THE OCCURRENCE OF CONTAMINANTS OF EMERGING CONCERN IN SLOVENE AND CROATIAN WASTEWATERS AND RECEIVING SAVA RIVER

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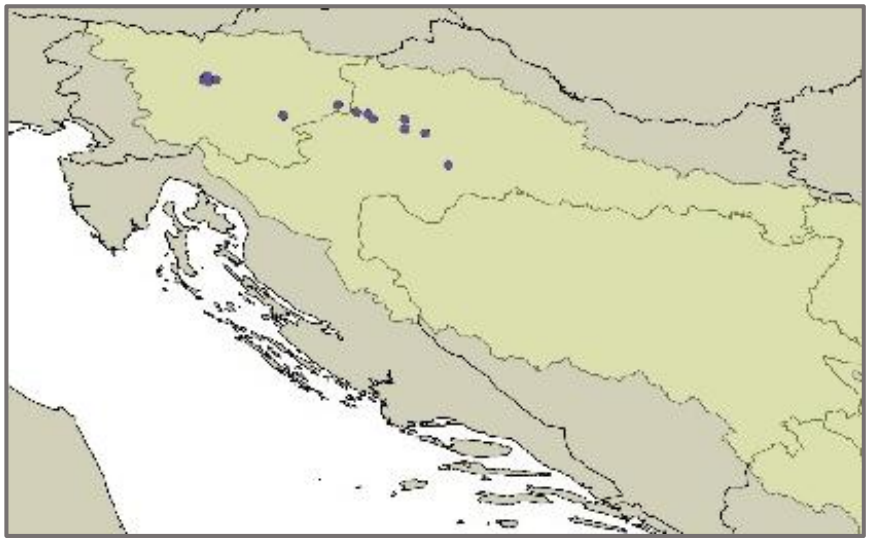
*Graphical Abstract

CONTAMINANTS OF EMERGING CONCERN IN SAVA RIVER BASIN



Sampling: WW effluents + Sava River

***Highlights (for review : 3 to 5 bullet points (maximum 85 characters including spaces per bullet point)**

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1. Bisphenols E and B were detected > LOQ in European SW for the first time.
2. Bisphenols AP, CL2, P and Z were detected > LOQ in European WW for the first time.
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Bisphenol A (BPA), 2-ethylhexyl 4-methoxycinnamate (CNM), 2-hydroxy-4-methoxybenzophenone (HM-BP), 2,2'-methylenediphenol (BIS2), 4,4'-biphenol (BP4,4), 4,4'-dihydroxydiphenyl ether (DHDPE), bisphenol AF (BPAF), bisphenol AP (BPAP), bisphenol C (BPC), bisphenol E (BPE), bisphenol F (BPF), bisphenol FL (BPFL), bisphenol M (BPM), bisphenol BP (BPBP), bisphenol P (BPP), bisphenol S (BPS), bisphenol Z (BPZ), 4-cumylphenol (HPP), 2,4-dihydroxybenzophenone (DH-BP), estrone (E1), 17β-estradiol (E2), 17α-ethynyl estradiol (EE2), CAF, 4-hydroxybenzophenone (H-BP), 2,2'-dihydroxy-4-methoxybenzophenone (DHM-BP), clofibric acid (CLA), ibuprofen (IB), naproxen (NP), ketoprofen (KP), diclofenac (DF) and its three transformation products (TPs), diazepam (DZP), methyl paraben (MePB), ethyl paraben (EtPB), propyl paraben (PrPB), butyl paraben (BuPB), iso-butyl paraben (iBuPB), benzyl paraben (BePB), nonylphenol (NONPH) and triclosan (TCS), carbamazepine (CBZ), iso-propyl paraben (iPrPB), bisphenol B (BPB), mecoprop (MEC), bisphenol Cl (BPCL2), bisphenol PH (BPPH), wastewater (WW), wastewater treatment plant (WWTP), surface water (SW), contaminant of emerging concern (CEC), active pharmaceutical ingredients (APIs), personal care products (PCPs), risk quotient (RQ), environmental risk assessment (ERA), transformation products (TPs), solid-phase extraction (SPE), N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA), N-(tert-butyldimethylsilyl)-N-methyltrifluoroacetamide with 1% tert-butyldimethylchlorosilane (MTBSTFA with 1% TBDMCS), Predicted No-Effect Concentration (PNEC), assessment factor (AF),

which is 1000 in the case of acute toxicity. When using the data for chronic toxicity, PNEC derives from the ratio between the No-Effect Concentration (NOEC), detection frequency (DFr), limit of quantification (LOQ)

ABSTRACT

This study investigated the occurrence of 48 contaminants of emerging concern (CEC) in wastewater effluents from three Slovenian and three Croatian waste water treatment plants (WWTP) representing the major inputs into the upper and middle course of the Sava River and simultaneously in the Sava River itself. Two sampling campaigns were carried out in May and July 2017. Samples were extracted using SPE and analysed by GC-MS. In effluents, 23 CEC were >LOQ with caffeine (<49,600 ng L^{-1}) and the UV-filter 4-hydroxybenzophenone (H-BP, <28,900 ng L^{-1}) present in the highest concentrations and being most frequently detected (DFr > 83.3 %). Bisphenol B and E were detected for the first time in wastewater (WW) from Velika Gorica (May) and Zaprešić (July), respectively. In surface water (SW), 19 CEC were detected >LOQ with CAF being the most abundant and most frequently detected (DFr = 92.9 %). Bisphenols AP, CL2, P and Z were detected >LOQ for the first time in European SW. Active pharmaceutical ingredients (API; naproxen, ketoprofen, carbamazepine and diclofenac; the preservative methyl paraben; CAF and UV-filter HM-BP were the most abundant CEC in SW and WW. An increasing trend in the total CEC load downstream the Sava River was observed, indicating cumulative effects of individual sources along the river. The Croatian Zaprešić, Zagreb and Velika Gorica WWTP effluents contributed the most towards the enhanced loads of the CEC studied probably due to their size (WWTP-ZG) or insufficient treatment efficiency (e.g. mechanical treatment at WWTP-Zaprešić). HM-BP was the only compound found at a levels exhibiting high environmental risk (RQ = 1.13) downstream from Ljubljana and Domžale-Kamnik WWTPs. Other SW samples that contained HM-BP, ibuprofen (API) and/or benzyl paraben (preservative) posed a medium risk for the environment. The results suggest the need for further monitoring of CEC in the Sava River Basin.

Keywords: Sava River, contaminants of emerging concern, surface water, wastewater, mass loads, risk assessment

Highlights:

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1. INTRODUCTION

Contaminants of emerging concern (CEC) occur in the environment on a global scale and encompass active pharmaceutical ingredients (APIs), personal care products (PCPs), life-style compounds like caffeine (CAF) and other substances, that appear in the environment due to human activities and have the potential to harm biota and human population (Sauvé and Desrosiers, 2014). Their widespread occurrence in wastewaters (WWs) and surface waters (SWs) has been continuously reported. In addition, some CEC with known effects on aqueous biota remain recalcitrant during WW treatment and under environmental conditions and new CEC with unknown effects are being reported with time (Bueno et al., 2012; Petrie et al., 2016).

It is hard if not impossible to monitor the presence of all potentially harmful compounds in the environment and even harder to control their release in the environment, e.g. by an appropriate treatment technology of wastewater treatment plants (WWTPs). Therefore, providing data on CEC occurrence is essential and serves as a basis for prioritizing candidates that have to be monitored and consequently regulated in terms of their emission. This has already been done for certain CEC in the EU by establishing the WFD Watch list (CEC that are to be monitored) and Priority list (CEC for which Environmental Quality Standards in SWs have been set) (EU Decision 495/2015; Tousova et al., 2017; Sousa et al., 2018). Additionally, some areas within Europe still remain to be investigated in terms of environmental CEC occurrence. An example is Central and South Europe, where the Sava River, the largest tributary (by flow) of the Danube River flows. The Sava River springs in the Slovenian mountains and flows a distance of 945 km through Croatia, Bosnia and Herzegovina eventually to join the Danube in Serbia. Since it supplies the groundwater aquifers, which are an important source of potable water for inhabitants living in this area, it is important to monitor its quality especially due to surrounding agricultural and industrial activities (Milačič et al., 2017). There were several attempts so far to perform a comprehensive region-specific prioritization of contaminants in the Sava River, which covered a wide spectrum of possible contaminants (Smital et al., 2013; Tousova et al., 2017), however, given the extremely high number of possible contaminants, the data on numerous CEC are still missing.

The aim of this study was to collect and analyse SW and WW from WWTPs at locations in Slovenia and Croatia along the Sava River and analyse them for 48 CEC including APIs and their selected transformation products (TPs), preservatives, bisphenol compounds, and estrogenic hormones. In addition, an environmental risk assessment based on the concentrations of CEC detected in Sava River was performed for the first time in the Sava River catchment.

2. EXPERIMENTAL

2.1 Materials for organic analysis

Information on reagents, solvents and analytical standards of CEC (Table 1) and surrogate standards used for the chemical analysis is given in details Supplementary Information (SI-I).

Table 1: Commercial names and abbreviations used for the studied CEC.

Commercial name	Abbreviation
EDCs: Bisphenols and related compounds, estrogens	
Bisphenol A	BPA
Bisphenol AF	BPAF
Bisphenol AP	BPAP
Bisphenol B	BPB
Bisphenol BP	BPBP
Bisphenol C	BPC
Bisphenol Cl	BPCL2
Bisphenol E	BPE
Bisphenol F	BPF
Bisphenol FL	BPFL
Bisphenol M	BPM
Bisphenol P	BPP
Bisphenol PH	BPPH
Bisphenol S	BPS
Bisphenol Z	BPZ
4,4’-biphenol	BP4,4
2,2’-methylenediphenol	BIS2
4,4'-dihydroxydiphenyl ether	DHDPE
4-cumylphenol	HPP
4-nonyl-phenol	NONPH
Estrone	E1
17β-estradiol	E2
17α-ethynyl estradiol	EE2
UV-filters: benzophenons and other	
2,4-dihydroxybenzophenone	DH-BP (BP1)
4-hydroxybenzophenone	H-BP
Oxybenzone	HM-BP (BP3)

Dioxybenzone	DHM-BP (BP8)
2-ethylhexyl 4-methoxycinnamate	CNM
APIs and metabolites/TPs	
Carbamazepine	CBZ
Clofibric acid	CLA
Diazepam	DZP
Diclofenac as sodium salt	DF
2-[(2,6-Dichlorophenyl)amino]-5-nitrophenyl-acetic acid	DFtp1
2-anilinophenylacetic acid	DFtp2
2-[(2-Chlorophenyl)amino]-benzaldehyde	DFtp3
Ibuprofen	IB
Ketoprofen	KP
Naproxen	NP
Preservatives	
Methyl paraben	MePB
Ethyl paraben	EtPB
Propyl paraben	PrPB
Iso-Propyl paraben	IPrPB
Butyl paraben	BuPB
Iso-Butyl paraben	IBuPB
Benzyl-paraben	BePB
Irgasan, triclosan	TCS
Other compounds	
Caffeine	CAF
Mecoprop	MEC

2.2 Sampling

Samples of wastewater (WW) effluent from three Slovenian and three Croatian WWTPs were collected during a dry period on two occasions (23rd May and 12th July 2017). The WWTPs differ in their sizes (population equivalents; P.E.), treatment technology, type of receiving WW and daily flow rates (Table 2). Additional information on each WWTP is given in the SI-II.

Table 2: Characteristics of the studied Slovene and Croatian WWTPs.

Location	Capacity (P.E.)	Treatment	WW type (I-industrial; M-municipal)	Hydraulic retention time (h)	Flow rate (m^3 day^{-1})

Ljubljana (LJ)	360,000	Mechanical-biological (suspended biomass)	I (≈11%); M	18	May	66,994
					July	69,916
Domžale-Kamnik (DK)	149,000	Mechanical-biological (SBR)	I (≈11%); M	16	May	17,935
					July	18,240
Novo mesto (NM)	55,000	Mechanical-biological (ultrafiltration)	I (≈12%); M	22	May	4,636
					July	4,337
Zaprešić	60,000	Mechanical	I (≈38%); M	24	May	6,665
					July	5,789
Zagreb (ZG)	1,200,000	Mechanical-biological (activated sludge)	I; M	10 – 12	May	261,126
					July	234,177
Velika Gorica (VG)	35,000	Mechanical-biological (activated sludge)	M	20	May	6,610
					July	5,928

With the exception of grab WW samples from WWTP-DK (in May), WWTP-ZG (in July) and Zaprešić (May and July), all samples were collected as 24 h time-proportional samples.

The Sava River *prior* or after WW discharges was also sampled at seven locations (three in Slovenia and four in Croatia) on the same day as the equivalent WW samples (Table 2). The Slovene samples were collected *prior* to WWTP-LJ discharge (Ljubljana), after WWTP-LJ and WWTP-DK discharge (Jevnica) and *prior* to Krka River tributary, into which the WWTP-NM discharges its effluent (Brežice). The First Croatian sample was collected after the Krka River

tributary (Otok Samoborski), the second after the discharge point of WWTP-Zaprešić and Krapina River tributary (Jankomir), the third after WWTPs ZG and Velika Gorica discharge points (Oborovo) and the fourth after the city of Sisak and tributary of Kolpa River (Crnac). All SWs were collected as a grab samples. The daily flows of Sava River used for mass load calculations are given in SI-II.

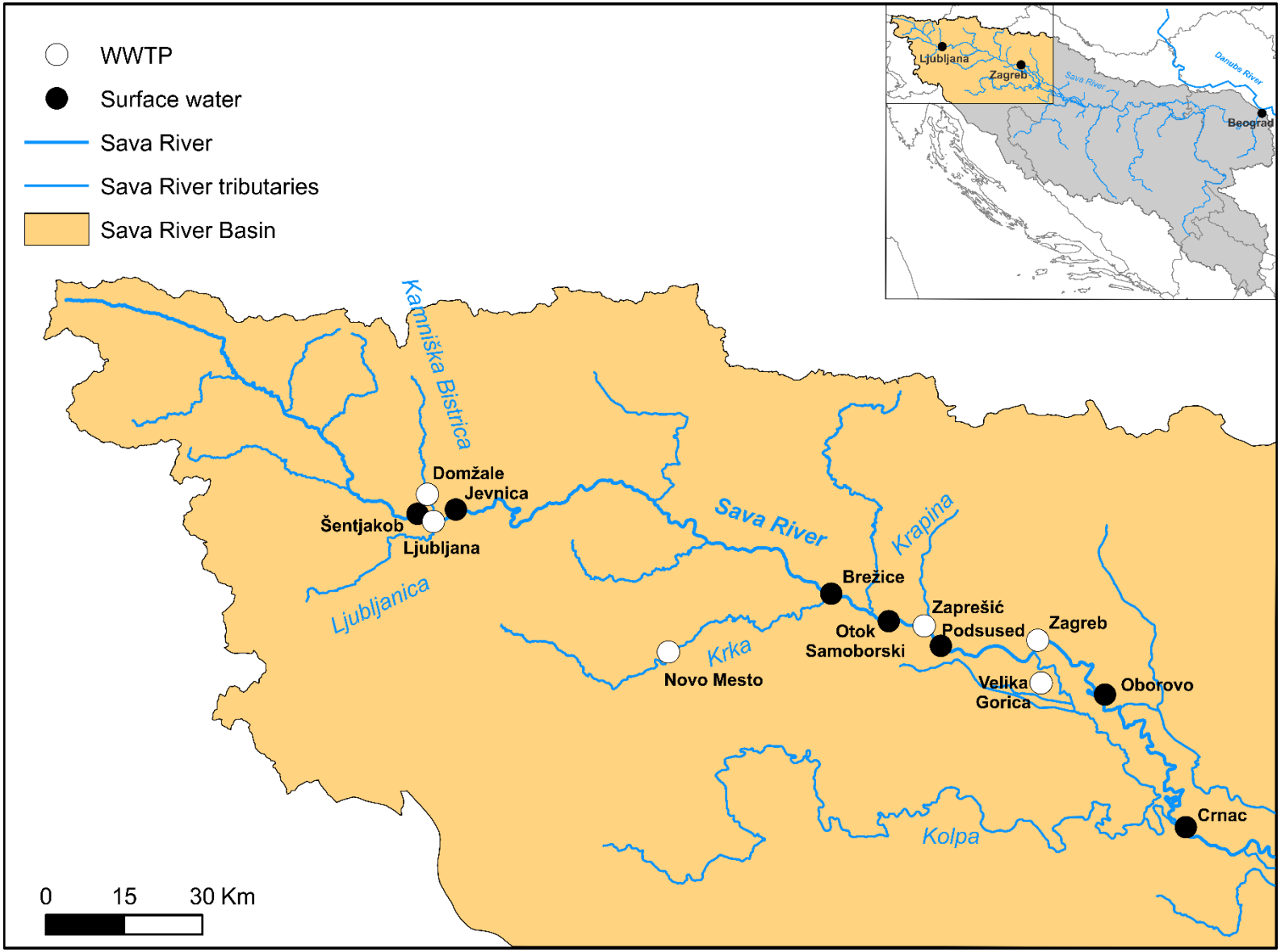


Figure 1: Sampling locations on Sava River (2-column fitting image).

All samples were filtered through a glass-microfiber (Machery Nagel, Düeren, Germany) and a cellulose nitrate membrane filter (0.45 µm; Sartorius Stedim Biotech GmbH, Göttingen, Germany) and stored at -20 °C *prior* to analysis.

2.3 Sample preparation and analysis

Table 3 shows the procedure of solid-phase extraction (SPE). Each SW and WW sample was pre-concentrated using an Oasis HLB Prime cartridge (Waters, Massachusetts, USA). After loading, the sorbent was washed and/or dried under vacuum (-10 mm Hg, 20 min) and eluted with the optimal solution (Table 3). The solvent was evaporated under nitrogen *prior* to derivatization.

Table 3: SPE conditions for SW and WW analysis.

SW (V_{sample} = 400 mL)	**WW (V_{sample} = 300 mL)**
Filtration (glass-microfibre and 0.45 µm cellulose nitrate filter)	
Acidification with hydrochloric acid (pH = 2)	
Spiking with surrogate standards	
Loading (≈ 5 mL min^{-1})	
/	Wash: 3 mL of 5 % methanol/water
Elution: 1,800 µL of 5 % formic acid/ethyl acetate	Elution: 1,800 µL of 5 % ammonia/methanol

The dried extracts were then halved (Group A: CAF, HPP, NONPH, BIS2, BPAF, DFtp3, H-BP, HM-BP, DHDPE, DH-BP, BP4,4, BPF, BPE, BPA, DHM-BP, BPC, BPB, CNM, BPCL2, BPZ, E1, BPS, E2, BPAP, EE2, BPM, BPP, BPBP, BPPH, BPFL; Group B: MePB, CLA, EtPB, iPrPB, IB, MEC, PrPB, iBuPB, BuPB, DFtp1, NP, TCS, KP, BePB, DZP, CBZ, DF, DFtp2). Group A was derivatized with 50 µL N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) in 50 µL pyridine at 60°C for 1 h, whereas Group B was silylated with 30 µL N-(tert-butyldimethylsilyl)-N-methyltrifluoroacetamide with 1% tert-butyldimethylchlorosilane (MTBSTFA with 1% TBDMCS) in 70 µL ethyl acetate at 60°C for 16 h. Samples were analysed using GC-MS

(Agilent 7890B/5977A, USA). Separation was achieved on a DB-5 MS capillary column (30 m × 0.25 mm × 0.25 µm; Agilent, USA) with helium as the carrier gas (1 ml min^{-1}). Each sample (1 µL) was injected in the splitless mode at 250 °C. Two different oven programs were used to give optimal chromatographic separation of compounds. For Group A from an initial temperature 65 °C (held 2 min), the oven was ramped at 30 °C min^{-1} to 100 °C (held 2 min), then at 10 °C min^{-1} to 200 °C (2 min), at 10 °C min^{-1} to 280 °C (10 min) and finally at 30 °C min^{-1} to 300 °C (3.5 min). Total GC-MS runtime was 39.3 min. For Group B, the initial oven temperature was set to 65 °C for 2 min, then ramped at 30 °C min-1 to 100 °C (2 min), at 10 °C min-1 to 200 °C (2 min), at 10 °C min-1 to 280 °C (2 min) and finally at 30 °C min-1 to 300 °C (5 min). Total GC-MS runtime was 32.8 min. Additional details of the chemical analysis and method validation are given elsewhere (Česen et al., 2018).

2.4 Environmental risk assessment

Environmental risk assessment (ERA) was assessed by determining the risk quotient (RQ) using the following equation:

$$RQ = \frac{MEC}{PNEC}$$

where MEC represents the average concentration (RQs were calculated for the SW data). The Predicted No-Effect Concentration (PNEC) was calculated by dividing the compound-specific EC_{50}/LC_{50} values with the assessment factor (AF), which is 1000 in the case of acute toxicity. When using the data for chronic toxicity, the PNEC (predicted no-effect concentration) is derived from the ratio between the No-Effect Concentration (NOEC) and the AF. When only one NOEC value is available (for one trophic level), an AF of 100 is used, when two NOEC values are available, an AF of 50 is used and when data for all three trophic levels exist, an AF of 10 is applied. A RQ ≥ 1 indicates a potential “high risk”, a value between 0.1 and 1 means a “medium risk” and a RQ between 0.01 and 0.1 means a “low/negligible risk” (Papageorgiou et al., 2016).

3. RESULTS AND DISCUSSION

3.1 CEC occurrence in WWs

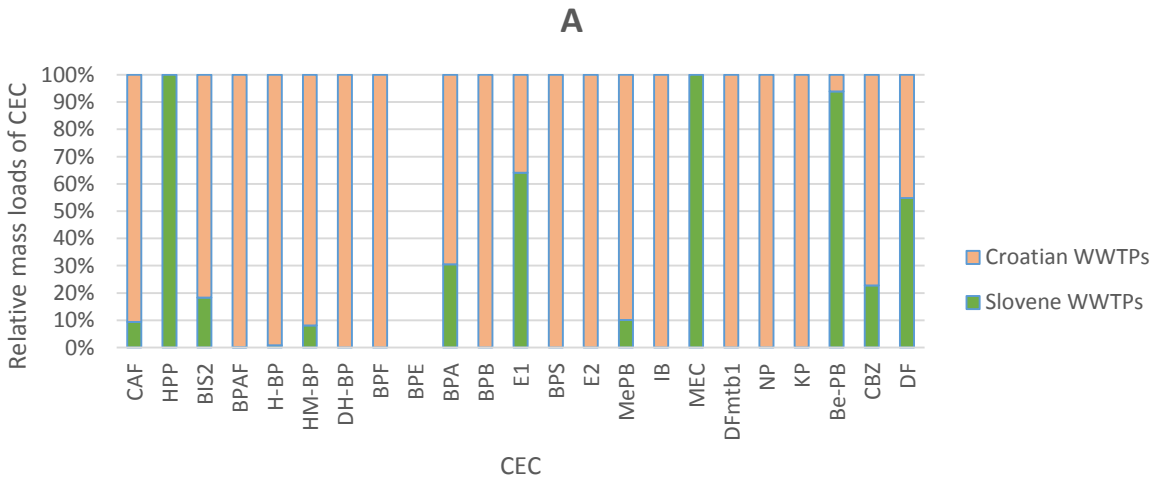
Table 4 gives the average, minimal and maximal values of CEC concentrations in WW samples and their detection frequency (DFr) (individual concentrations are given in SI-III). The variation in concentrations are likely due to the extent of certain CEC used in a given sampling area, the treatment technology and the characteristics of a particular WWTP, which affect CEC removal efficiency. Of the 48 target compounds, 23 were detected in levels above the limit of quantification (LOQ) at least once. Caffeine and H-BP were found in the highest concentrations (49,600 ng L^{-1} and 28,900 ng L^{-1}, respectively) and the average concentration of CAF (8,190 ng L^{-1}) was the highest among all of the analytes. The CEC were then divided into 3 groups according to their detection frequency (DFr; Table 4). Caffeine and H-BP had the highest detection frequencies (> 83.3 %), whereas HPP, BPE, BPB and E2 were the least frequently detected (DFr at 8.33 %).

Table 4: The average, minimum and maximum CEC concentration (>LOQ) and DFr in WW samples.

CEC	Concentration (ng L^{-1})			DFr
	Average	Min.	Max.	
CAF	8,190	133	49,600	100.0%
HPP	51.9	51.9	51.9	8.33%
BIS2	20.4	4.94	36.4	58.3%
BPAF	1.47	0.0367	3.40	41.7%
H-BP	3,000	7.50	29,900	83.3%
HM-BP	15.1	2.47	48.5	50.0%
DH-BP	306	36.1	563	33.3%
BPF	44.3	2.54	117	41.7%
BPE	476	476	476	8.33%
BPA	971	44.3	2,620	66.7%
BPB	27.1	27.1	27.	8.33%
E1	840	88.5	1,980	41.7%

BPS	316	108	435	25.0%
E2	713	713	713	8.33%
MePB	379	11.8	1,910	66.7%
IB	5,340	4,330	6,130	33.3%
MEC	38.0	8.05	67.9	16.7%
DFtp1	3,250	781	5,720	16.7%
NP	966	81.6	2,190	50.0%
KP	1,210	53.8	2,460	53.8%
Be-PB	409	23.6	676	28.6%
CBZ	809	86.2	5,320	75.0%
DF	480	113	812	70.6%

Further, mass loads using determined CEC concentrations were calculated taking into account the daily flow rates at the studied WWTPs (Table 2; SI-III).The Spatial variation in sums of mass loads at Slovene *vs.* Croatian WWTPs revealed that higher level of contamination derives from the latter for the majority of detected CEC in May (17 out of 22) and in July (16 out of 20; Figure 2). This can be related to either greater use of CEC in Croatia and/or lower removal efficiency of Croatian WWTPs if compared to Slovene WWTPs. Indeed, WWTP-Zaprešić includes only mechanical treatment, whereas WWTP-VG is planned to be reconstructed in the near future due to its current poor biological treatment efficiency (Table 2).



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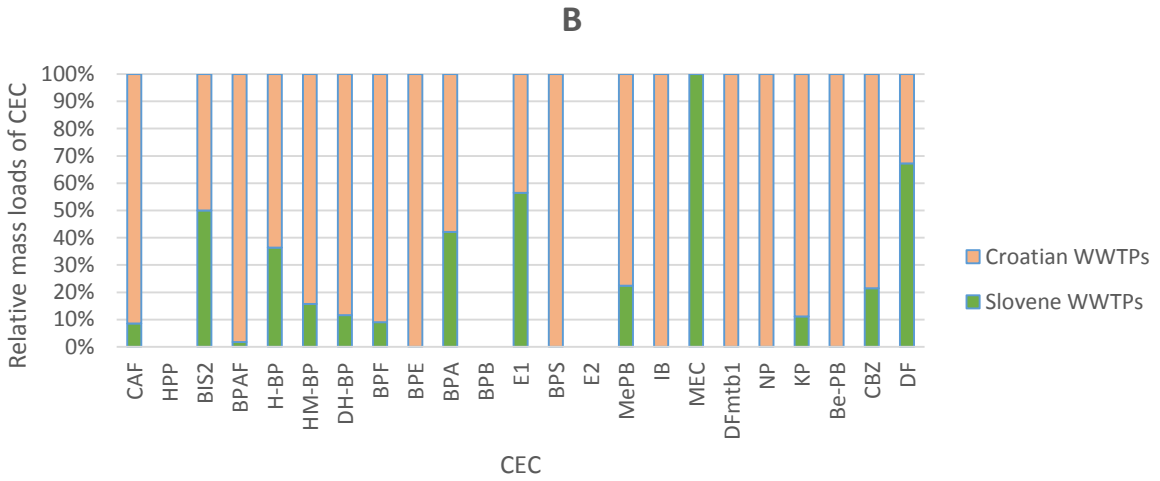

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204 **Figure 2: Total CEC mass loads in Slovene and Croatian WWTPs (stacked columns; A = May; B = July) (2-**
205 **column fitting image).**

206 Figure 3 shows the overall CEC contamination from each WWTP in May and in July. The
207 highest amounts of CEC were released from Croatian WWTPs with one exception, i.e.
208 WWTP-LJ on May had lower total mass load than WWTP-VG (182 g day^{-1} vs. 174 g day^{-1}). The
209 obvious difference in mass loads between the two abovementioned WWTPs (Zaprešić and
210 VG) and Slovene WWTP-NM is also clear despite the fact that they all have comparable sizes
211 and daily flow rates (Table 2). The latter is probably more efficient since advanced biological
212 treatment, i.e. biofiltration, is applied. In fact, for highly biodegradable compounds like e.g.
213 CAF the major factor determining the mass loads in WWTP effluents is not the size of a
214 certain WWTP but rather its removal efficiency.

215 Additional clear difference in the calculated total mass loads between both capital WWTPs
216 was also observed. In this case it derives from varying sizes of both WWTPs rather than

removal efficiencies since both apply the mechanical-biological treatment (Table 2; Figure 3). Therefore, higher mass loads of individual CEC were expected and mostly confirmed for WWTP-ZG. Surprisingly, few exceptions were observed, i.e. Be-PB and BPA in May, MePB in July and E1 and DF on both samplings. This could be explained by a significantly higher use and occurrence of these contaminants in Slovene WWs or by a highly inconsistent removal which was already reported in the literature for e.g. DF (Archer et al., 2017).

In particular case of WWTP-Zaprešić, the effluent had considerably higher total mass loads in May (599 g day^{-1}) than July (192 g day^{-1}; Figure 3). High overall mass load in May mainly origins from the high mass loads of CAF and H-BP, that accounted for 88.5 % of the total mass load (SI-III). CAF was the most abundant also in WWTP-Zaprešić sample from July since it accounted for 53.6 % of overall mass load (SI-III). The opposite phenomenon was revealed in the case of WWTP-VG with higher total mass load observed in July (250 g day^{-1} *vs.* 174 g day^{-1}), where 57.8 % of the total mass load belonged to CAF. Interestingly, the sample from May contained only 22.5 g day^{-1} of CAF, which represents 12.9 % of overall mass load (SI-III).

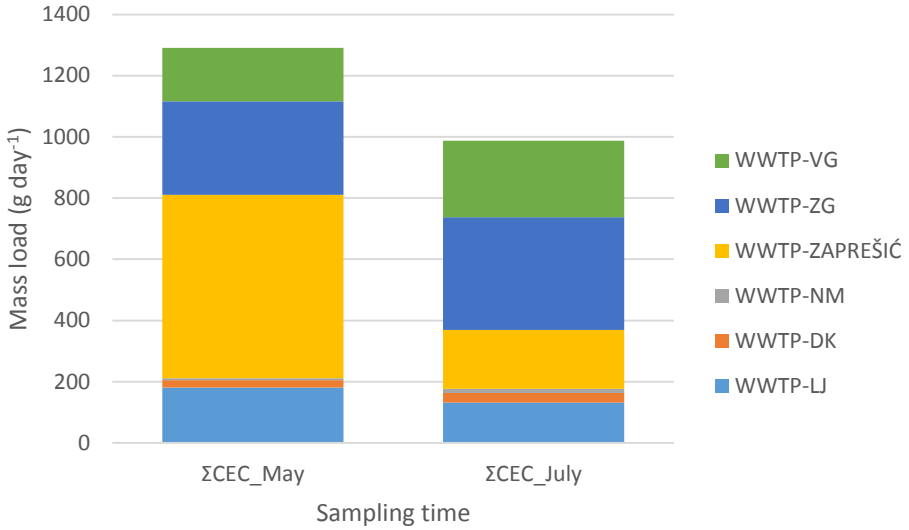


Figure 3: Total mass loads of detected CEC for each WWTP in May and July 2017 (1-column fitting image).

Comparison with the literature data

The data on detected CEC concentrations was compared with five other studies (SI-IV) including a EU-wide study by Loos et al. (2013), one study of the Western Balkans by Terzić et al. (2008), a Slovene study by Česen et al. (2018), a Spanish study by Osorio et al. (2014) addressing the occurrence of DF TPs and a review paper addressing the occurrence of CEC in

238 the UK WWs by Petrie et al. (2015). The findings reveal higher average concentrations of
239 CAF, BIS2, DH-BP, BPF, BPA, E1, BPS, E2, MePB, NP and KP (e.g. up to few orders of
240 magnitude in the case of KP – this study: 1,210 ng L^{-1}; Petrie et al. (2015) > 23.0 ng L^{-1}) in the
241 present study. Only IB and DF levels in the UK (Petrie et al., 2015) and MEC and CBZ in EU-
242 wide study (Loos et al., 2013) were comparable with the concentrations of CEC detected in
243 Slovenia and Croatia (SI-IV). One TP of DF, namely DFtp1, was present in WWTP-VG1 and
244 WWTP-VG2 at 5,720 ng L^{-1} and 781 ng L^{-1}, respectively. These values are considerably higher
245 than the ones found in the literature, i.e. 20.0 – 29.0 ng L^{-1} and <LOQ – 6.64 ng L^{-1} in Spanish
246 and Slovene effluents, respectively (Osorio et al., 2014; Česen et al., 2018). Interestingly, a
247 comparison with Česen et al. (2018) revealed the presence of similar positive hits for
248 bisphenols, e.g. BIS2, BPAF, BPF, BPA and BPS were detected in both studies, whereas BPE
249 and BPB were detected only in this study. The average concentrations of BIS2, BPF, BPA and
250 BPS were considerably higher in this study (SI-IV). In addition, we calculated average values
251 of bisphenols separately for Slovene and Croatian WW samples. Except for BIS2, all other
252 bisphenols were present in higher concentrations in samples collected at Croatian WWTPs
253 (Table 5). Slovene WW analysed within this study contained higher concentrations of BIS2
254 and BPA if compared to that reported by Česen et al. (2018). Finally, WW samples contained
255 also higher BPA concentrations than reported by Terzić et al. (2008).

256 **Table 5: Comparison of average bisphenol concentration (in ng L^{-1}) detected in Slovene and Croatian WWs.**

	Average / median concentrations in WW samples – this study		(Česen et al., 2018)
	Slovenia	Croatia	
BIS2	27.1 / 35.6	15.4 / 15.4	4.03
BPAF	0.0367 / 0.0424	2.43 / 1.95	2.24
BPF	3.77 / 3.77	71.2 / 58.9	3.39
BPE	< LOQ	476 / 476	< LOQ
BPA	540 / 177	1,690 / 2,340	58.7
BPB	< LOQ	27.1 / 27.1	< LOQ
BPS	< LOQ	316 / 404	28.0

257

3.2 CEC occurrence in SWs

Table 6 gives the average, minimal and maximal concentrations and DFr of CEC in SW (for individual values see SI-V). Out of 48 CEC, 19 were quantified (>LOQ) at least once. Similar to WW, CAF was present in the highest concentrations among all the studied CEC and had the highest average concentration among all of the analysed samples (1,390 ng L^{-1} and 283 ng L^{-1}, respectively), followed by Be-PB (457 ng L^{-1} and 246 ng L^{-1}, respectively; Table 6). The highest DFr in SW was observed for CAF; the UV-filter HM-BP, the bisphenol BPS, the preservatives MePB, EtPB, PrPB, BePB and the pharmaceuticals NP, KP, and CBZ (> 87.5 %).

Table 6: The average, lowest and highest CEC concentration (>LOQ) along with DFr in collected SW samples.

CEC	Concentration (ng/L)			DFr
	Average	Min.	Max.	
CAF	283	37.0	1,390	92.9%
BIS2	9.79	2.44	17.1	14.3%
HM-BP	11.3	4.48	44.2	100%
BPA	61.5	1.53	215	35.7%
BPCL2	0.884	0.365	2.09	71.4%
BPZ	4.68	0.250	9.11	14.3%
BPS	9.00	1.68	35.2	85.7%
BPAP	0.704	0.540	0.903	21.4%
BPP	6.45	6.45	6.45	7.14%
MePB	26.3	14.8	139.50	100%
EtPB	11.12	4.79	67.2	85.7%
IB	46.2	1.46	262	50.0%
MEC	10.4	10.4	10.4	7.14%
PrPB	4.31	0.815	23.4	92.9%

NP	7.95	2.67	20.4	100%
KP	6.10	0.897	52.7	100%
Be-PB	246	175	457	100%
CBZ	8.60	2.69	18.4	100%
DF	2.30	0.0649	4.62	42.9%

Similar to WW, mass loads in SW were calculated using the daily Sava River flows on both sampling campaigns (SI-V). The majority of CEC were more abundant in Croatian samples from May (17 out of 19) and from July (10 out of 15; Figure 4), which might be correlated to the fact that only 3 samples of Sava River were collected in Slovenia, whereas 4 were sampled in Croatia. Regardless, the higher mass loads in Croatia could be also correlated with the findings for WW analysed within this study, where Croatian samples contained higher number of positive hits and CEC mass loads regardless of the sampling time.

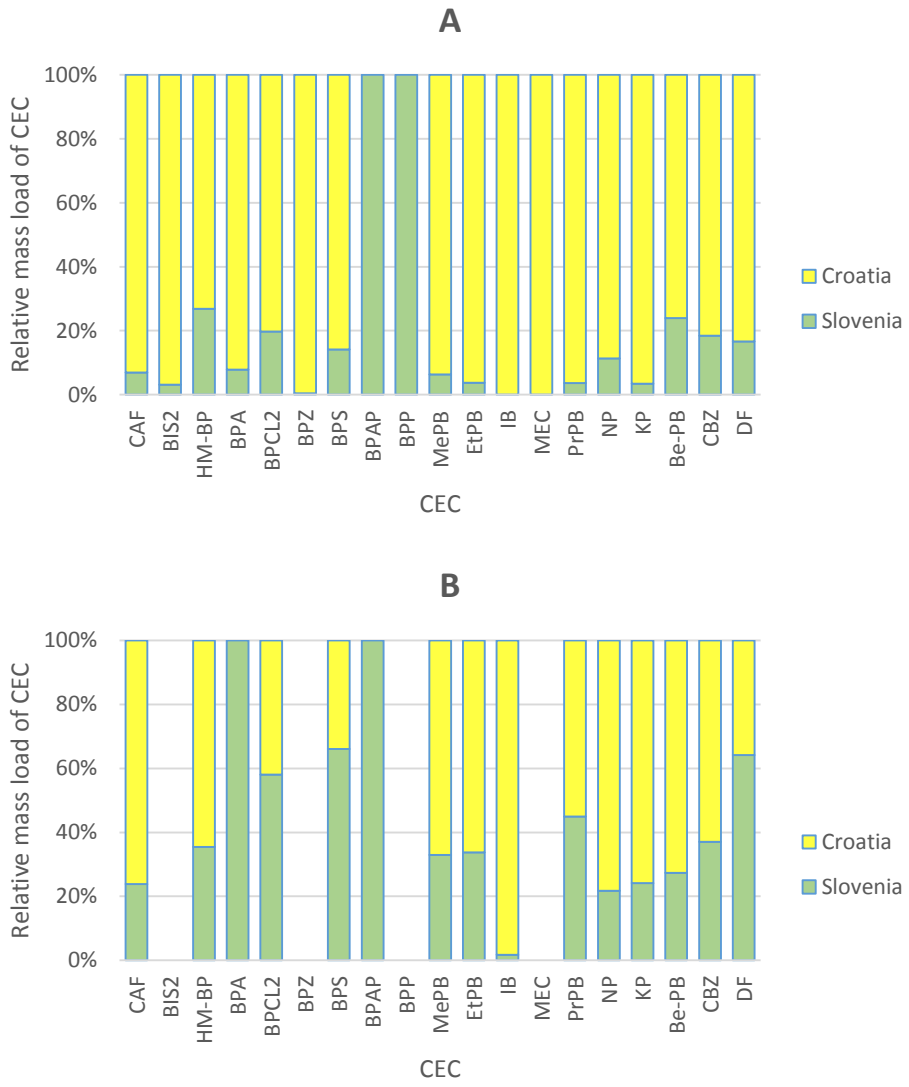
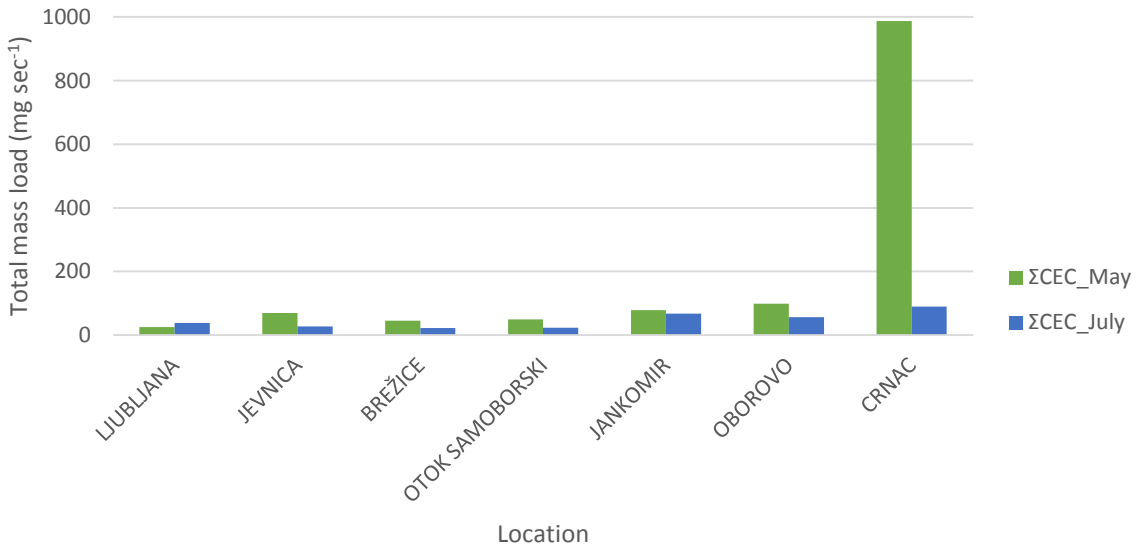


Figure 4: CEC mass loads in Slovene and Croatian samples of Sava River (stacked columns; A = May; B = July) (2-column fitting image).

In general, total CEC mass loads were slightly higher on May if compared to July, which could be explained by potentially increased environmental degradation (e.g. biodegradation and photolysis) during Summer month (Figure 5). A noteworthy result was observed for sample from Crnac on May, that contained the highest amounts of CEC with CAF (529 mg sec^{-1}), BPA (81.6 mg sec^{-1}), BPP (53.0 mg sec^{-1}), IB (99.4 mg sec^{-1}) and KP (20.0 mg sec^{-1}) being the most abundant. Possible explanation for this deviation is given in section 3.3., where also trend in contamination of Sava River is discussed in details.



286

287 **Figure 5: Total mass loads of detected CEC in Sava River in May and July.**

288 *Comparison with the literature data*

289 Concentrations in SW were also compared with the available literature data in four
290 publications addressing the same issue in Macedonia, Slovenia, Italy and UK (Meffe and de
291 Bustamante, 2014; Petrie et al., 2015; Stipaničev et al., 2017; Česen et al., 2018). In general,
292 comparable values among all these studies were found for CAF, BIS2, HM-BP, BPS, MePB,
293 EtPB, PrPB, NP, KP and CBZ (SI-IV). DF was the only compound found in lower concentrations
294 within this study (2.30 ng L^{-1}) compared to the others (up to 154 ng L^{-1}; SI-IV). BPA (61.5 ng L^{-1})
295 and IB (46.2 ng L^{-1}) were more abundant compared to the Slovene study (BPA: 7.50 ng L^{-1} –
296 27.9 ng L^{-1}; IB: 4.07 ng L^{-1} – 11.6 ng L^{-1}), but comparable with all other European studies.
297 Interestingly, BPCL2, BPZ, BPAP, BPP, MEC and Be-PB were > LOQ only in this study, where
298 BPCL2, BPZ, BPAP and BPP were, to the author's knowledge, quantified (0.884 ng L^{-1} for
299 BPAP to 6.45 ng L^{-1} for BPP) in European SW for the first time.

300 **3.3 Trend in CEC mass loads along the Sava River and correlation between SW and WW analysis**

301 Among all investigated CEC, only CBZ and CAF were detected in all SW and WW samples
302 collected with the absence of CAF in SW sample Otok Samoborski on July as the only
303 exception. The ubiquitous presence of CBZ could be explained by its known poor
304 biodegradation and deconjugation of CBZ metabolites during WW treatment (Archer et al.,
305 2017). On the contrarily, CAF is readily biodegradable (Tran et al., 2018), yet its presence in

306 WW and SW can be correlated to the high global consumption of CAF-containing beverages
307 (Gracia-Lor et al., 2017).

308 Figure 6 shows the total mass loads at each sampling location from Ljubljana prior to WWTP-
309 LJ and WWTP-DK effluents to Crnac as the last sampling point on Sava River (Figure 1). In the
310 case of WWTP-LJ and WWTP-DK as well as WWTP-ZG and WWTP-VG, the sums of mass loads
311 are given since both pairs of WWTPs influence the corresponding SW Sample, i.e. Jevnica
312 and Oborovo, respectively. Generally, the increasing mass loads were observed for SW
313 samples (blue) downstream Sava River flow for both samplings with the few exceptions. The
314 drop in mass loads between Jevnica and Brežice can be related to possible degradation of
315 CEC in the river itself in this relatively long river section. Additionally, Ljubljana sample from
316 July contained higher mass loads than Jevnica, where WWTP-LJ and WWTP-DK discharges
317 contribute to overall pollution. This can be explained by the fact that CAF was highly
318 abundant in Ljubljana sample, i.e. it represented 53.1 % of total mass load in this sample,
319 whereas in Jevnica sample, CAF accounted only for 16.4 % of total mass load (SI-V). The third
320 exception was observed for a pair of samples from Jankomir and Oborovo on July, where
321 higher mass load in Jankomir can be also related to a higher BePB abundance, i.e. 66.2 % *vs.*
322 only 32.7 % in a sample from Oborovo.

323 In general, relatively constant increase in CEC contamination downstream Sava river can be
324 correlated to the calculated mass loads coming from the studied WWTPs despite their
325 varying inputs (from 6.83 g day^{-1} for WWTP-NM in May to 619 g day^{-1} for WWTPs ZG and VG
326 in July; Figure 6). The significantly higher increase was observed among the last two Sava
327 River samples, i.e. Oborovo and Crnac especially in May.

328 The reason for such an increase derives from the additional inputs of the Kupa River and the
329 city of Sisak prior to the Crnac sampling location. Higher increase than expected, could be a
330 consequence of the fact that the sampling point at Crnac was possibly situated before the
331 full mixing of wastewaters of the nearby city of Sisak with main flow of the Sava River.

332

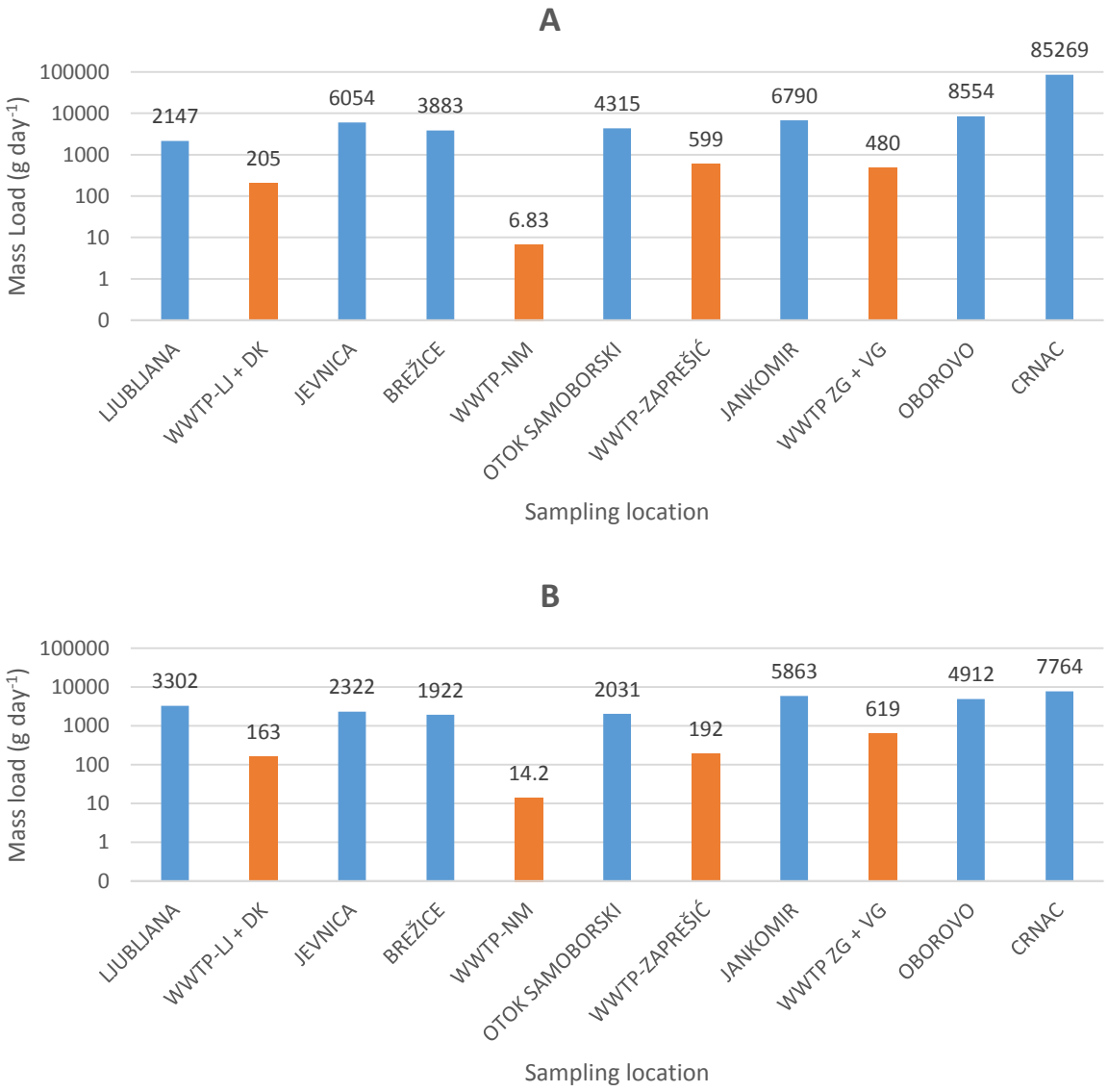


Figure 6: Total CEC mass loads at all sampling locations in May (A) and July (B). Sava river is marked in blue, whereas WWTP effluents are marked in orange.

3.4 Environmental risk assessment

An ERA was determined by establishing RQs using the average concentrations of CEC determined in SW (Table 7). Toxicity data (lowest values of EC/LC_{50} or NOECs were taken for "worst-case scenario") were either taken from the literature or calculated using the ECOSAR software V2.0 (SI-VI). Only HM-BP, BPP and Be-PB represented a medium risk according to their average concentrations (Table 7).

344 **Table 7: Determined RQ values based on the average and highest CEC concentrations in SW samples.**

	$RQ_{aver.}$	$RQ_{highest}$
CAF	0.00188	0.00922
BIS2	0.00530	0.00929
HM-BP	0.289	**1.13**
BPA_1	0.0117	0.0410
BPA_2	0.00615	0.0215
BPCL2	0.000645	0.00152
BPZ	0.0387	0.0753
BPS	0.000164	0.000641
BPAP	0.00228	0.00293
BPP	0.120	**0.120**
MePB	0.00235	0.0125
EtPB	0.00111	0.00672
IB	0.0280	**0.159**
MEC	8.37E-05	8.37E-05
PrPB	0.00560	0.0304
NP	0.00304	0.00780
KP	0.000391	0.00338
Be-PB	0.337	**0.626**
CBZ	0.000623	0.00133
DF	0.000237	0.000476

345 The RQ values taking into account the highest determined concentrations revealed that HM-
346 BP (44.2 ng L^{-1}) in a sample Jevnica-1, which is a sample of Sava River, collected after WWTP-

LJ and WWTP-DK discharges, represented a high environmental risk (SI-V). Interestingly, this compound was associated with a medium environmental risk also in a study by Česen et al. (2018), who addressed the occurrence of CEC in various other Slovene SWs. In addition, BPP (detected only on one occasion), IB and Be-PB posed a medium risk taking into account the highest determined concentrations in the samples from Brežice-1, Crnac-1 and Jankomir-2, respectively (Table 7). Further, RQs of all the samples that contained HM-BP, IB and Be-PB posed at least a medium risk to the environment since they contained either of these CEC (SI-VII). It is clear that high concentrations of CEC detected in water body like rivers do not necessarily pose a significant risk to the environment. However, one must take into account that mixture of various CEC occurs in the environment, hence, individually-derived calculations of RQ values might be misleading. Therefore, investigation of the toxicity of naturally occurring mixtures must be considered in future studies.

4. CONCLUSIONS

The occurrence of 48 CEC was assessed in Slovenian and Croatian WWTP effluents and in Sava River for the first time. In total, 23 and 19 CEC were above the LOQ in WW and SW, respectively, with CAF being the most abundant in both matrices. Several bisphenols (B and E in WW and AP, CL2, P and Z in SW) were quantified for the first time in Europe. CEC mass loads from the studied Croatian WWTPs contributed more towards overall contamination of Sava River if compared to Slovene WWTPs. Levels of the UV-filter HM-BP represent a high risk in the Sava River collected after WWTP-LJ and WWTP-DK discharges, whereas other SW samples containing either HM-BP, IB or BePB pose a medium risk based on RQ determination. The obtained data within this study can serve as a good basis for future monitoring studies that will cover the whole Sava River catchment, i.e. from its origins in Slovenian Alps until its confluence with the Danube River in Serbia.

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