Impact of a megacity on a tropical estuary assessed using a combination of chemical analysis and *in-vitro* bioassays

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Abstract

Tropical estuaries are threatened by rapid urbanisation, which leads to the spread of thousands of micropollutants and poses an environmental risk to such sensitive aqueous ecosystems. In the present study, a combination of chemical and bioanalytical water characterization was applied to investigate the impact of Ho Chi Minh megacity (HCMC, 9.2 million inhabitants in 2021) on the Saigon River and its estuary, in the South of Vietnam, and provide a comprehensive water quality assessment. Water samples were collected along a 140 km stretch integrating the river-estuary *continuum* from upstream HCMC down to the estuary mouth in the East Sea. Additional wastewater impacted samples were collected at the mouth of the four main canals of the city center. Chemical analysis was performed targeting up to 217 micropollutants (pharmaceuticals, plasticizers, PFASs, hormones, pesticides). Bioanalysis was performed using six *in-vitro* bioassays for hormone receptor-mediated effects, xenobiotic metabolism pathways and oxidative stress response, respectively, all accompanied by cytotoxicity measurement. A total of 120 micropollutants were detected and displayed high variability along the river *continuum* with total concentration ranging from 0.25 to 78 µg L^{-1}. Among them, 59 micropollutants were ubiquitous (detection frequency ≥ 80%). An attenuation was observed in concentration and effect profiles towards the estuary. The urban canals were identified as major sources of micropollutants and bioactivity to the river, and one canal (Bến Nghé) exceeded two effect-based trigger values defined for the bioassays. Iceberg modelling apportioned the contribution of the quantified and the unknown chemicals to the measured effects. Diuron, metolachlor, chlorpyrifos, daidzein, genistein, climbazole, mebendazole and telmisartan were identified as main risk drivers of the oxidative stress response and xenobiotic metabolism pathway activation. Our study reinforced the need for improved wastewater management and deeper evaluations of the occurrence and fate of micropollutants in tropical estuary environments.

Keywords

Emerging contaminants

Mixture toxicity

Untreated wastewater

Tropical river

Estuarine environment

Vietnam

1. Introduction

Tropical estuaries provide essential services and are among the most productive and biodiverse ecosystems in the world (Ayyam et al., 2019; Bayen, 2012; Poynton et al., 1998). Their ecosystem health is, however, threatened by intense urbanization. Tropical areas are projected to host more than 50% of the world population by 2050 (Marcotullio et al., 2021). By that time, 1.95 billion people will be concentrated in the tropical coastal zone, and the number of tropical coastal megacities, nine currently, will continue to rise (UN, 2018; Sale et al., 2014). Higher densities of human populations inherently result in the spread of a broad range of pollutants to the environment. Among them, countless organic micropollutants are emitted from diverse anthropogenic point and diffuse sources, especially domestic and hospital wastewaters, urban areas, agriculture, aquaculture, industry and maritime activities. They include pharmaceuticals and personal care products (PPCPs), pesticides, per- and polyfluoroalkyl substances (PFASs), plasticizers, among others. These compounds may show one or several alarming properties such as mobility, persistence or pseudo-persistence, bioaccumulation, toxicity; leading to potential or proven detrimental effects on human and ecological health (Farré et al., 2008; Tijani et al., 2016).

Organic micropollutants and their transformation products remain largely unregulated and hence unmonitored in many regions of the world. While many studies on their occurrence and fate have so far been conducted in North America, Europe and Eastern Asia (Castiglioni et al., 2018; Rehrl et al., 2020; Richardson and Kimura, 2020; Yang et al., 2014), such studies are still missing in tropical zones, especially in areas under strong anthropogenic pressure. Only a paucity of studies have focused on the occurrence and behavior of micropollutants in tropical aquatic systems (Chaves et al., 2021; Dsikowitzky et al., 2018; Guruge et al., 2019; Kandie et al., 2020; Tran et al., 2019) and the integration of the whole water system, from sources through the river basins down to estuaries is rarely considered. Contrasting environmental conditions occur in tropical environment in comparison to temperate zones including high water temperature, microbial activity, different precipitation patterns and high solar radiation (Lewis et al., 2016; Syvitski et al., 2014). The environmental fate of micropollutants may then

differ significantly in terms of degradation, transport and ecotoxicity, which is a strong incentive to better understand the occurrence of micropollutants in tropical systems.

The Saigon-Dongnai River basin and its estuary in the South of Vietnam are a perfect example of a tropical estuarine ecosystem impacted by the growth of a coastal megacity, Ho Chi Minh City (HCMC, 9.2 million inhabitants). To date, limited evaluation has been conducted on the impact of HCMC on the Saigon River Estuary and on the Can Gio mangrove, a UNESCO biosphere reserve located at the river mouth. Three studies provided first scattered data on some micropollutants but were constrained to the urban area of HCMC (Chau et al., 2018; Duong et al., 2015, 2014). Only one study was conducted upstream of the megacity on endocrine disrupting compounds (Le et al., 2016) and none downstream. Therefore, so far, no attention has been given to the spatial variation in level and pollution profile of micropollutants along the river-estuary *continuum*. A dedicated strategy was developed to characterize the multitude of micropollutants present in this system and to assess the impact of the megacity on the water quality along the estuary and the mangrove.

In the present work, trace analysis and bioanalytical tools were combined for a comprehensive water quality assessment of the whole *continuum* from the river upstream the megacity through the urban areas and down to the mangrove and the estuary mouth, covering a stretch of 140 km. Quantitative target analysis was performed for 217 micropollutants using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The targeted micropollutants included chemicals that can be used as source indicators (wastewater markers, agricultural markers, industrial markers) and process indicators (micropollutants known to be sensitive to biodegradation or photodegradation processes). The chemical analyses were complemented with *in-vitro* bioassays covering 6 different toxicity endpoints (hormone receptor-mediated effects, xenobiotic metabolism and adaptative stress responses, respectively) to provide an integrative measure of toxicity of all the bioactive micropollutants present in the surface water (Escher, 2011; Neale et al., 2015). The predicted effects of the mixtures of detected chemicals were calculated based on the concentration obtained from the chemical analysis and compared to the measured bioactivity of the chemical mixture extracted from the water samples (Escher et al., 2020b). The contribution of targeted chemicals (so-called “tip of the iceberg”) to the mixture effects

observed along the river *continuum* were then apportioned to better identify their individual contribution to the observed toxicity.

The present paper aims (1) to determine the occurrence and spatial variation of multi-class micropollutants along a heavily urbanized tropical river-estuary *continuum*; and (2) to assess the impact of the urban center of HCMC on the water quality by combining the results from the chemical analysis and from the bioassays.

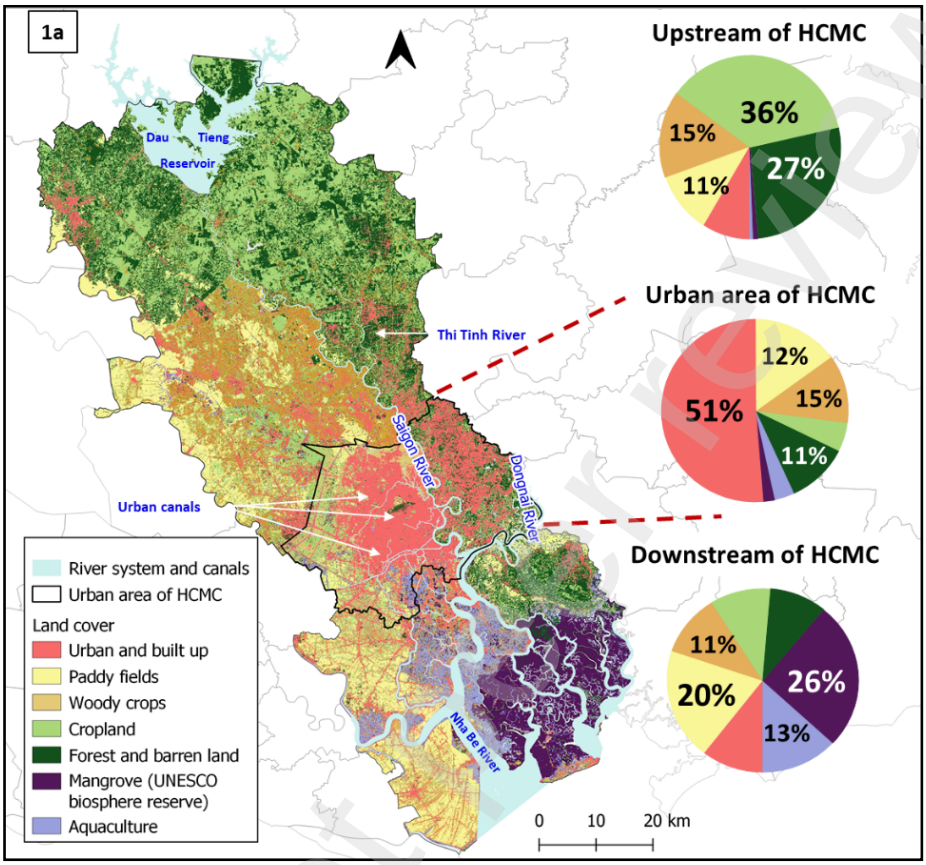
2. Material and methods

2.1 Study area

The Saigon River is part of the Dongnai River basin, one of the largest basins in the country (MONRE, 2006). The river (18 ± 14 $m^3 s^{-1}$) runs over 250 km from its source in Southeastern Cambodia to the estuary mouth in the East Sea. On the way, it connects to the Thi Tinh River (mean annual discharge 20 ± 11 $m^3 s^{-1}$), its main tributary in the upstream region, crosses HCMC and then joins the Dongnai River (632 ± 446 $m^3 s^{-1}$) (Nguyen et al., 2022). The resulting confluence, located about 40 km from the East Sea, forms the Nha Be River that divides into several estuary arms and flows in the Can Gio Mangrove Biosphere Reserve. The study area falls under a tropical monsoonal climate. The annual mean temperature is 28°C and the average annual rainfall is 1940 mm (Khoi and Trang, 2016), mainly distributed during the wet season (Norwine, 2014; Vachaud et al., 2019). Due to the proximity of the estuary, the Saigon and Dongnai Rivers are subject to strong semi-diurnal tide cycles with a tidal range oscillating between -2 m to 1.50 m and discharge oscillations from -1000 and 1000 $m^3 s^{-1}$ in the Saigon River (Camenen et al., 2021).

The province of HCMC has a diverse land use (Fig. 1a). In addition to being the most populated province, it is a very productive agricultural region (Aygun et al., 2019). Upstream the megacity, forest, croplands and woody crops dominate with a small percentage of paddy fields and urban settlements. The megacity covers the middle part of the province. It is the economic center of the country and the most populated city with 9.2 million inhabitants (Norwine, 2014; van Leeuwen et al., 2016). The total amount of wastewater emitted by HCMC was estimated to more than 2 million m^3 / day in 2015 (Tran Ngoc et al., 2016). Three centralized sewage treatment plants (STPs) receive only 13 % of the total daily domestic wastewater flow generated from HCMC: the Binh Hung Hoa STP (30,000 $m^3 d^{-1}$; 136,000 Population Equivalent or PE), Bing Hung WWTP (141,000 $m^3 d^{-1}$; 641,000 PE) and Tham Luong-Ben Cat WWTP (131,000 $m^3 d^{-1}$; 595 000 PE) (Ministry of Construction, 2021). The major part of the megacity wastewater is therefore discharged directly to the Vam Thuat River (4 $m^3 s^{-1}$) and the Nhieu-

Loc (NL), Ben Nghe (BN) and Kenh Te (KT) canals (5.5 m^3 s^{-1}), which downstream connect to the Saigon River (Nguyen et al., 2022, 2019). Downstream of the megacity, the estuary is covered by the mangrove preserved area and aquaculture farms (East bank), and paddy fields and harbours (West bank).



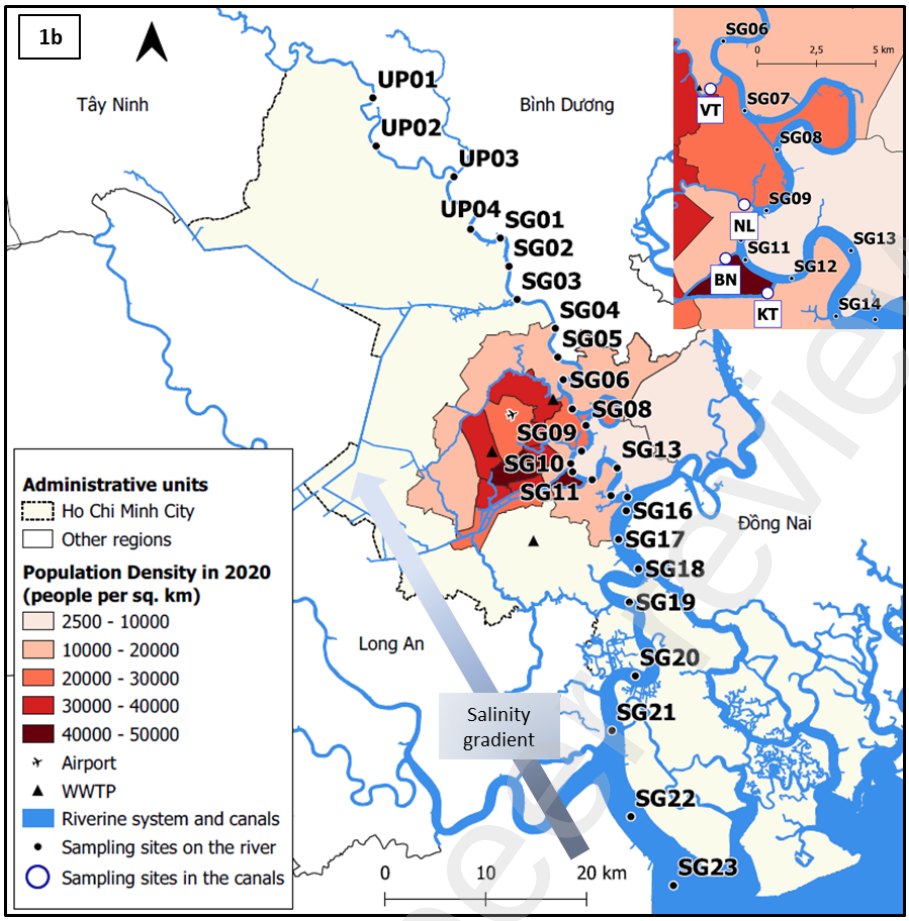


Figure 1:

1a. Land use distribution of HCM province (adapted from Nguyen et al., 2022 and Phan et al., 2021).

1b. Sampling points and population density of HCM province (adapted from Nguyen et al., 2020)

2.2 Sampling collection

The sampling campaign was organised in March 2020 during the dry season. Grab samples of 500 mL were collected along the river at 26 locations forming a 140-km longitudinal profile (Fig. 1b). Additional samples were collected at the outlets of the four main canals of the city center, namely from North to South: VT, NL, BN and KT (Figure 1b, upper right corner). The outlet of BN canal was closed due to the construction of a dam, preventing the interactions between this canal and the Saigon River (Fig. A.1a and A.1b in the supplementary material). All the samples were collected at low tide and at each sampling site, two samples were collected, one for chemical analysis and one for bioassays.

Samples UP1 to SG10 were collected on March 11th, and samples SG11 – SG23 on March 12th. The canal samples were collected on March 10th. Field blanks were also added, as well as duplicates for quality control. More details can be found in the supplementary material on the date and location of the samples (Appendix A.1). After collection, the samples were kept in cool box and brought back to the CARE lab and stored at -20°C. The samples were then transferred to France in isothermal (-20°C) boxes in less than 48 h and arrived frozen.

2.3 Target micropollutants

High purity solvents (LC-MS grade) methanol and acetonitrile were purchased from Biosolve (France). Acetic and formic acid (LC-MS grade) were purchased from LiChropur (Germany) and ammonium acetate (ULC-MS grade), from Biosolve (France). Ultra-pure Milli-Q water was obtained from Veolia ELGA Purelab chorus. Native and internal standards were purchased from Sigma-Aldrich (USA), Fisher Scientific (Germany), AccuStandard (USA), Cayman Chemicals (USA), DR. Ehrenstorfer (Germany) and ITW Reagents (USA). Whatman Filters (GF/F) were purchased from Sigma-Aldrich (China) and OASIS HLB cartridges (6 cc, 200 mg, 30 µm) from Waters (Ireland).

A total of 217 micropollutants were targeted (Fig 2a). This study focused on emerging contaminants (Richardson and Kimura, 2020). This included various pollution source indicators, among which wastewater tracers, pesticides, industrial chemicals. Chemicals known to be sensitive to photodegradation (diclofenac, hydrochlorothiazide, propranolol) and biodegradation (acetaminophen, salycilic acid) where also targeted and used to investigate the capacity of the system to attenuate such micropollutants. Some pesticides known to be frequently used in the region of HCMC were also selected (based on available literature). Analytical working solutions were prepared in methanol at concentrations of 500 ppb for internal standards and 1000 ppb for pesticides, pharmaceuticals and PFASs mixtures. All standards were preserved in adequate conditions and working solutions were stored at -20°C.

The chemicals used as reference compounds for the *in vitro* bioassays (Table 1) were purchased from Sigma Aldrich (Germany) and Dr. Ehrenstorfer (TCDD; Germany).

2.4 Sample preparation

Prior to sample preparation, all samples were filtered through Whatman glass microfiber filters (GF/F).

For chemical analysis, all samples including procedural blanks, field blanks and duplicate samples were then spiked with 25 µL of a mixture of 61 internal standards (500 ppb). The samples were enriched using solid phase extraction on OASIS HLB cartridges (6cc, 200 mg, 30 µm). The cartridges were conditioned with 6 mL MeOH followed by 6 mL of MilliQ water, and then, the samples were loaded and passed through the cartridges. 6 mL of MilliQ water were used as a wash solution to remove any ions followed by vacuum drying of the cartridges. The analytes were eluted using 6 mL of methanol. The eluates were concentrated down to 125 µL at 30°C under a gentle stream of nitrogen and then reconstituted by adding 375 µL of pure water for chemical analysis.

For bioassays, the same protocol was applied except the spiking of internal standards. The dried loaded cartridges were sent by express shipment at low temperature to Germany. The samples were reconstituted with 1 mL of methanol and aliquots of each sample extract were then added to a dosing vial. The solvent was blown down to dryness and the sample was resolubilized with cell assay media. Therefore, the bioassays contained no residual solvent. A solvent control using the same amounts of solvent was also run to ensure that there were no interferences from residuals in the solvents. The samples were transferred from the dosing vial into a 96-well plate and serially diluted in test media to obtain a concentration-effect curve. The calculations of the relative enrichment factors of the samples can be found in the supplementary material (section A.2).

2.5 Chemical analysis and quality control

The samples extracts were analysed using high-performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS), using the Thermo-Fischer TSQ Quantiva system and the Exion-LC and Sciex 5500 QTRAP MS/MS system. Instrumental blanks, procedural blanks and field blanks were added to each batch of samples to check for potential contamination along the sampling and analytical process. Calibration curves covered 8 levels of concentration: 0, 0.25, 0.5, 1, 5, 10, 25, 50 and 100 ppb. The assignment of the 61 internal standards was done based on matrix effects and absolute recoveries for each chemical (Tables B.2 and B.3). The main parameters of the analytical methods can be found in section A.3 of the supplementary material. The data obtained was processed using TraceFinder 3.3 (Thermo Fischer) and Sciex OS (Sciex Now) softwares. The concentrations of all detected chemicals can be found in Table B.1.

2.6 *In-vitro* bioassays

Six *in-vitro* bioassays covering previously identified priority endpoints for surface water (Escher et al., 2014; Escher and Neale, 2021) were applied (Table 1). Three bioassays targeted hormone-mediated modes of action for endocrine disruption: estrogenicity (ERα GeneBLAzer), progestogenic activity (PR GeneBLAzer) and glucocorticogenic activity (GR GeneBLAzer). The other bioassays looked at wider explained effects: xenobiotic metabolism pathway (AhR CALUX for activation of the aryl hydrocarbon receptor and PPARγ GeneBLAzer binding to the peroxisome proliferator-activated receptor gamma) and oxidative stress response (AREc32 gene reporter assay). Cell viability was quantified in parallel in all assays by quantifying the reduction of cell confluency during exposure using an IncuCyte S3 live cell imaging system (Essen BioScience, Ann Arbor, Michigan, USA) (Escher et al., 2019). Detailed methods for these bioassays are available in the references of Table 1. More details on the different bioassays and their QA/QC can be found in section A.5 of the supplementary data.

The concentration causing 10% effect (EC_{10}) and 10% inhibition (IC_{10}) were determined from the linear concentration-effect curves plotted up to 30% of effects. In the case of AREc32, the

concentration causing an induction ratio IR of 1.5 ($EC_{IR1.5}$) was derived. The data which caused more than 10% cytotoxicity was not included in the data evaluation. The EC values were reported as relative enrichment factor (REF) in units of $L_{water}/L_{bioassay}$, considering the sample enrichment via SPE and the dilution of the assay.

To compare with the Effect-Based Trigger (EBT) values, the effect measured were expressed as 17β-estradiol equivalent concentrations (EEQ) for ERα GeneBLAzer, benzo[a]pyrene equivalent concentrations (B[a]P-EQ) for AhR CALUX, rosiglitazone-EQ for PPARγ GeneBLAzer and dichlorvos-EQ for AREc32.

Table 1: Bioassays applied in the current study and literature reference for detailed method description. Effect concentration (EC) of the reference compounds (mean of three experiments, details in the SI, Table B.4) in comparison to literature and effect-based trigger values expressed as bioanalytical equivalent concentration (EBT-BEQ)

Endpoint	Bioassay	Experimental method	QA/QC reference compound	Effect descriptor	EC reference compound (this study)	EC reference compound (literature)	Bioanalytical equivalent (BEQ)	EC_{10} reference compound for BEQ (literature)	EBT-BEQ	Literature EBT
Activation of the estrogen receptor (ER)	ERα GeneBLAzer	König et al., 2017	17β-Estradiol	EC_{10}	$(8.91 \pm 0.03) \times 10^{-12}$ M	$9.87\ 10^{-12}$ M (Nivala et al., 2018)	17β-Estradiol-EQ (EEQ)	See left	EBT-EEQ 0.34 ng_{E2}/L	(Beate I Escher et al., 2018)
Activation of the progesterone receptor (PR)	PR GeneBLAzer	König et al., 2017	Promegestone	EC_{10}	$(8.86 \pm 0.14) \times 10^{-12}$ M	$1.22\ 10^{-11}$ M (Leusch et al., 2018)	n/a		n/a	

Activation of the glucocorticoid receptor (GR)	GR GeneBLAzer	König et al., 2017	Dexamethasone	EC_{10}	$(3.5 \pm 0.04) \times 10^{-11}$ M	$2.08\ 10^{-10}$ M (Nivala et al., 2018)	Dexamethasone-EQ		n/a	
Activation of the arylhydrocarbon receptor (AhR)	AhR CALUX	Brennan et al., 2015	2,3,7,8-Tetrachlordibenzodioxin (TCDD)	EC_{10}	$(1.42 \pm 0.01) \times 10^{-14}$ M	$3.3\ 10^{-10}$ M (Nivala et al., 2018)	Benzo[a]pyrene-EQ (B[a]P-EQ)		EBT-B[a]P-EQ 250 $ng_{B[a]P/L}$	(Escher and Neale, 2021)
Activation of the peroxisome proliferator activated receptor (PPARγ)	PPAR γ GeneBLAzer	König et al., 2017	Rosiglitazone	EC_{10}	$(7.38 \pm 0.05) \times 10^{-12}$ M	$9.87\ 10^{-12}$ M (Jia et al., 2015)	Rosiglitazone-EQ		EBT-Rosiglitazone-EQ 1.2 $\mu g_{Rosiglitazone}/L$	(Escher and Neale, 2021)
Activation of Nrf2-ARE pathway	AREc32 gene reporter assay (based on MCF7)	Escher et al., 2012; Wang et al., 2006	t-Butyl hydro quinone (tBHQ)	$EC_{IR1.5}$	$(9.50 \pm 0.17) \times 10^{-6}$ M	$1.32\ 10^{-6}$ M (Escher et al., 2012)	Dichlorvos-EQ		EBT-Dichlorvos-EQ 1.4 $mg_{Dichlorvos}/L$	(Escher and Neale, 2021)

2.7 Data evaluation and iceberg modelling

Iceberg modelling was performed using the bioanalytical equivalent concentration (BEQ) approach for AhR CALUX, PPARγ GeneBLAzer and AREc32 assays. This approach assumes that most chemicals act in a concentration-additive manner when they are found as a mixture, which has been justified in numerous designed mixture studies at concentration ratios how chemicals typically occur in the aquatic environment (Escher et al., 2020; Neale et al., 2020a). Bioanalytical equivalent concentrations from bioanalysis (BEQ_{bio}) were expressed in effect concentration of the sample (EC_y(sample)) divided by the effect induced by the assay reference compound (EC_y(ref)), where y refers to the effect level, typically 10% of maximum effect or induction ratio (IR)1.5 (Equation 1).

$$BEQ_{bio} = \frac{EC_y(ref)}{EC_y(sample)} \quad (1)$$

On the other hand, the predicted bioanalytical equivalent concentrations of the mixture of detected chemicals (BEQ_{chem}) were obtained from the sum of the BEQ_i of each quantified chemical (Equation 2). To calculate the BEQ_i, the concentration (C_i) in molar units was multiplied by the relative effect potency (REP_i) of the chemical (i) (Equation 3). The REP is used to quantify the relative effects of the individual chemicals compared with the reference and are independent of effect levels. The EC_y values of the detected chemicals were collected from own previous measurements and the US EPA Tox21 database (Escher et al., 2020a).

$$BEQ_{chem} = \sum_{i=1}^{n} BEQ_i = \sum_{i=1}^{n} REP_i \cdot C_i \quad (2)$$

$$REP_i = \frac{EC_y(ref)}{EC_y(i)} \quad (3)$$

The mixture effect caused by unknown chemicals can be calculated from the effect balance Equation 4.

$$BEQ_{unknown} = BEQ_{bio} - BEQ_{chem} \quad (4)$$

The contribution of the detected chemicals to the mixture effects can be estimated by Equation 5.

$$\text{Effect explained by detected chemicals} = \frac{BEQ_{chem}}{BEQ_{bio}} \cdot 100\% \quad (5)$$

3. Results and discussion

3.1 Occurrence of micropollutants

Out of 217 micropollutants analysed, 120 were detected at least once and 111 were quantifiable (Table B.1). Along the Saigon River, the number of quantified chemicals increased from 43 micropollutants at the first sampling point (UP1) to more than 90 in the city center (SG6 to SG9) (Fig. 2b). Then, a slow decrease was observed from SG11 and 58 chemicals were still quantifiable at the river mouth (SG23). In the four city canals, 83 to 95 chemicals were quantified. There were 30 chemicals found at all sampling points and 59 micropollutants had detection frequencies equal to or higher than 80% (Fig A.2a).

Upstream of the megacity, the first two sampling points (UP1 and UP2) carried a lower burden of concentration adding up to 260 ng L^{-1} and 390 ng L^{-1} (Fig. 2c). Total concentration increased gradually from 1,100 ng L^{-1} (UP3) to reach a maximum of 2,900 ng L^{-1} in the urban center (SG6 – SG8) and then, decreased sharply to 680 ng L^{-1} in SG11. The total concentration decreased then over the last 60 km from 680 ng L^{-1} (SG11) to 250 ng L^{-1} in the river mouth (SG23). The total concentrations obtained in the city canals were significantly higher than those measured along the river: 5,000 ng L^{-1} (VT canal), 4,400 ng L^{-1} (NL canal), 78,000 ng L^{-1} (BN canal) and 9,900 ng L^{-1} (KT canal).

The highest concentrations were reported for caffeine (40,000 ng L^{-1}), its metabolite paraxanthine (14,000 ng L^{-1}), laureth-5 (4,400 ng L^{-1}) and daidzein (2,300 ng L^{-1}), all measured in the BN canal. The highest concentrations measured in the river were obtained for paraxanthine (780 ng L^{-1} in SG8), isoprothiolane (300 ng L^{-1} in SG5), daidzein (280 ng L^{-1} in SG7), iopromide (250 ng L^{-1} in SG8) and carbendazim (230 ng L^{-1} in UP3).

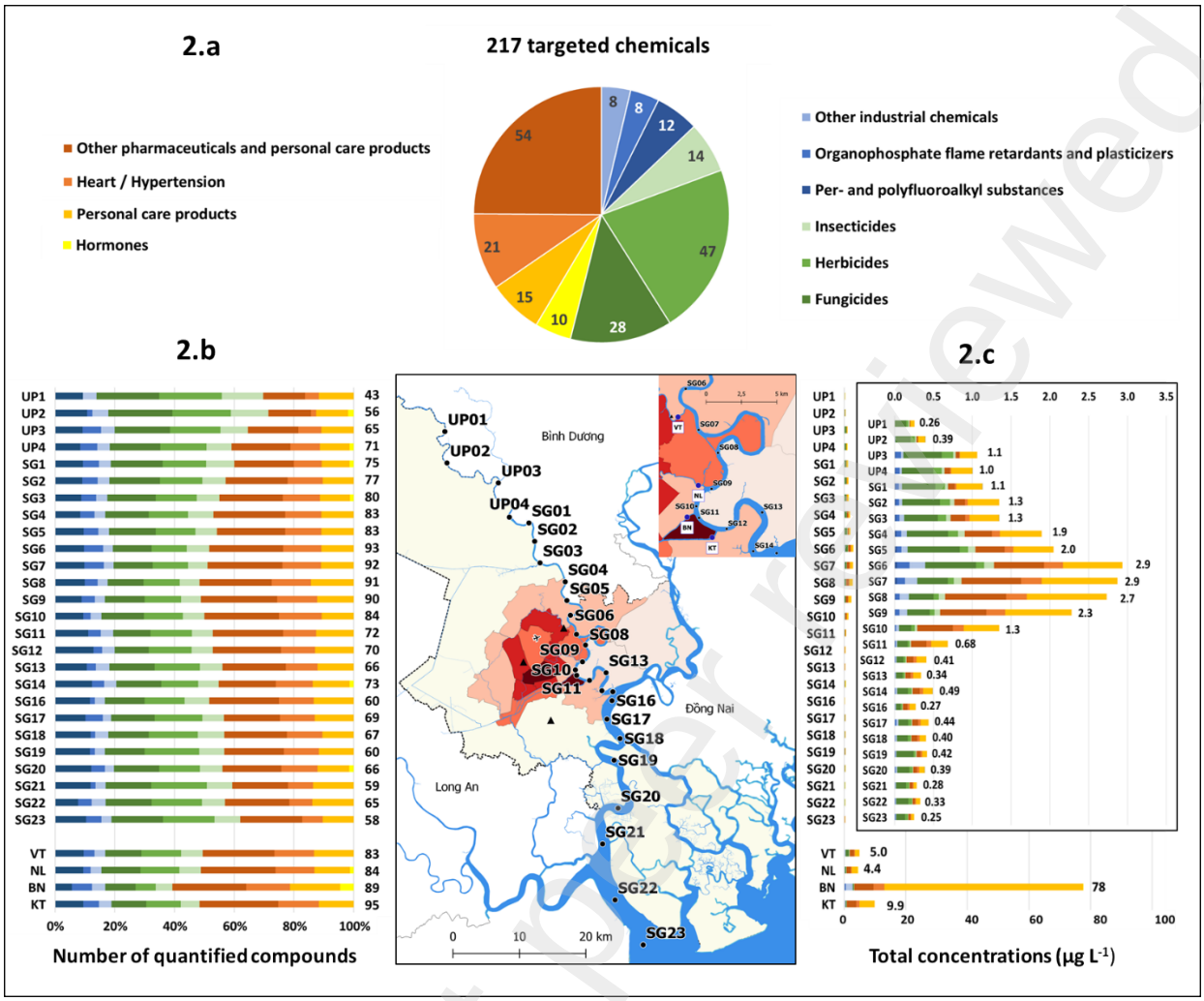


Figure 2:

a Classification of the 217 targeted chemicals,

b Number of quantified chemicals per sampling site (out of 111) with respective contribution (%) of each chemical class,

c Total concentrations (µg L^{-1}) with separate scale for river samples (scale 0 - 3.5 µg L^{-1}) and the 4 city canals VT, NL, BN and KT (scale 0 – 100 µg L^{-1}).

3.1.1 PPCPs and hormones

Among the 100 targeted PPCPs, 6 hormones and 54 PPCPs belonging to different therapeutic groups (painkillers, antihistaminic, antihypertensive drugs, antidiabetics, UV filters, food additives)

were detected at least once at all sampling sites. Four metabolites were also found including paraxanthine and theophylline, *O*-desmethylvenlafaxine and 10,11-dihydro-10-hydroxycarbamazepine, respectively, corresponding to caffeine, venlafaxine and carbamazepine use. The number of quantifiable PPCPs and metabolites doubled from 23 upstream (UP3) to 47 in the city center (SG8) and later decreased to 22 in the river mouth (SG23). Similarly, the total concentration of all the quantified PPCPs increased from the upstream region (UP3, 280 ng L^{-1}) to reach a maximum in the city center (SG6 to SG9: 1,700 – 2,100 ng L^{-1}). Then, the concentrations decreased sharply to SG11 due to nearby confluence with the Dongnai River, and to a value of 75 ng L^{-1} in the final sampling point (SG23) with seawater mixing. The city canals had higher total concentrations adding up to 3,200 ng L^{-1} (VT canal), 3,600 ng L^{-1} (NL canal), 74,000 ng L^{-1} (BN canal) and 9,000 ng L^{-1} (KT canal), highlighting that they are a direct source of PPCPs to the Saigon River. PPCPs were the dominant group of chemicals in the city center and accounted for 52 up to 76% of the total concentrations (SG6 – SG14) (Fig. 2c). Their contribution was even higher in the canals with 63% to 96% of the total concentrations.

Ten PPCPs were ubiquitous (listed from highest to lowest concentration range): paraxanthine, caffeine, fexofenadine, cetirizine, fluconazole, nicotine, irbesartan, lidocaine, metformin and thiabendazole. Iopromide, DEET, valsartan, telmisartan, mefenamic acid, losartan, sulisobenzone, carbamazepine, bisoprolol, atenolol, mebendazole, codeine, niflumic acid and levamisole also predominated with detection frequency ≥ 80%. The main family of drugs identified were the antihypertensive drugs, which accounted for a quarter of the PPCPs quantified (15 chemicals). Caffeine and its metabolite paraxanthine had the highest concentrations at many sampling locations and contributed largely (27% to 78%) to the total concentrations of PPCPs measured along the Saigon River. Daidzein and iopromide were other major contributors to the total concentrations, especially in the city center (SG5 – SG11), followed by cetirizine and fexofenadine, two widely used and detected antihistamines (Kosonen and Kronberg, 2009; Kristofco and Brooks, 2017). The four hypertensive drugs irbesartan, losartan, telmisartan and valsartan also exhibited high detection rate and relatively high concentration in comparison to other PPCPs. Their combined total concentrations exceeded 200 ng L^{-1} in the city center (SG6 – SG9) and the canals, with a maximum of 3,000 ng L^{-1} measured at the mouth

of BN canal. The use of sartans is expanding quickly worldwide and they are gaining market in Asia (Bayer et al., 2014; Ladhari et al., 2021). Only losartan had been previously reported in the canals of HCMC with similar levels as this study (16 – 230 ng L^{-1}; Chau et al., 2018) but was <LOQ in the river.

3.1.2 Industrial chemicals

Among the 28 industrial chemicals targeted, 9 PFASs, 4 plasticizers, 2 flame retardants and 4 other diverse industrial chemicals were quantified along the river-estuary continuum. 13 chemicals were found in UP3, 16 in SG8 and 11 in SG23 (Fig. 2b). Only the first sampling point UP1 carried a smaller number of chemicals (n=6). The total concentrations directly increased from the sampling point UP3 (110 ng L^{-1}) and remained quite stable until SG9 (160 ng L^{-1}) with the exception of the sites SG6 (390 ng L^{-1}) and SG7 (290 ng L^{-1}) that carried a higher burden of chemicals. The total concentrations decreased at SG10 (56 ng L^{-1}) and remained low in the downstream part of the river (21 ng L^{-1} in SG23). The concentrations measured in the canals were 410 ng L^{-1} (VT canal), 260 ng L^{-1} (NL canal), 2,800 ng L^{-1} (BN canal) and 450 ng L^{-1} (KT canal). Dibutyl phosphate, a catalyst and antifoaming agent, triisopropanolamine, a chemical intermediate and surfactant, and the perfluorocarboxylic acids PFHpA and PFOA were found at all the sampling points. Bisphenol A and S, PFBS, PFHxS, PFHxA and 6:2 FTSA had detection frequency equal to or higher than 80%.

The total concentrations measured for perfluorocarboxylic and sulfonic acids (ΣPFCAs and ΣPFSAs) were relatively low (0.1– 2.5 ng L^{-1}). The fluorotelomer 6:2 FTSA was the dominant PFASs with the highest concentrations, up to 21 ng/L in the North of the city center (SG7). These results are coherent with the range of values reported previously for individual PFASs by Duong et al. (2015) and Lam et al. (2017) (0.10 - 18 ng L^{-1} and <0.03 – 6.4 ng L^{-1}, respectively). The main plasticizers quantified were bisphenol A and bisphenol S found in most of the samples. Bisphenol F had lower detection frequency and was found only near the city center (SG6 and SG7; three city canals). Bisphenol A was previously reported in the city canals with up to 140 ng L^{-1} (Le et al., 2016) and ranging 19 - 93 ng L^{-1} in the rivers (Chau et al., 2018) during the dry season of 2013. Its analogues were not reported yet in water at the study site. The other compounds quantified (triisopropanolamine, DEHPA, dibutyl

phosphate) and the organophosphate flame retardants (OPFRs) were reported for the first time in surface water at the study site.

3.1.3 Pesticides

Among 89 pesticides targeted, 14 fungicides, 10 insecticides, 11 herbicides and 2 transformation products (desethyl and deisopropyl atrazine) were detected at least once. In contrast to PPCPs, the number and type of pesticides quantified was consistent along the river: 29 were found upstream (UP3), 28 in the city center (SG8) and 25 at the estuary mouth (SG23). The samples collected upstream carried similar range of total concentrations as the samples taken in the city center (Fig. 2c). The total concentrations increased from the two upper sampling points (UP1 and UP2, 190 ng L^{-1} and 280 ng L^{-1}) to reach a range of 550 to 890 ng L^{-1} between UP3 and SG6 above the megacity. The concentrations started to decrease from SG7 (570 ng L^{-1}) to reach a minimum of 90 ng L^{-1} in SG16 after the confluence with the Dongnai River. Interestingly, despite the dilution occurring with the sea and the tidal effect, the pesticide concentration increased slightly in the downstream part of the river (SG17 to SG23) to a mean concentration of 200 ng L^{-1}, highlighting an input of pesticides in this area. The canals did not carry a significantly higher burden comparing to the river (410 – 490 ng L^{-1}) except for the VT canal (1,390 ng L^{-1}). Isoprothiolane (500 ng L^{-1}), hexaconazole (260 ng L^{-1}) and carbendazim (130 ng L^{-1}) had the highest concentrations at the mouth of this canal. Pesticides were the dominant group of chemicals in the upstream part (40 – 74% of the total concentrations, UP1 – SG5) and the downstream part of the river (41 – 64%, SG17 – SG23).

The majority of the pesticides (n = 24) had detection frequencies ≥ 80% and were detected all along the profile. 16 pesticides were ubiquitous including carbendazim, isoprothiolane, fenobucarb, tricyclazole, hexaconazole, azoxystrobin, propiconazole, metalaxyl, atrazine, terbutryn, bentazon, fipronil, acetamiprid, thiamethoxam, desethyl atrazine and clothianidin. The remaining pesticides identified were found at a limited number of sampling points. Most of the pesticides quantified are used for paddy fields and other crops, reflecting the presence of important agricultural lands upstream and downstream of the urban center (Fig 1a).

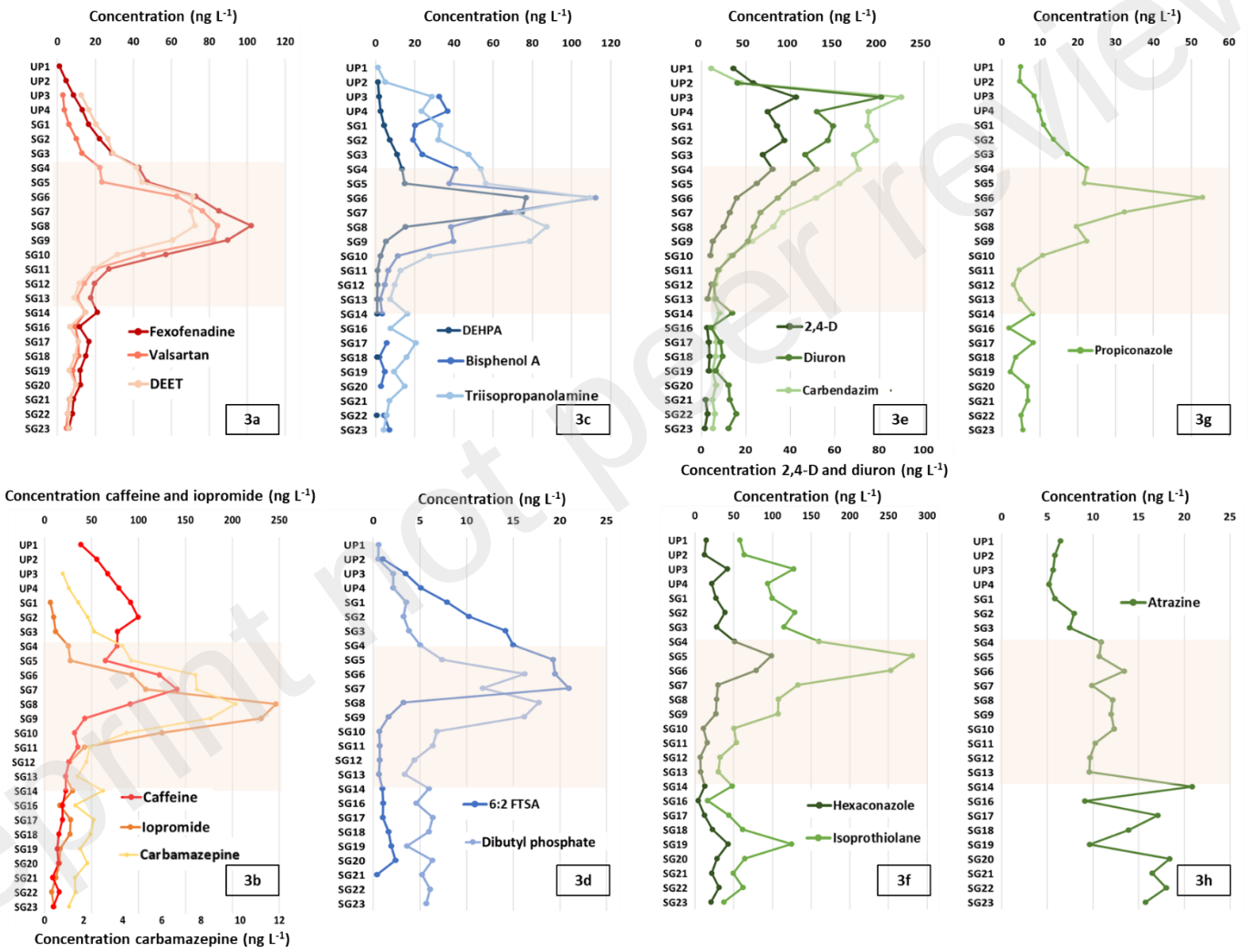
Fungicides accounted for more than 60% of the total concentrations at each sampling point with carbendazim, isoprothiolane, hexaconazole and tricyclazole showing the highest concentrations. Carbendazim is a toxic pesticide banned in the USA with low potential for degradation and long half-life in water (Cuppen et al., 2000). In Vietnam, it appears to be used frequently on paddy fields and also vegetables and fruits (Berglöf et al., 2002; Houbraken et al., 2016; Nguyen et al., 2018). It was detected with high frequency in fruits and vegetables imported from Vietnam and was also reported in surface and drinking water from Hanoi and other northern provinces (Skretteberg et al., 2015; Wan et al., 2021). Isoprothiolane, hexaconazole and tricyclazole are widely used in paddy fields (Berg and Tam, 2018; Sattler et al., 2018) and were detected with high detection frequency and concentrations in surface water and drinking water from the Mekong Delta (Chau et al., 2015; Toan et al., 2013; Trinh et al., 2017).

The carbamate insecticide fenobucarb represented 49% to 89% of the total concentrations of the insecticides detected. It was already monitored in several studies as a dominant pesticide due to its wide use in paddy fields in Vietnam (Duong et al., 2014; Toan et al., 2013; Trinh et al., 2017).

The dominant herbicides identified were 2,4-D, diuron, and atrazine with maximum concentrations of 42, 21 and 81 ng L^{-1} respectively. Atrazine and diuron were found at similar concentration ranges (18 – 23 ng L^{-1} and 27 – 50 ng L^{-1}) in samples from the Saigon and Dongnai Rivers (Chau et al., 2018). 2,4-D is a banned herbicide that entered in the composition of the Agent Orange and was also widely used by farmers to remove weeds in paddy fields (Berg and Tam, 2018; Nguyen et al., 2018).

Figure 3: Concentration profiles of selected micropollutants expressed in ng L^{-1} along the river-estuary *continuum*

(the orange shade indicates the urban section of the estuary)



3.2. Spatial dynamics of micropollutants

The majority of quantified micropollutants, especially PPCPs and industrial chemicals displayed higher concentration values in the sampling points located in the urban center (especially SG4-SG11) as illustrated in Figure 3. Several micropollutants such as bisphenol A, carbamazepine, caffeine, codeine, DEET and iopromide, known as indicator compounds for the presence of treated and untreated wastewater (Carpenter and Helbling, 2018; Dickenson et al., 2011; Nakada et al., 2008; Nguyen et al., 2018), were particularly elevated at these sites. Releases of untreated wastewater also occurred upstream of the megacity as caffeine concentration started to increase from UP1 (Fig. 3b). However, the concentrations of these sewage indicator compounds were the highest in the city canals, especially in the southern canals BN and KT which drain the water from the densest residential areas. In these canals, some compounds were found with comparable concentration levels as those measured previously in the influents of the WWTPs of HCMC (Nguyen et al., 2018), indicating that the canals received important micropollutant loads of untreated wastewater discharge. The concentrations of caffeine (40,000 ng L^{-1}) measured in BN canal were in the same range (20,000 and 38,000 ng L^{-1}) as the influents of Bing Hung Hoa and Binh Hung WWTPs while the concentrations of paraxanthine (14,000 ng L^{-1}), DEET (1,500 ng L^{-1}) and carbamazepine (88 ng L^{-1}) were even higher (4,800 and 5,700 ng L^{-1}; 800 and 720 ng L^{-1} and 40 ng L^{-1} respectively). Hormones were also detected in BN canal with similar levels as raw wastewater (Ying et al., 2002). The percentage of wastewater present in the canals and in the Saigon River can be roughly assessed by comparing the concentrations of persistent carbamazepine (Clara et al., 2004) found in our samples to those measured in the influents of the two WWTPs mentioned above (Daneshvar et al., 2012; Dickenson et al., 2011; Kiguchi et al., 2016). The resulting ratio, converted into percentage, was around 200% in BN canal, 30 – 50% in the other canals and 10 – 30% in the samples located in the city center (SG4 – SG10). The percentage measured for BN canal is coherent with the observations collected on this sampling site (Fig A.1a and 1b), suggesting less mixing with the Saigon River compared to the other canals and therefore an accumulation of micropollutants. These different results illustrated that the urban center of HCMC and its four canals were a major source of

micropollutants to the river system *via* significant discharges of treated and untreated wastewaters in comparison to the upper and lower parts of the province. The results reflect the higher population density and the higher number of medical facilities and industrial clusters found inside the urban area.

The concentrations of pesticides measured upstream (UP3 – SG4) reflected the dominance of croplands, woody crops and paddy fields upstream the megacity (Fig. 1a). Some of the pesticides (2,4-D, azoxystrobin, carbendazim, dimethoate, diuron, and metolachlor) had maximum concentrations in upstream and remained low in the rest of the profile (Fig. 3e). Pesticide concentration in VT canal (1,390 ng L^{-1}) reflected its proximity to agricultural zones located at the outskirts of the northern urban area in comparison to other canals draining less pesticides (410 – 490 ng L^{-1}). The VT canal may centralise important inputs of pesticides and contribute significantly to the contamination of the Saigon River for these compounds. Some pesticides also had clear increase in concentrations along the estuary (atrazine, diuron, hexaconazole and isoprothiolane) despite the mixing with seawaters. This reveals the presence of important inputs of pesticides downstream consistent with a greater share of agricultural land (41%), among which paddy fields (20%), and aquaculture (13%) are dominant (Fig. 1a). Atrazine had a distinct distribution along the profile with concentrations increasing from upstream to downstream and was the only pesticide to have its maximum of concentrations in the estuary (Fig. 3h). Atrazine and other herbicides like diuron have been used in the shrimp farms near the Can Gio mangrove to remove weeds and disinfect the ponds (Nguyen et al., 2016). In the case of diuron, the increase observed in its curve could also be linked to the presence of harbours in the downstream region as it is used as an antifouling agent (Martínez et al., 2000; Sapozhnikova et al., 2007). The increase in concentration measured near the river mouth for the fungicides isoprothiolane, hexaconazole and tricyclazole reflects the high percentage of rice paddies found downstream and highlight that those chemicals could be potential tracers of the emissions of pesticides from paddy fields (Fig 3f).

A general attenuation of the chemical burden was first observed from SG11 which most likely is due to the dilution effect occurring nearby the confluence with the Dongnai River. The chemical concentrations kept decreasing for most of the chemicals down to the estuary mouth due to mixing process with seawater. Attenuation could be well observed with persistent chemicals such as

carbamazepine or iopromide (Fig. 3b). Other processes than dilution can also contribute to the natural attenuation of micropollutants such as photodegradation, biodegradation and/or sorption to particulate matter. Indeed, several highly used pharmaceuticals were quantified occasionally and at low concentrations close to their potential sources (high density residential areas) like acetaminophen (detected in five samples with a mean of 2 ng L^{-1}) which was below LOQ at almost all sampling points. Other identified chemicals were: ranitidine (one detection of 300 ng L^{-1} in KT canal), diclofenac (mean concentration of 14 ng L^{-1}), salicylic acid (28 ng L^{-1}), propranolol (0.8 ng L^{-1}) and HCTZ (18 ng L^{-1}) which were quantified mostly in city canals and in the city center (SG4 to SG10). Their low detection frequencies and concentrations could potentially be linked to their high sensitivity to photodegradation and biodegradation (Baena-Nogueras et al., 2017; Latch et al., 2003; Yu et al., 2006).

3.3 Biological effects

Most of the samples showed low effect with cytotoxicity. The IC_{10} values did not differ much between the different cell lines and ranged from REF 10 to 30 (**Error! Reference source not found.**4 and Fig A.3). Cytotoxicity increased from UP1 (mean IC_{10}= 17) to UP3 (mean IC_{10}= 11) and then plateaued until SG8 (mean IC_{10}= 13) before it lowered again in SG10 (mean IC_{10}= 22) with relatively stable values towards the estuary (mean IC_{10}= 33 in SG23). This distribution reflects the decrease observed in the concentrations of the quantified chemicals from SG11 and suggests that the attenuation observed downstream for most of the micropollutants contributes to reduce the cytotoxic burden of the samples. The city canals had similar IC_{10} values as the first half of the profile except for the BN canal, which was highly cytotoxic with a mean IC_{10} of 1. This IC_{10} value is comparable with those of highly contaminated samples taken downstream of WWTPs and indicates the presence of a high chemical burden (König et al., 2017; Müller et al., 2018; Neale et al., 2020b).

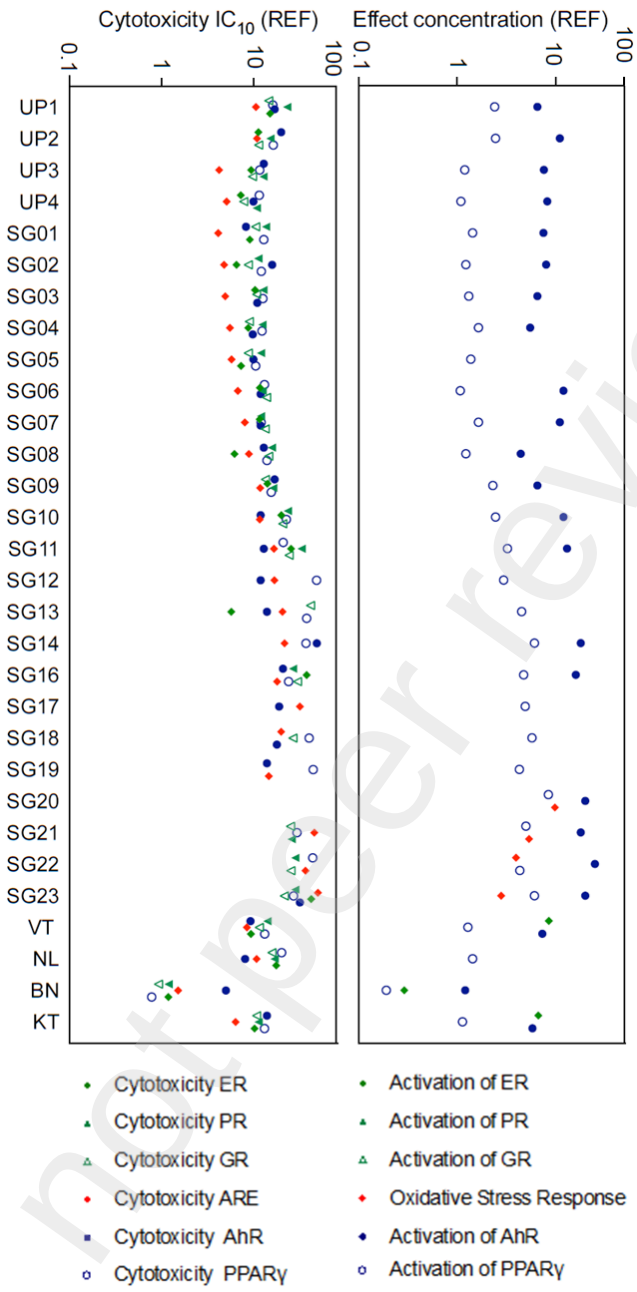


Figure 4: Course of cytotoxicity IC_{10} (bottom) and effect concentrations (top) for all samples along the river. Note that the y-axis was plotted inverse as a high EC and IC10 refers to a low toxicity

PR, GR and ERα were activated by many samples, but the effects were observed close to cytotoxic concentrations. Therefore, most of the activation measured were invalid (see example for SG8 in Fig. A.4). Indeed, cytotoxicity can lead to the so-called cytotoxicity burst phenomenon (Escher et al., 2020b; Fay et al., 2018; Judson et al., 2016); the activation at cytotoxic concentrations might be an

artifact and have to be omitted from the evaluation. The activation of PR and GR was masked by cytotoxicity in all cases and no EC_{10} values could be derived for activation. The river samples showed no estrogenic activity with the ERα-GeneBLAzer, but the estrogenic effect could only be quantified in three city canals (VT, KT, BN). The estradiol equivalent concentration EEQ of these three samples was 0.28 ng_{E2} L^{-1} for VT canal, 0.37 ng_{E2} L^{-1} for KT canal and 8.40 ng_{E2} L^{-1} for BN canal (Table B.4). The results of the KT and BN canals exceeded the effect-based trigger EBT-EEQ of 0.34 ng_{E2} L^{-1} (Escher et al., 2018), meaning that the water quality of these canals was poor and could cause a risk for environmental health. In the case of BN canal, the EEQ measured was four times higher than a EEQ measured for surface water samples collected downstream of a WWTP in Germany (Müller et al., 2018). Estrogenicity is mainly driven by hormones, phytoestrogens and endocrine disruptors chemicals (König et al., 2017; Neale et al., 2015). The predominance of the BN canal is coherent with the accumulation of such chemicals from untreated sewage as discussed in 3.2.

The samples from the upstream region and the city center (UP1 - SG11) were clearly inactive in AREc32 bioassay for oxidative stress response. The samples from the downstream region (SG12 – SG23) were activated, but most of them were masked by cytotoxicity. Only the four sampling points located close to the estuary mouth were active with dichlorvos-EQ of 174 $\mu g_{Dichlorvos}$ L^{-1} for SG20, 317 $\mu g_{Dichlorvos}$ L^{-1} for SG21, 432 $\mu g_{Dichlorvos}$ L^{-1} for SG22 and 610 $\mu g_{Dichlorvos}$ L^{-1} SG23. All four samples were below the EBT-dichlorvos-EQ of 1.4 $mg_{Dichlorvos}$ L^{-1} (Escher and Neale, 2021). They were in the upper range but aligned well with surface water samples from previous studies (Fig. A.5a).

Despite substantial cytotoxicity, most of the samples showed activation of the AhR. Their B[a]P-EQ values ranged from 8.5 to 49 ng L^{-1} and showed no clear spatial trend. These results were below the EBT-B[a]P-EQ of 250 ng L^{-1} (Escher and Neale, 2021) and the effects measured in the samples were clearly lower than wastewater influents and effluents (Fig. A.5b). The BN canal had the highest B[a]P-EQ value with 180 ng L^{-1}. This value was still below the effect-based trigger value but close to outlier values of surface water impacted by wastewater discharge.

Finally, PPARγ was activated by most samples at the lowest enrichments and was the most sensitive assay. The Rosiglitazone-EQ values ranged from 0.11 to 0.25 $\mu g_{Rosiglitazone}$ L^{-1} from UP1 to

SG10 while remaining below 0.1 $\mu g_{Rosiglitazone}$ L^{-1} in the second half of the profile (SG11 – SG23). Only the city canal BN (1.4 $\mu g_{Rosiglitazone}$ L^{-1}) exceeded the EBT_rosiglitazone-EQ of 1.2 $\mu g_{Rosiglitazone}$ L^{-1} and was close to the range of two WWTPs effluents that only received primary treatment and two surface water samples impacted by wastewater (Neale et al., 2020a, 2020b). All other samples were below the EBT and in a similar range as previously detected effects in river samples during rain events (Fig A.5c) (Neale et al., 2020a).

Despite the limitations brought by cytotoxicity, the derived results from bioassays were found to be consistent with the results of the chemical analyses. They confirmed that the first two sampling points of the profile carried a lower burden of chemicals and that the major inputs of chemicals start from UP3 towards the city center. The results for BN canals raised concern about the toxicity of the waters at the canal mouth where untreated sewage get discharged into the river water. There might be consequently associated with hazardous effects on aquatic organisms and human health. Finally, lower effect values were measured near the estuary mouth reflecting the attenuation observed on micropollutant concentrations. It suggests that the toxic burden is reduced near the estuary and the risk for the sensitive mangrove ecosystem might be lower.

Less dynamics were observed for the bioassays in comparison with chemical analyses, probably due to the important number of potential inducers present in the system and the large size of the river system. The effect dynamics observed along the Saigon River were similar to those observed along the Danube, another large river system (König et al., 2017).

3.4 Iceberg modelling

Iceberg modelling was performed to determine the contribution of the quantified chemicals to the effects measured with the bioassays. Out of the 111 quantified chemicals, 76 (68%) was included in the mixture toxicity modelling based on the effect data available (Table B.5). Among them, a total of 32 chemicals (29%) were found to be active in one of the AhR, PPARγ and AREc32 assays. As expected, these active chemicals explained a very small fraction (< 0.5%) of the average measured effects (Fig. 6, Tables B.7, B.9, B.11) which is consistent with previous studies (König et al., 2017; Neale et al., 2020b; Neale et al., 2015). These endpoints are known to be triggered by a wide range of chemicals with different specific modes of action, the majority of which remains unidentified to date (Escher et al., 2018; Escher et al., 2020b; Escher and Neale, 2021). If we extrapolate the relationship between number of detected chemicals and % of effect explained, we would need to measure > 7000 chemicals for explaining 100% effect. In contrast, bioassays with indicative of receptor-mediated effects can typically be explained by a small number of chemicals with a common specific mode of action. For estrogenicity, these are mainly estrone, 17b-estradiol and 17a-ethinylestradiol. Since estrogenic chemicals were not included in the chemical analysis list, these assays were consequently omitted from the modelling.

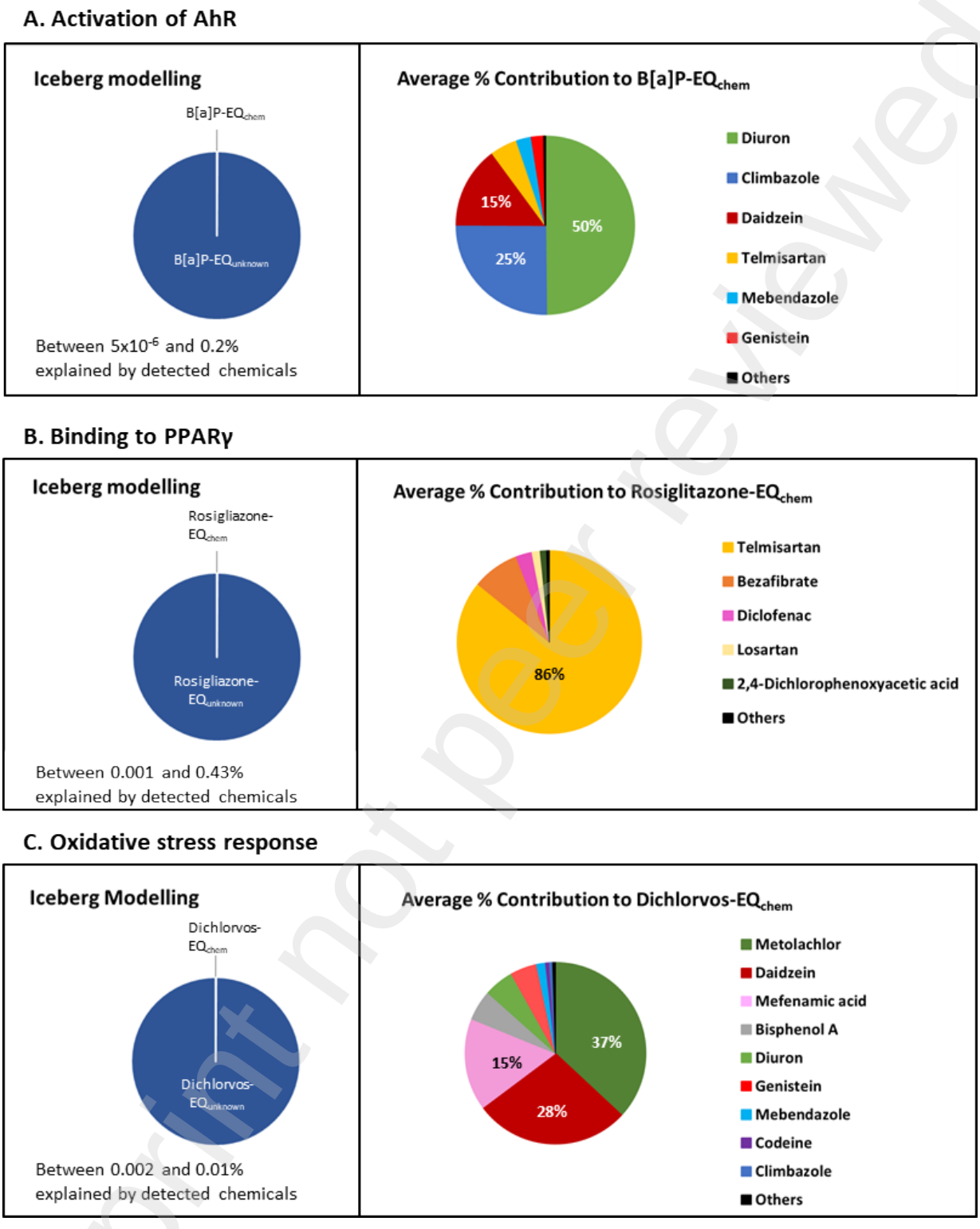


Figure 5: Mean contribution of BEQ_{chem} to BEQ_{bio} (left) and top chemicals contributing on average to BEQ_{chem} (right) for assays indicative of activation of the (5.a) AhR (data in Table B.6), (5.b) binding to PPARγ (data in Table B.8), and (5.c) oxidative stress response (data in Table B.10).

There were 11 quantified chemicals contributing to the effects of AhR CALUX (Table B.6). On average, diuron explained 50% of the B[a]P-EQ_{chem} measured in the water extracts followed by climbazole (25%), daidzein (15%) and telmisartan (5%) (Fig 5a). The herbicide was the most potent chemical with the highest Relative Effect Potency (REP) followed by chlorpyrifos and climbazole (Table S3). The predicted effects calculated for diuron almost accounted for the entire BEQ_{chem} along the river except for the city center (SG6 to SG11) (Fig A.6). The contributions of climbazole, daidzein and telmisartan were higher at these sites, reflecting the inputs from the city's wastewater. In the southern city canals, climbazole dominated over diuron. Diuron had also been identified as a major contributor (to an average of 15%) in the samples collected in agricultural streams (Neale et al., 2020a) and in influents and effluents of WWTPs in Australia (Neale et al., 2020b). The maximum percentage of effect explained by the quantified active chemicals was 0.2% for the KT canal (Table B.7). The AhR CALUX assay aims to identify "non-classical" ligands that may have dioxin-like adverse effects (Denison and Nagy, 2003). Nevertheless, it is mainly activated by hydrophobic organic chemicals (dioxin-like chemicals) which were not analysed here. They are supposed to be mostly removed by the filtration step due to their affinity with particulate matter (Neale et al., 2020a). It is possible that a part of these chemicals still remains in the samples and contribute to the mixture effects, especially polycyclic hydrocarbons (PAHs), which have very high REP (Neale et al., 2020a; Neale et al., 2020b).

Moreover, eight chemicals were active in the PPARγ GeneBlazer assay (Table S3). Telmisartan was dominant and contributed to 86% of the effect measured with the highest REP (Fig 5b, Table S3). Bezafibrate (8%) and diclofenac (3%) were active to a lower extent. Similarly, telmisartan had the higher REP value in Neale et al., 2020b and contributed significantly to the effects measured. These results confirm that sartans are prioritized substances as discussed in session 3.1.1. In Neale et al., 2020a, diclofenac was the main contributor (35%) and among the most potent chemicals. Bezafibrate, 2,4-D and losartan had a small contribution to the total effects similarly to the results showed here. The maximum percentage of effect explained was 0.43% for KT canal (Table B.9).

Twenty of our quantified micropollutants were found active in the AREc32 (Table S3). Many chemicals can induce an oxidative stress response as it is part of defense mechanisms and the response

is no specific to a certain group of chemicals with a distinct mode of action (Escher et al., 2013; Martin et al., 2010). This assay can be seen as a warning indicator of potential adverse effects and suitable for sensitive general toxicity assessment (Escher et al., 2013, 2012). The major contributors were metolachlor which in average explained 37% of dichlorvos-EQ_{chem}, followed by daidzein (28%) and the NSAID drug Mefenamic Acid (16%) (Fig 5c). As observed in the AhR assay, the dominance of Metolachlor to the total BEQ_{chem} for this assay was reduced in the city center (38% to 55% from SG4 to SG11) due to a larger contribution from Daidzein and Mefenamic acid (Fig A.7). Diuron and Bisphenol A also contributed to the BEQ_{chem} mainly in the upstream region (UP3 – SG2). Metolachlor had the highest REP for this assay followed by dichlorvos, genistein and mefenamic acid (Table S3). The herbicide was already reported in other studies as a major contributor to the oxidative stress response (Martin et al., 2010; Neale et al., 2017; Neale et al., 2020a). Daidzein was the main contributor in influent samples of 10 Australian WWTPs (Neale et al., 2020b). As expected, the percentage of effects explained remained low (maximum of 0.2%) (Table B.11). Similar percentages were measured in small streams impacted by WWTPs in Switzerland (1.9%; Neale et al., 2017) for 26 active chemicals and in the Australian WWTPs (0.2%; Neale et al., 2020b) for 46 active chemicals.

Even if not all the quantified chemicals could be included in the modelling, its outcome supported the results obtained with the chemical analysis as they highlighted the presence of inputs from wastewater in the city center. The iceberg modelling revealed that one to three identified compounds dominated in each assay and they were different among assays. This illustrates the importance of combining different bioassays for the identification of the main chemical risk drivers to integrate a wider range of effects potentially generated by the chemicals present in a sample. Among the main active chemicals, the herbicide diuron, the insecticide chlorpyrifos, the antifungals climbazole and mebendazole, the phytoestrogens daidzein and genistein and the antihypertensive telmisartan were active in two of the three assays. The two pesticides are already known hazardous compounds. Diuron is toxic for aquatic life and suspected to be harmful for humans as well (Huovinen et al., 2015) and chlorpyrifos is a possible carcinogen already highly toxic to amphibians and other organisms (John and Shaike, 2015). Therefore, despite the lower toxic burden measured near the mangrove area, there might

be a risk induced by the presence of important inputs of pesticides in this area, especially those identified as risk drivers.

4. Conclusions

The megacity of HCM is one of the most dynamic cities in the world and constitutes a perfect example of the fast-growing cities located in the tropical coastal zone. Tropical estuarine systems are currently coping with increasing inputs of micropollutants often discharged without any preliminary treatment. The integrative bioanalytical approach has been applied in this study to obtain a complete overview of the contamination of the Saigon River and its estuary and revealed a diversity of chemical mixtures along the river *continuum*. These results illustrate the need for adapted wastewater management taking into consideration the specificities of such dense urban areas. As expected, iceberg modelling confirmed the low impact of the quantified micropollutants to mixture toxicity which highlights the need to combine both strategies, chemical analysis for relevant indicators and bioassays to provide a meaningful and comprehensive assessment of surface water quality.

This study contributes to a better understanding of the occurrence and impact of micropollutants in tropical regions as many were quantified for the first time at the study site. Among the contaminants detected, seven - estrone, diclofenac, and the neonicotinoids imidacloprid, thiacloprid, thiamethoxam, clothianidin and acetamiprid - are listed in the first Watch List of the EU (2015/495 and 2015, 2015), while 2 PPCPs - fluconazole, o-desmethylvenlafaxine - are listed on the most recent watch list from 2020 (Decision 2020/1161/EU, 2020). Iceberg modelling enabled the identification of several risk drivers (diuron, chlorpyrifos, metolachlor, climbazole, mebendazole, daidzein, genistein and telmisartan) which could be added to further monitoring plan. Additional data are under process to investigate the influence of tropical seasonality on the occurrence and concentration of micropollutants along this tropical estuary.

Acknowledgements

This work was supported by the International Joint Laboratory LECZ-CARE. The authors would like to thank all the staffs and students at CARE laboratory for their contribution/support during field trip.

We gratefully acknowledge access to the platform CITEPro (Chemicals in the Environment Profiler) funded by the Helmholtz Association for bioassay measurements and financial support of the work at UFZ from the Helmholtz POF IV Topic 9 "Healthy Planet- towards a non-toxic environment". We thank Maria König (UFZ) for experimental assistance.

Sample preparation and part of the chemical analysis have been performed at the AirOSol platform (IGE, Grenoble, France). The Sciex LC-MS/MS 5500 QTRAP was funded by Labex OSUG@2020 (investissements d'avenir, ANR10-LABX56). This work was partially funded by Labex CECs-FATE.

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