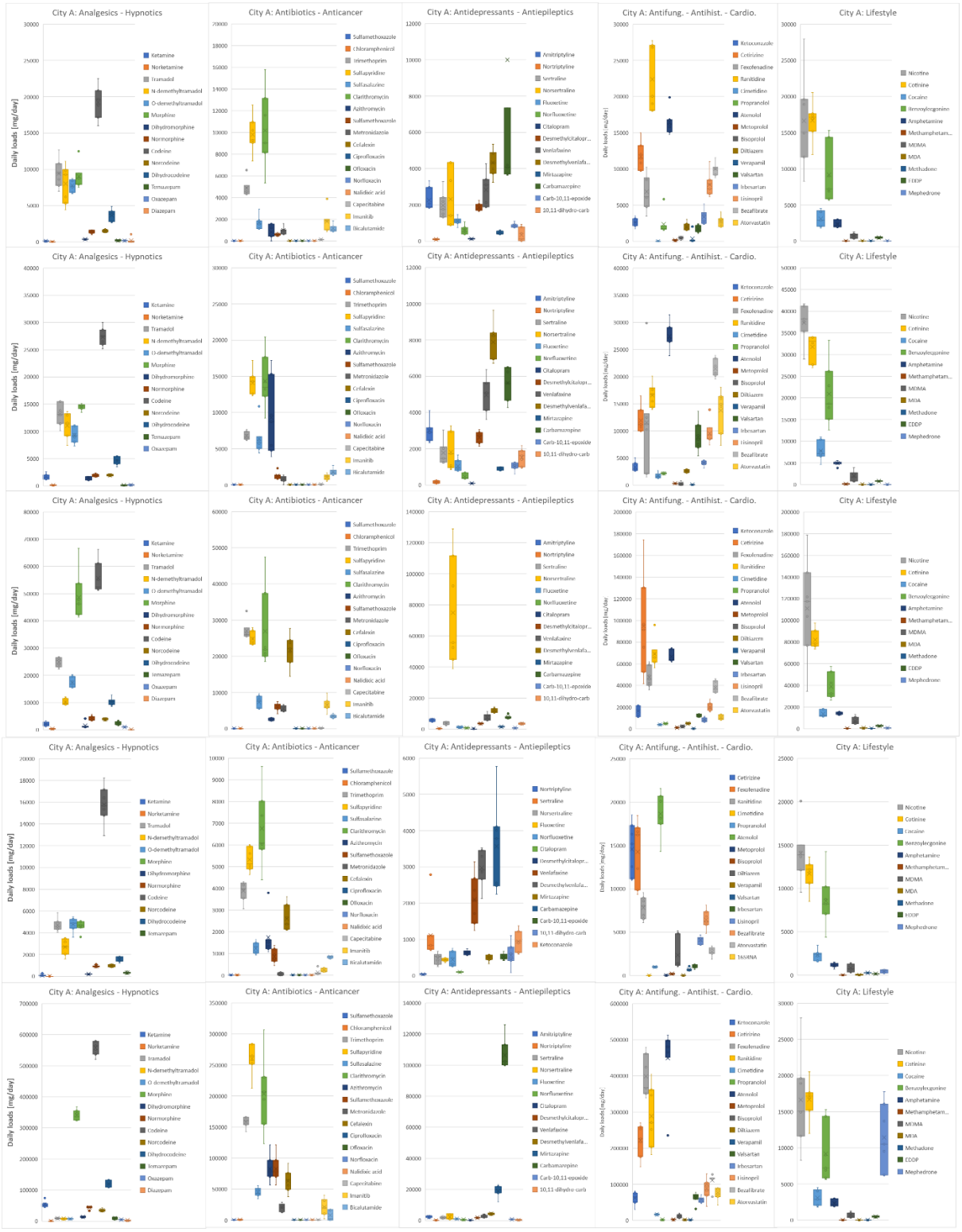

Figure S3. Daily loads of BCIs

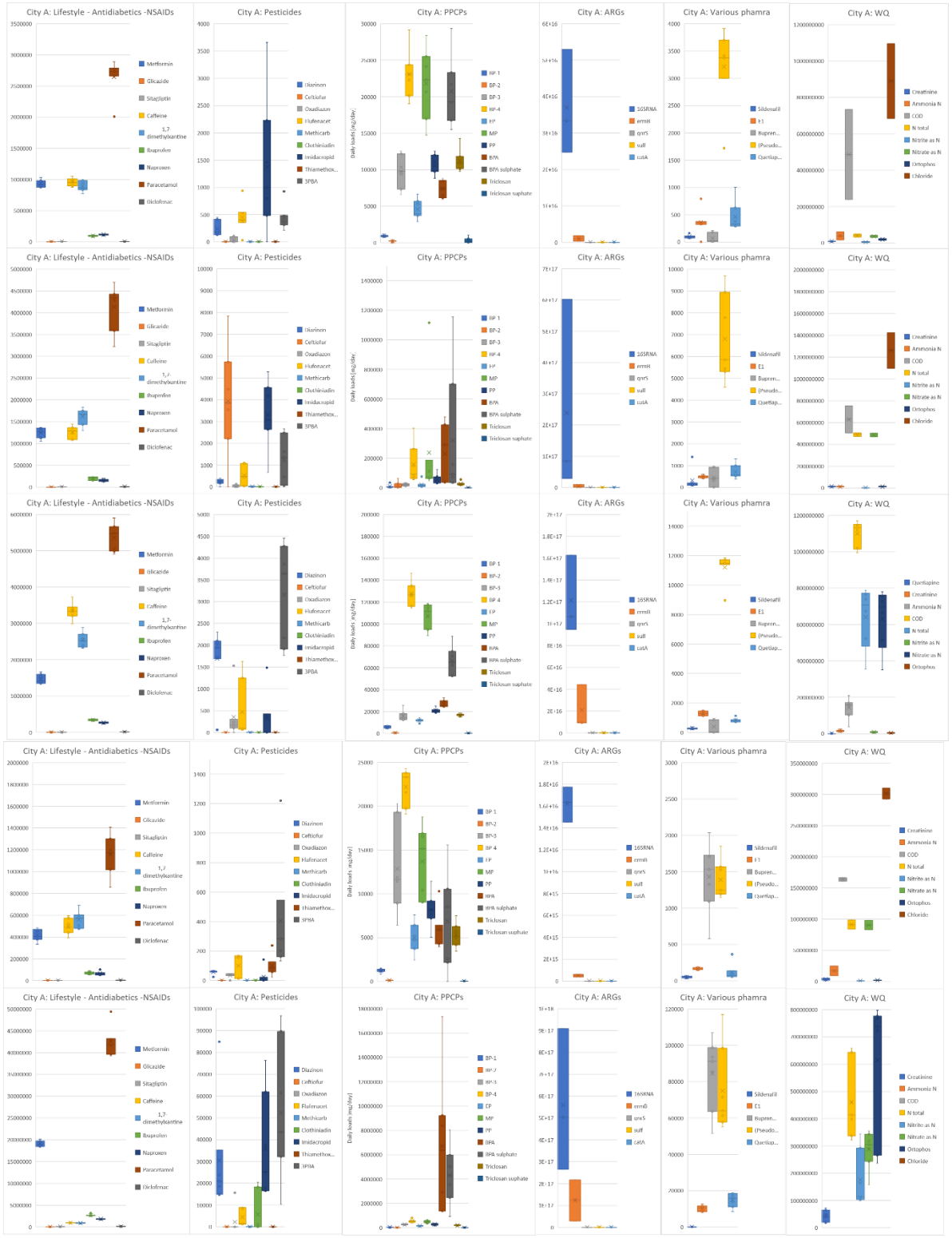

Figure S3. Daily loads of BCIs continued

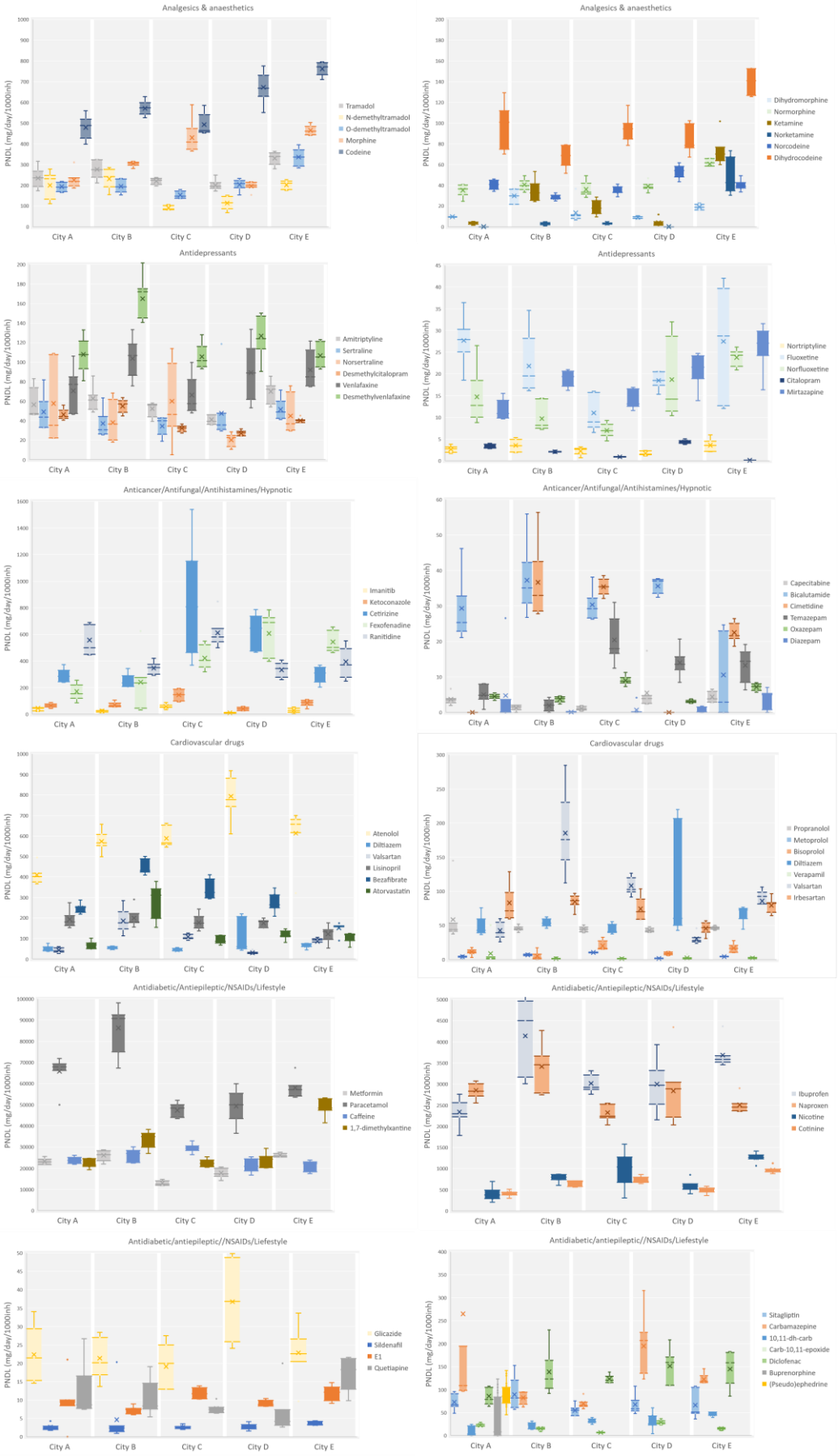

Figure S4. PNDLs of pharmaceuticals (calculated using WW-PE)

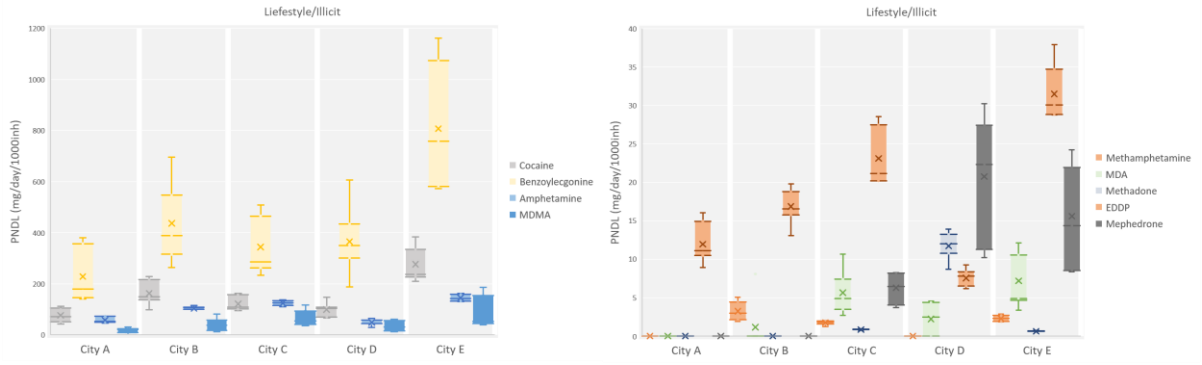

Figure S5. PNDLs of lifestyle chemicals (calculated using WW-PE)

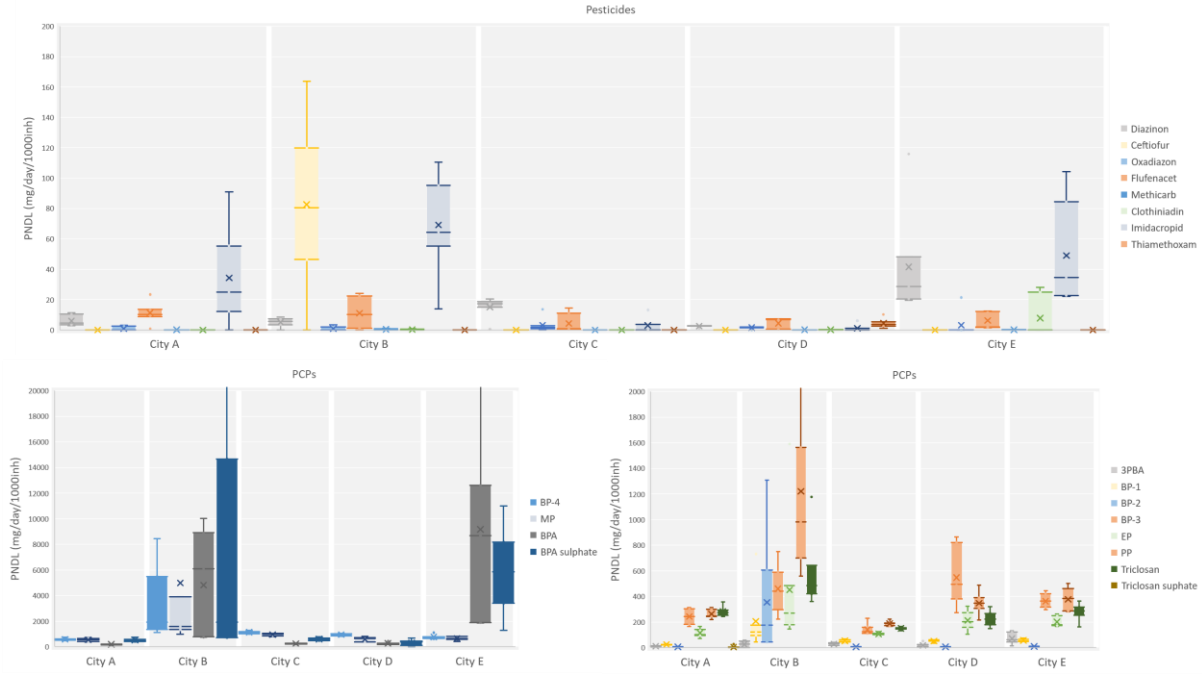

Figure S6. PNDLs of pesticides and industrial chemicals (calculated using WW-PE).

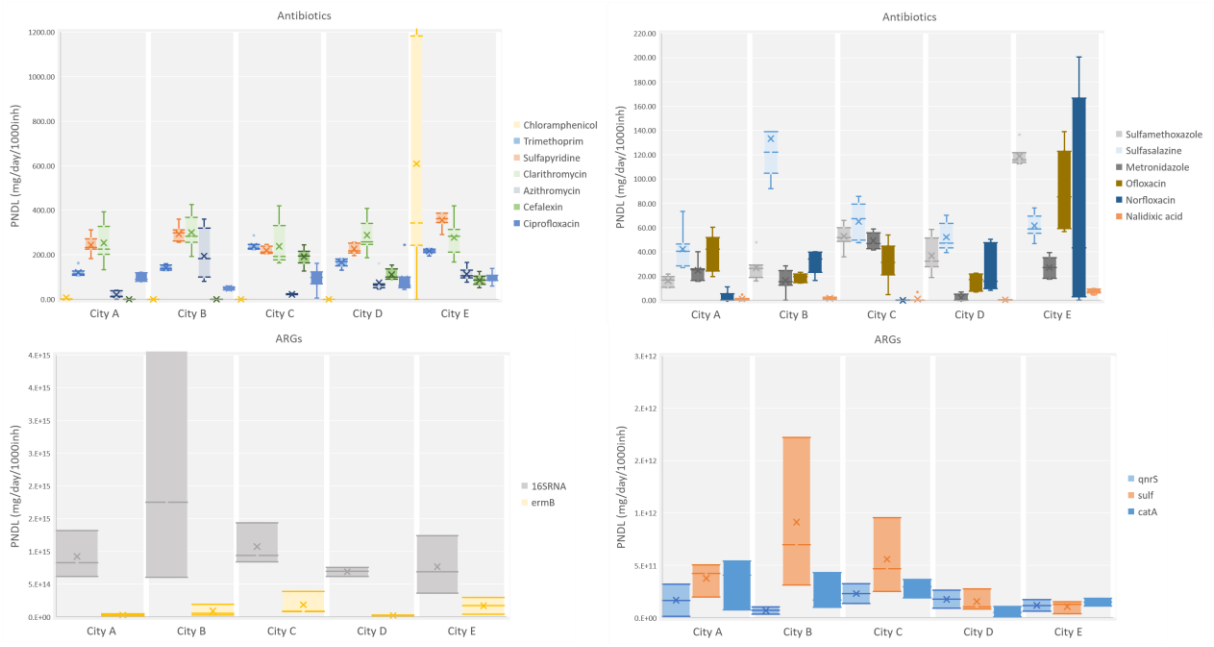

Figure S7. PNDLs of antibiotics and ARGs (calculated using WW-PE)

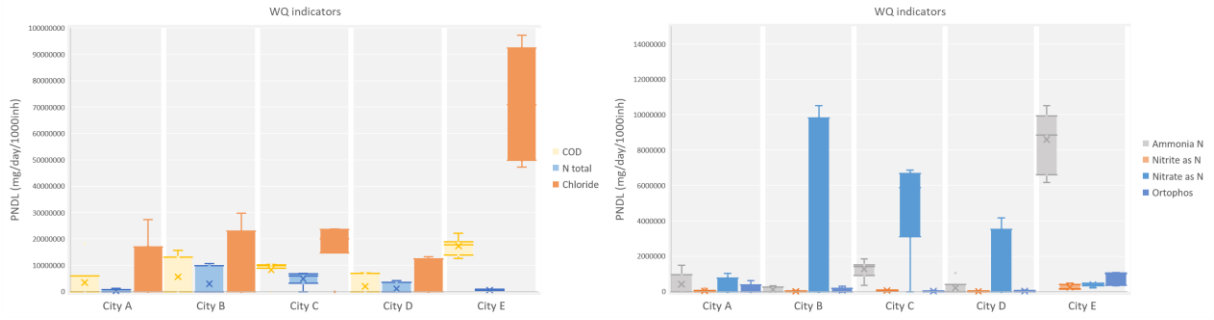

Figure S8. PNDLs of WQIs (calculated using WW-PE).

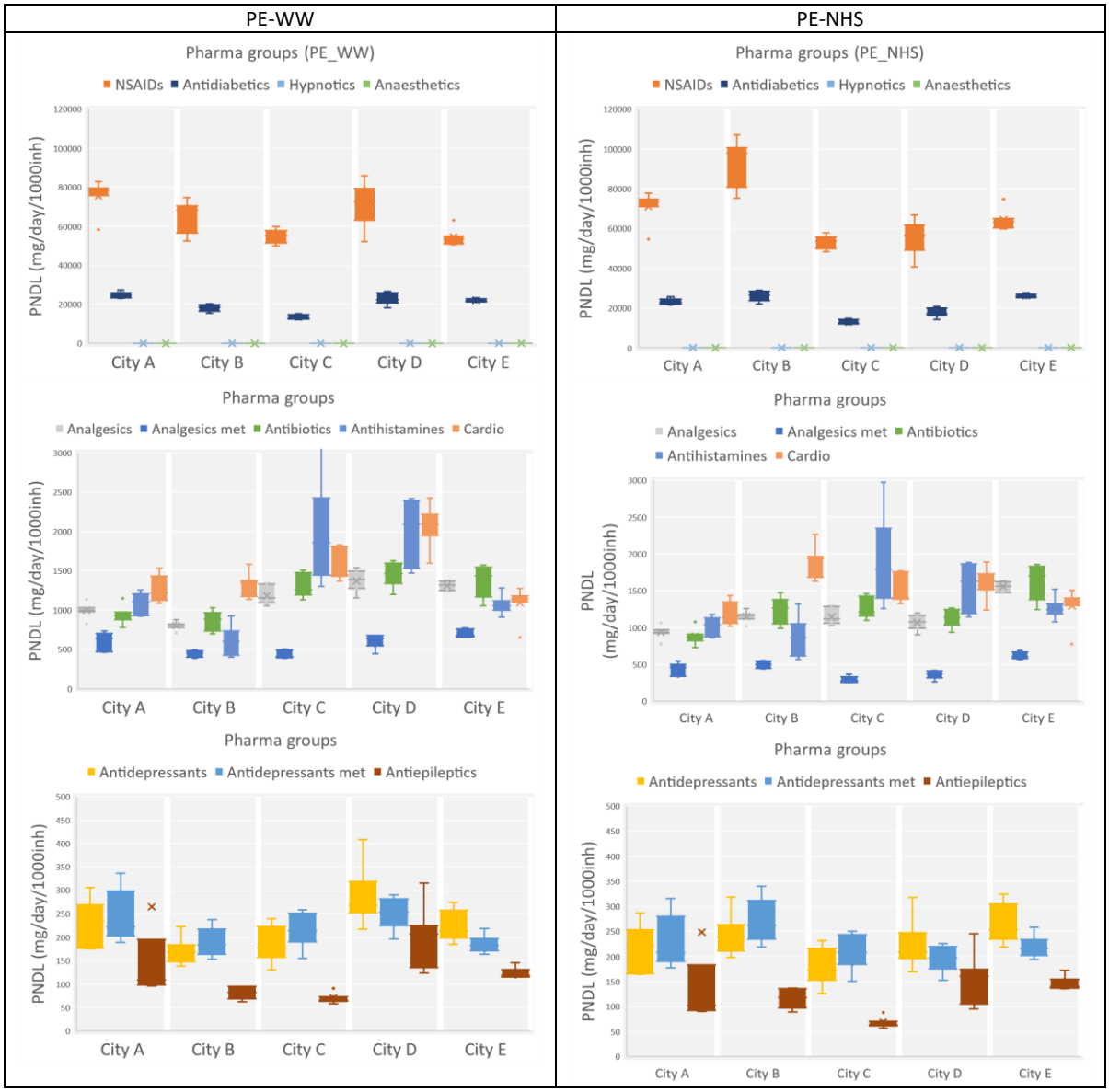

Figure S9. PNDLs for pharmaceuticals

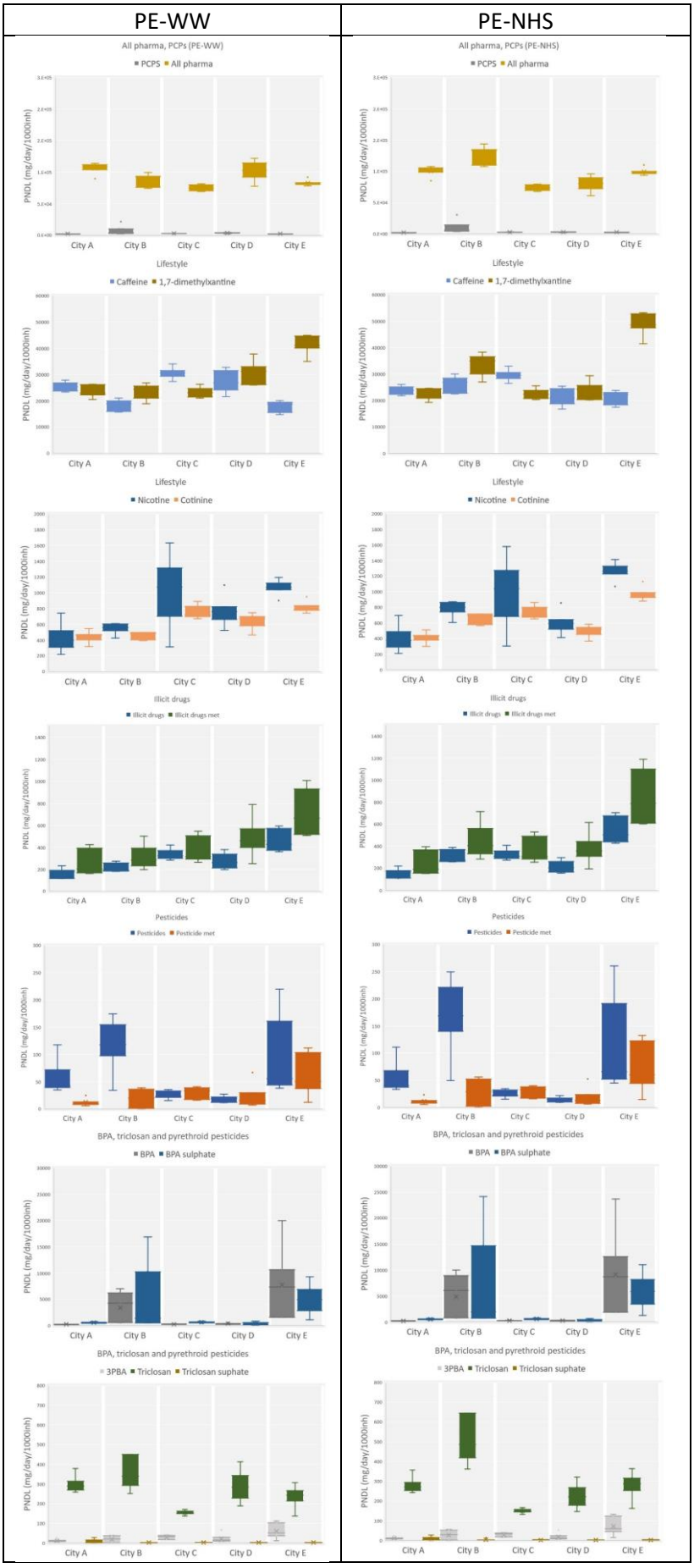

Figure S10: PNDLs of BCIs driven by city function

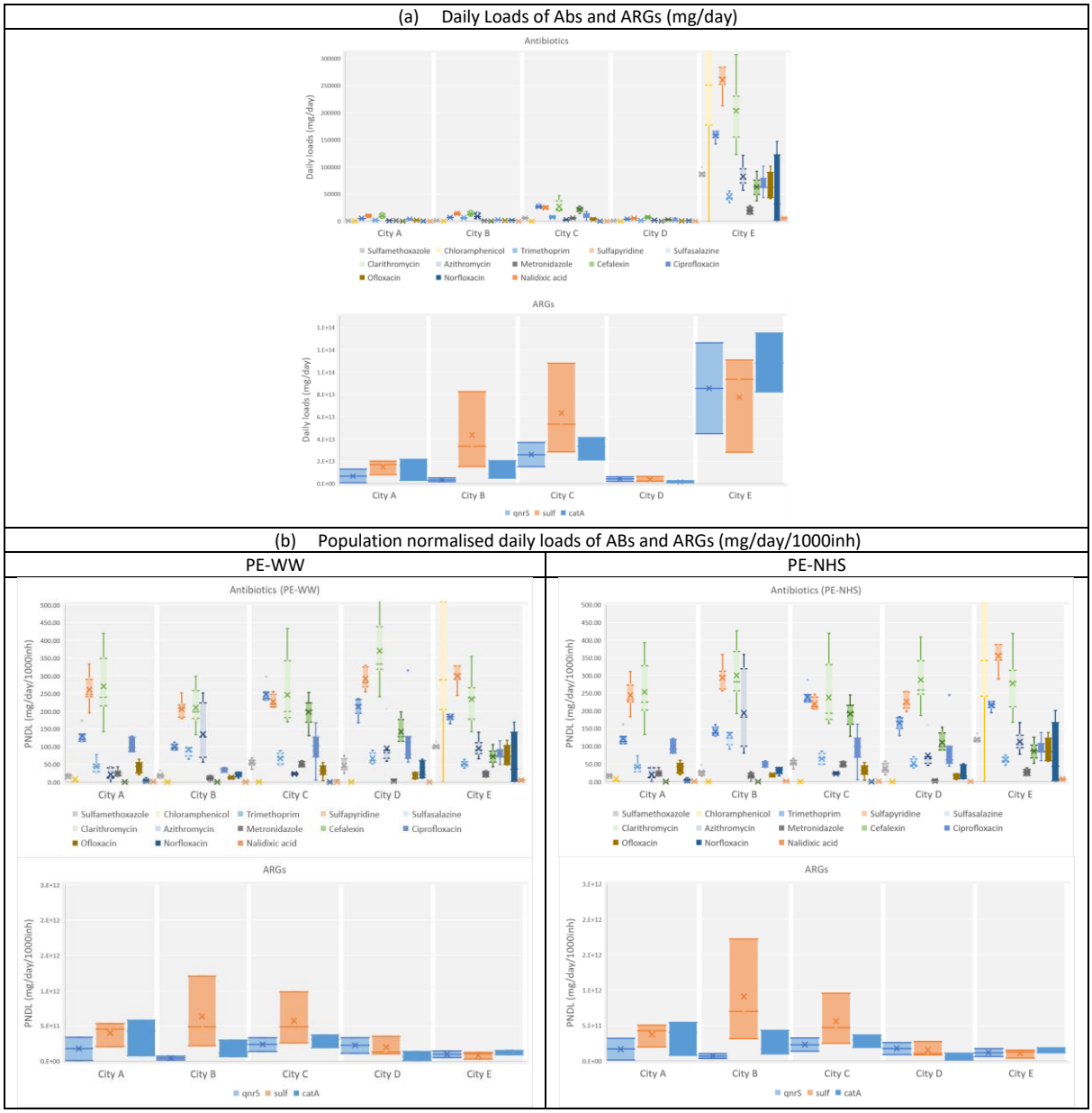

Figure S11. AB and ARGs: (a) daily loads and (b) population normalised daily loads - PNDLs (chloramphenicol was excluded in total AB calculation).

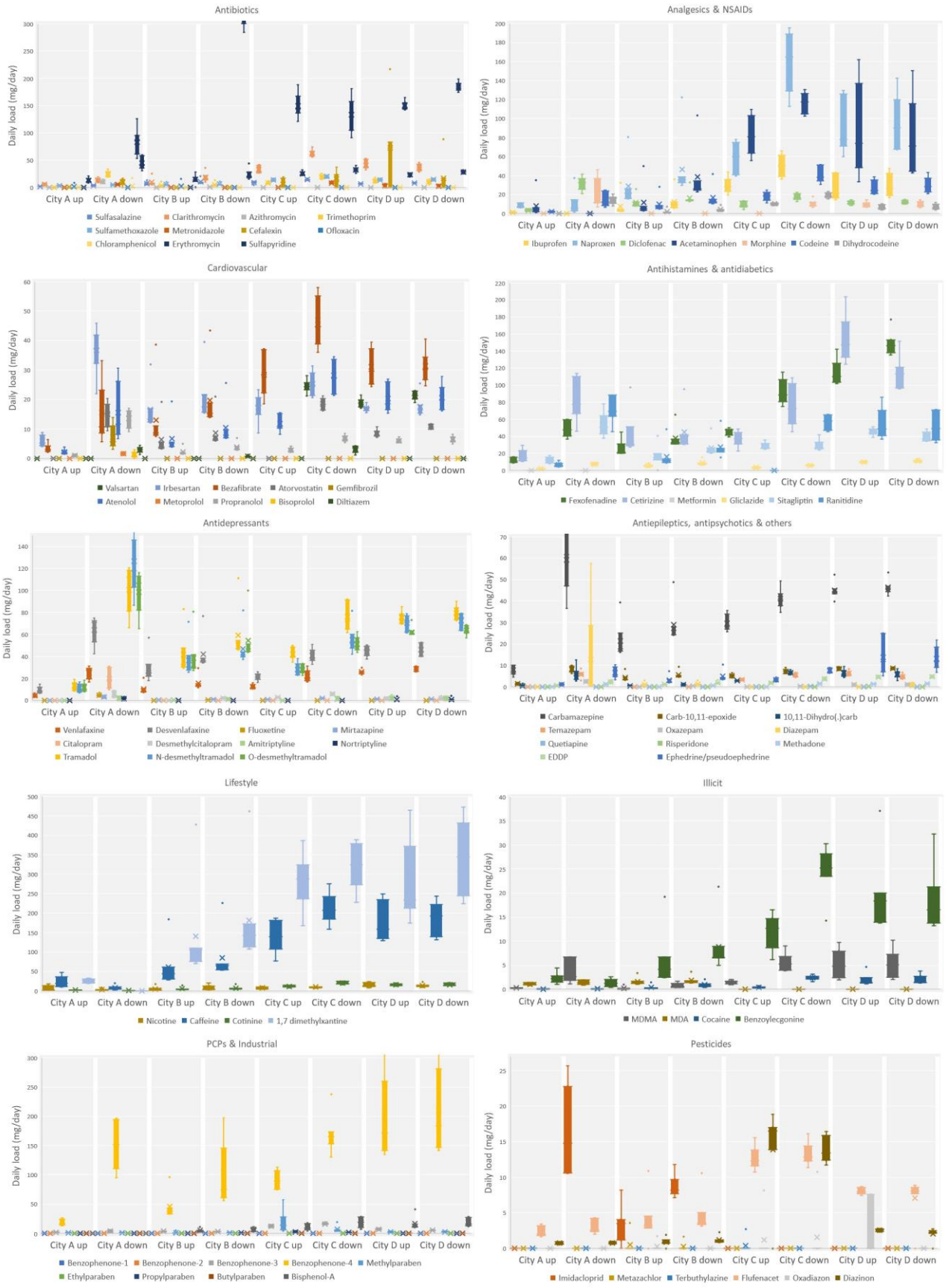

Figure S12. Daily loads of BCIs in receiving waters.

Proctor, K., B. Petrie, R. Barden, T. Arnot, and B. Kasprzyk-Hordern. 2019. "Multi-residue ultra-performance liquid chromatography coupled with tandem mass spectrometry method for

comprehensive multi-class anthropogenic compounds of emerging concern analysis in a catchment-based exposure-driven study." *Anal Bioanal Chem* 411 (27):7061-7086. doi: 10.1007/s00216-019-02091-8.