Joint Danube Survey 3

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Chapter (summary report) on:

Chemical and immunochemical analysis of anthropogenic markers and organic contaminants

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Table of content

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1 Introduction

Municipal wastewater is a main point source for the input of xenobiotics into the river Danube. Raw wastewater contains many different potentially hazardous organic and inorganic contaminants. Many of these compounds require at least a secondary wastewater treatment for efficient removal. This high level of wastewater treatment is not available in all parts of the Danube basin, thus raw wastewater is continuously released into the Danube (Figure 1).

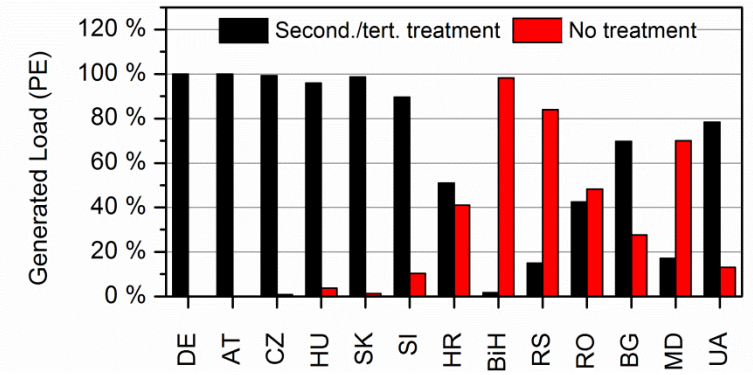

Figure 1: Population equivalents (PE) of treated urban wastewater in the Danube river basin in 2009/2010 (data source: ICPDR).

Different anthropogenic markers – indicative of human presence or activity – have been discussed to track the origin and type of contamination sources. Caffeine (CAF), the antiepileptic drug carbamazepine (CBZ) and the artificial sweetener acesulfame (ACE) have been proposed as possible markers for wastewater (Buerge, et al. 2003, Buerge, et al. 2009, Clara, et al. 2004). CAF is efficiently removed (> 99%) in wastewater treatment plants (WWTP), while CBZ and ACE are not significantly degraded by activated sludge, thus passing almost unchanged to the receiving water bodies. Therefore, CAF is suitable to indicate the presence of untreated wastewater, while the latter two indicate wastewater in general (treated or untreated). Untreated wastewater may contain very high levels of CAF up to 500 µg/L, while the concentrations of ACE and CBZ are usually below 50 and 5 µg/L, respectively.

All three compounds are likely to occur in all countries in the Danube basin. Beverages containing CAF and ACE are widely used in Europe. As far as CBZ is concerned, sales data indicate high amounts above 100 mg consumed per capita in Austria, Bulgaria, Germany, Hungary, Romania and Slovakia (Zhang and Geißen 2010).

Apart from effluents of WWTP, there are other inputs of domestic wastewater into the environment. During intense rainfall, combined sewers are likely to overflow releasing the runoff alongside raw sewage, sewer deposits and sewer slime into the receiving water body. Combined sewers are still in use for many cities in Europe. For well degradable contaminants such as CAF, combined sewer overflows are the main input pathway into surface water (Weyrauch, et al. 2010).

In addition to rainfall episodes, sewer leakage and damaged pipelines may also contribute to the CAF input into surface waters (Buerge, et al. 2003). At least 5% of the sewage is believed to exfiltrate from the sewers through broken pipes, joint failures and faulty connections (Reynolds and Barrett 2003).

2 Methods

CBZ and CAF were measured with two enzyme-linked immunosorbent assays (ELISA) at the BAM Federal Institute for Research and Testing in Berlin, Germany, according to previously published methods (Bahlmann, et al. 2009, Bahlmann, et al. 2012, Carvalho, et al. 2010). No sample pretreatment was applied; the limits of quantitation (LOQ) were 20 ng/L for CBZ and 30 ng/L for CAF.

The concentrations of ACE were measured with a newly developed method using liquid chromatography tandem mass spectrometry (LC-MS/MS) at the UFZ in Leipzig. No sample enrichment or other pretreatments were applied, except for the addition of isotopically labeled internal standards. The LOQs for CBZ, CAF and ACE were 1 ng/L, 80 ng/L and 20 ng/L, respectively. The same method was applied to determine the concentrations of 35 additional organic compounds, among them four of the priority compounds according to the water framework directive (atrazine, cybutryne, diuron, n-nonylphenol) as well as other pharmaceuticals, pesticides, personal care products and industrial chemicals. A detailed list of all analytes is shown in Table 1.

A total of 180 samples were measured with both the ELISA and the LC-MS/MS method, comprising left, middle and right profile samples of the 68 locations (see chapter Bacterial Faecal Indicators).

For CBZ and CAF, both methods showed a high level of correlation. For the discussion in this report, the mean value of the CBZ concentrations obtained by both methods was calculated. For CAF, only the ELISA results were used because the LC-MS/MS results suffered from a significantly higher LOQ and a lower repeatability.

Quality assurance included the analysis of blank samples that were taken at six locations along the Danube. Using distilled water produced in situ from drinking water stored in the ship tank, these blank samples were processed and stored in the same way as all other samples. Since previous studies had indicated frequent CAF contaminations in field blanks (Focazio, et al. 2008), special care was taken to minimize the risk of contamination. Still, a single blank sample contained detectable concentrations of CAF (240 ng/L) as well as the nicotine metabolite cotinine (49 ng/L). Presumably, this sample was contaminated by human contact.

Furthermore, the insect repellent diethyltoluamide (DEET) was found in all six blank samples at concentrations between 9 and 91 ng/L. Obviously, these samples were accidentally contaminated either during sampling or sample preparation. Since the concentrations of DEET found in the Danube samples were similarly elevated (6-110 ng/L), we omitted these findings.

A brief interlaboratory comparison was conducted using the analytical results obtained from the laboratories of Croatian Waters (CW, lab no. 4, see chapter "Survey preparation", CBZ and CAF), the Joint Research Centre of the European Commission (lab no. 9, CBZ only) and Zweckverband Landeswasserversorgung Langenau (ZLBF, lab no. 33, CBZ and CAF). Results from all 68 JDS sampling locations were provided, all of which were taken from the middle of the river except JDS1 (left side). Although taken approximately at the same time and location as the samples analyzed by us, these samples and the samples analyzed by us were no aliquots. Thus, small variations between the analytical results were to be expected.

In general, a sufficient correlation between our results and the results obtained in the three aforementioned labs was observed for most samples. For CAF, seven out of 68 samples differed by more than 50% compared to each of the labs. The biases found in these seven outliers were visible with both methodologies applied in our lab (ELISA and LC-MS/MS), hinting at an alteration of the sample (see above) rather than a methodological bias. The CAF results obtained from the middle of the river at stations 17, 19, 50, 51, 58, 62, 63 were therefore neglected in the discussion. For CBZ, no outlier matching the aforementioned criteria was found. No results for ACE were available to us.

Table 1: List of compounds analysed with LC-MS/MS

Analyte	CAS No.	Usage / description
Acesulfame	55589-62-3	Artificial sweetener
Acetyl-sulfamethoxazole	21312-10-7	Transformation product of sulfamethoxazole
Atenolol	29122-68-7	Pharmaceutical
Atrazine	1912-24-9	Pesticide
Bentazon	25057-89-0	Pesticide
1H-Benzotriazole	95-14-7	Corrosion inhibitor
Bisphenol S	80-09-1	Plasticizer
Caffeine	58-08-2	Stimulant
Carbamazepine	298-46-4	Pharmaceutical
Cetirizine	83881-51-0	Pharmaceutical
Chlorophene	120-32-1	Personal care product
Clofibric acid	882-09-7	Transformation product of clofibrate
Cotinine	486-56-6	Transformation product of nicotine
Cybutryne	28159-98-0	Antifouling agent
Desethylatrazine	6190-65-4	Transformation product of atrazine
Desethylterbutylazine	30125-63-4	Transformation product of terbutylazine
Desisopropylatrazine	1007-28-9	Transformation product of atrazine
Diazinone	333-41-5	Pesticide
2,4-Dichlorophenoxy acetic acid	94-75-7	Pesticide
Diclofenac	15307-86-5	Pharmaceutical
10,11-Dihydro-10,11-dihydroxycarbamazepine	35079-97-1	Transformation product of carbamazepine and oxcarbazepine
Diphenhydramine	58-73-1	Pharmaceutical
Diuron	330-54-1	Pesticide
2-Hydroxycarbamazepine	68011-66-5	Transformation product of carbamazepine
Mecoprop	93-65-2	Pesticide
Metazachlor	67129-08-2	Pesticide
5-Methyl-1H-benzotriazole	136-85-6	Transformation product of benzotriazole
2-Methyl-4-chloro-phenoxy acetic acid (MCPA)	94-74-6	Pesticide
Metolachlor	51218-45-2	Pesticide
Metoprolol	37350-58-6	Pharmaceutical
N-acetyl-4-aminoantipyrine	83-15-8	Transformation product of metamizole
4-n-Nonylphenol	104-40-5	Industrial chemical
Perfluorohexanoic acid	307-24-4	Industrial chemical
Sotalol	3930-20-9	Pharmaceutical
Sulfadimidin / Sulfamethazin	57-68-1	Pharmaceutical
Sulfamethoxazole	723-46-6	Pharmaceutical
Terbutylazine	5915-41-3	Pesticide
Tramadol	27203-92-5	Pharmaceutical

3 Results

3.1 Marker for untreated wastewater - caffeine

CAF was abundantly present in the river Danube and its tributaries (Figure 2). The median concentration found in the Danube was 93 ng/L, while a slightly higher median concentration of 132 ng/L was observed in the tributaries. These results are in good agreement with the median concentration of CAF found during the previous Danube expedition JDS 2 (80 ng/L) (Loos et al. 2008).

In Germany and most parts of Austria, CAF concentrations were below the median of the whole river at 60 and 39 ng/L, respectively. An enormous increase of the CAF concentration was observed at JDS11 (Hainburg, AT) and JDS13 (Bratislava, SK), which were both taken on the same day during intense rainfall. These high concentrations of CAF hint at the presence of large amounts of untreated wastewater, which was most likely discharged by an overflow of combined sewers in the upstream regions.

Interestingly, the CAF concentrations found at the right side of JDS13 (870 ng/L) and at the left side of JDS11 (470 ng/L) were among the highest of the entire campaign. Unfortunately, these results could not be confirmed by other participating laboratories because only samples from the middle of the river were measured. Still, the lab of Croatian Waters found a higher than average concentration of CAF of 198 ng/L at JDS11 (middle), slightly less than the result obtained by us (270 ng/L). The inhomogeneous distribution of CAF on the different banks of the river at these locations can be explained by a short-termed release of CAF from various potential point sources in the upstream regions. JDS13 was sampled six hours later than JDS11, thus a short and intense rainfall event may explain the observed differences between the two locations. Together with the input of CAF, increased concentrations of the pharmaceuticals CBZ (70 ng/L), diclofenac (120 ng/L), tramadol (80 ng/L) and others were detected in JDS11 (left), which confirms the increased presence of wastewater at this location.

The magnitude of the aforementioned rainfall event was also documented by the hydromorphological team. At these two locations, the river discharge was temporarily increased by approximately 60% compared to the discharge at JDS10 taken on the day before.

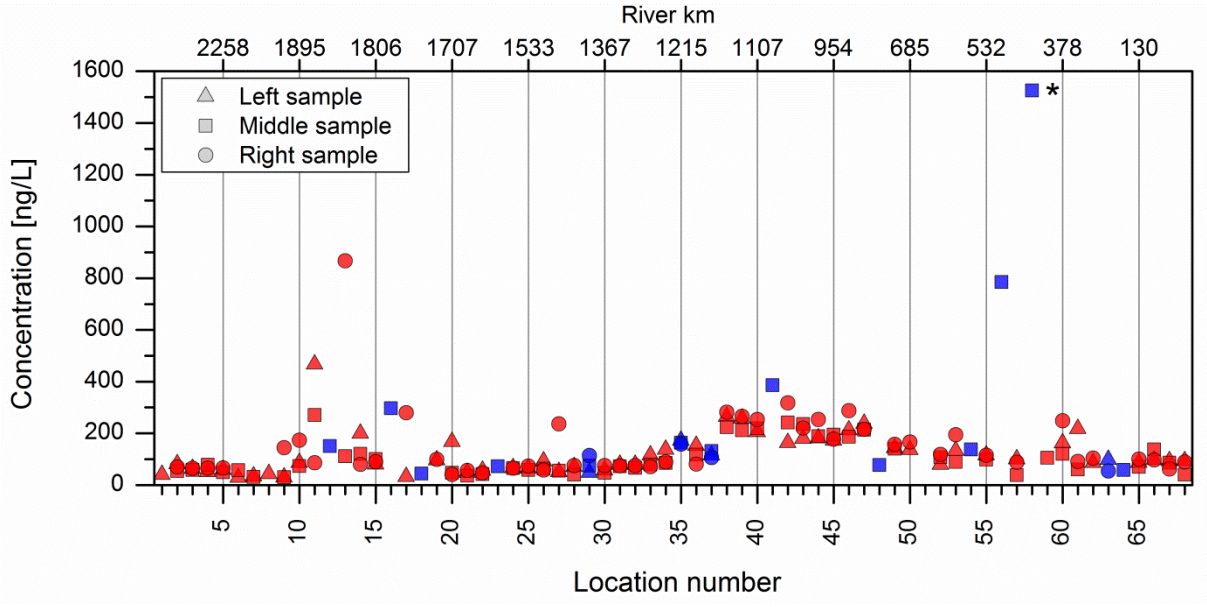

Figure 2: Caffeine concentrations in the Danube (red) and its tributaries (blue). *For JDS58, the mean result obtained by the laboratories of CW and ZLBF is shown.

Moreover, elevated concentrations of CAF were found at JDS17 (Klizska Nema, SK/HU) in the middle and on the right side of the river, while a low concentration was found on the left side. It is plausible that this increase is related to the Moson arm (JDS16, HU) re-entering the Danube from the right side 4 km earlier. The concentrations of CAF found in the Moson arm and in these Danube samples were similarly high at approximately 300 ng/L.

Beginning with JDS38 (Belgrade, RS), the CAF level raised again above 200 ng/L for the next several hundred river kilometres. This observation can be explained by the input of untreated wastewater from Belgrade, the largest Serbian city. The input of the river Sava (JDS37, RS), one of the main Danube tributaries, seems to play a minor role, as the CAF concentration was relatively low.

Furthermore, the concentration of CAF was elevated in the tributary Velika Morava (JDS41, RS). For the pharmaceutical metabolite N-acetyl-sulfamethoxazole, the highest concentration of the whole campaign (43 ng/L) was measured at this location. Like CAF, this compound is known to be well degradable during wastewater treatment confirming the presence of untreated wastewater. Entering the Danube from the right side, the input of Velika Morava resulted in increased concentrations of CAF on the right side of the Danube in the following sample JDS42 (RS).

A very high concentration of CAF (790 ng/L) was found in the tributary Russenski Lom (JDS56, BG). This river carried also high concentrations of various other compounds such as ACE, CBZ and N-acetyl-sulfamethoxazole. However, the relatively small Russenski Lom did not noticeably impact the following Danube samples.

The highest CAF concentration of the entire campaign was found in the river Arges (JDS58, RO). Concentrations of 1.25 µg/L and 1.8 µg/L were reported by the laboratories of CW and ZLBF (see section Methods), indicating a high level of untreated wastewater. In agreement with this observation, the highest load of microbiological faecal pollution in the whole river basin was found in this tributary (see chapter Bacterial Faecal Indicators). Furthermore, for numerous compounds the highest concentrations of the entire campaign were found in this river, such as N-acetlyaminoantipyrine (1500 ng/L), metoprolol (820 ng/L), diclofenac (320 ng/L), sulfamethoxazole (210 ng/L), CBZ (130 ng/L) and atrazine (70 ng/L). Therefore, the Arges was identifed as the river with the highest relative portion of untreated wastewater during this survey.

In conclusion, Figure 3 shows the median concentrations of CAF found in each country, in relation to the percentage of secondary and tertiary wastewater treatment. Due to the relatively low level of wastewater treatment, the highest median concentration was determined in the Serbian part of the river. The lowest median concentrations were found in Austria and Germany, owed to their high level of wastewater treatment.

Despite the high level of wastewater treatment, the concentrations of CAF in the Slovakian part of the Danube (SK) were relatively high. In this region, the input of wastewater was presumably temporally elevated due to a massive rainfall event during sampling resulting in combined sewer overflows.

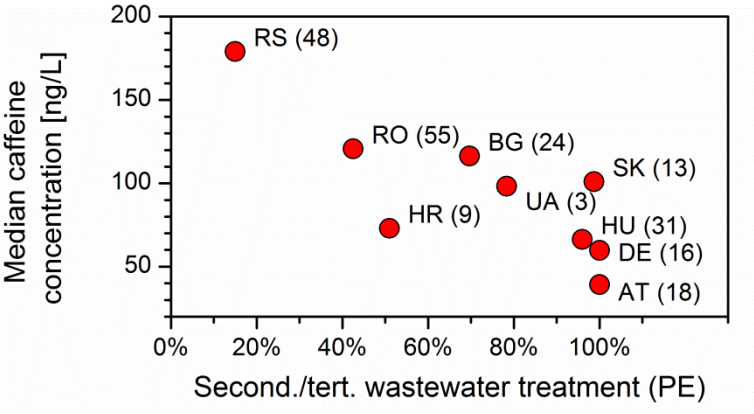

Figure 3: Median caffeine concentrations in the countries in the Danube basin (number of samples in brackets).

3.2 Markers for treated and untreated wastewater – carbamazepine and acesulfame

The two anthropogenic markers for treated wastewater, the pharmaceutical CBZ and the artificial sweetener ACE, were found in all analysed samples from the Danube and its tributaries. The median concentration of CBZ was 30 ng/L in the Danube and 40 ng/L in the tributaries (Figure 4). This is in good agreement with the results of the last expedition JDS 2, where a median of 37 ng/L was reported (Loos et al. 2008). The artificial sweetener, which was not analysed during the last expedition, was found at median concentrations of 460 ng/L in the Danube and 470 ng/L in the tributaries (Figure 5).

Along the whole river span, the level of CBZ stayed rather constant at concentrations between 20 and 50 ng/L, showing much less variation than the level of CAF, as described in the previous section. An exception to the homogeneous distribution of CBZ was found at JDS11 (Hainburg, AT) with an elevated concentration of 70 ng/L on the left side, probably as a result of a combined sewer overflow (see above). Interestingly, the concentration of ACE was not significantly increased at this site.

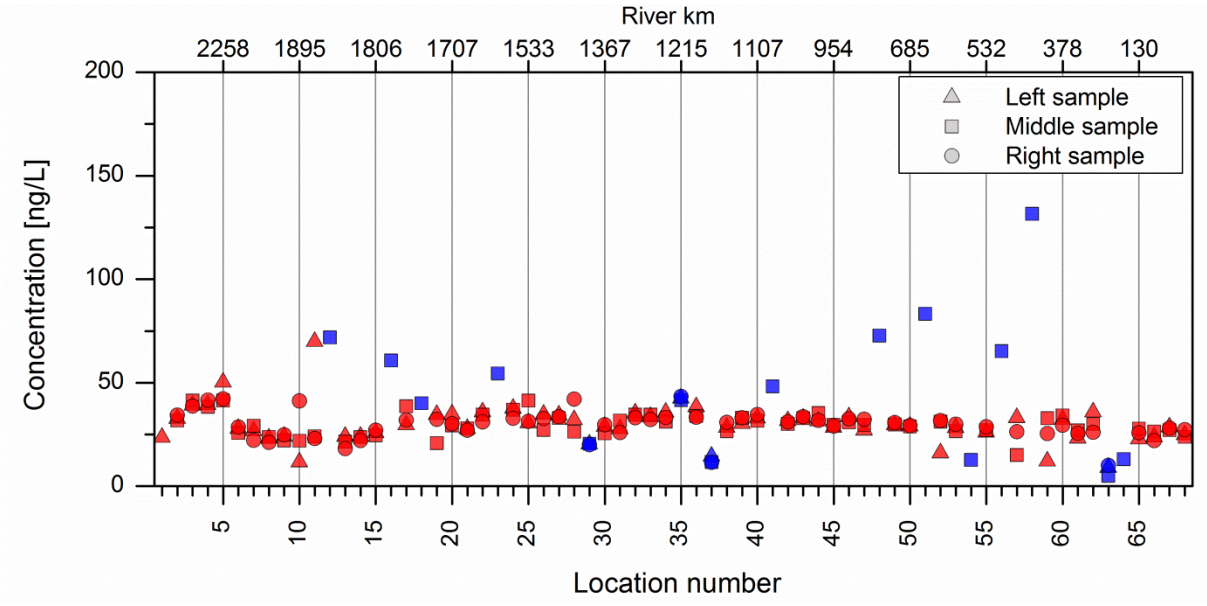

Figure 4: Carbamazepine concentrations in the Danube (red) and its tributaries (blue).

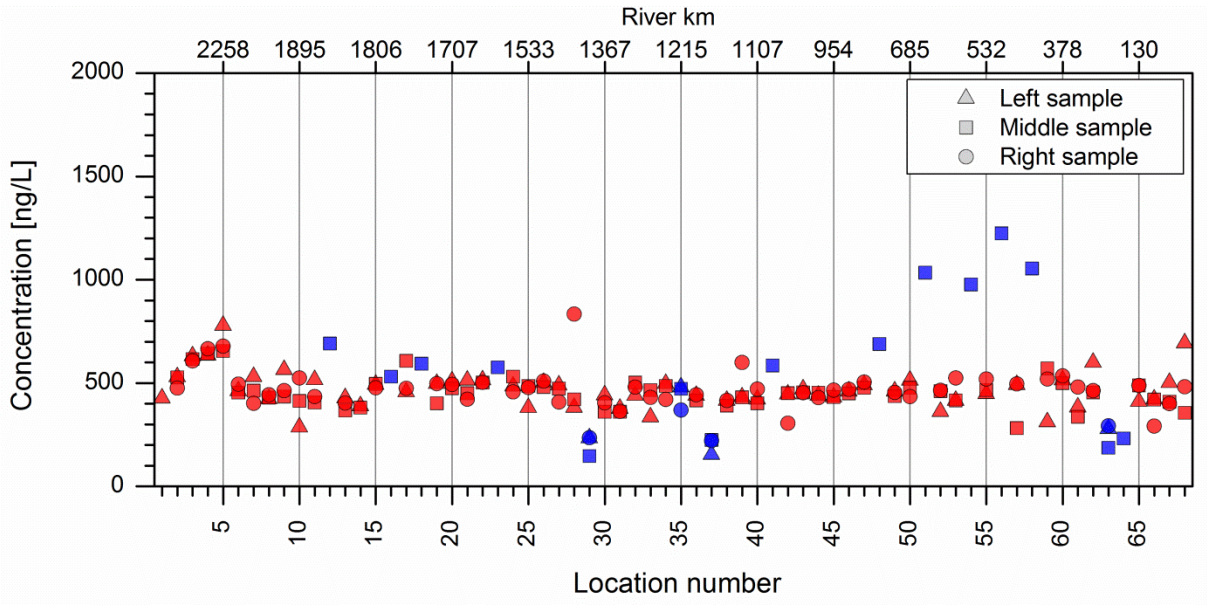

Figure 5: Acesulfame concentrations in the Danube (red) and its tributaries (blue).

The highest concentration of ACE in the Danube was found at JDS28 (HR) on the right side with a concentration of 830 ng/L, while the concentration of CBZ was only slightly increased to 42 ng/L at this location.

In the tributaries, the highest concentration of CBZ was found in the river Arges (JDS58, RO) with 130 ng/L. Elevated concentrations were also found in the rivers Morava (JDS12, SK), Timok (JDS48, RS/BG), Iskar (JDS51, BG) and Russenski Lom (JDS56, BG), while the concentrations in the rivers Sava (JDS37, RS), Jantra (JDS54, BG), Siret (JDS63, RO) and Prut (JDS64, RO/MD) were lower compared to the Danube.

The highest overall concentration of ACE was found in the river Russenski Lom (JDS56, BG) with 1200 ng/L, while the lowest concentrations were present in the rivers Drava (JDS29, HR), Sava (JDS37, RS), Siret (JDS63, RO) and Prut (JDS64, RO/MD).

In general, similar results were obtained for the two markers CBZ and ACE. For both compounds, high concentrations were found in several tributaries. This indicates a high percentage of wastewater in these rivers. In a few cases, the results obtained for ACE and CBZ seem to be contradictory, e.g. in JDS54 a low concentration of CBZ was found, while the concentration of ACE was high. However, variations in the ratio between CBZ and ACE can be explained by local differences, e.g. the presence (or absence) of hospitals in specific river segments, different usages in each country, etc. In conclusion, a low concentration of one of the markers does not necessarily mean the absence of wastewater.

4 Conclusions

The analytical results obtained for CAF and CBZ were in good agreement with the previous Joint Danube Survey. The concentrations obtained for CBZ and ACE were similar to other European streams like Elbe or Rhine. The concentrations of CAF, the marker for untreated wastewater, were considerably higher in the middle and lower sections of the Danube, compared to Elbe and Rhine. This indicates a higher amount of untreated wastewater present in the Danube which can be attributed to the lower level of wastewater treatment in this river basin. For the four priority compounds analysed (atrazine, cybutryne, diuron, n-nonylphenol), the EQS was not exceeded for any of the 180 samples. As there are currently no EQS defined for the three marker substances, the findings shown in this report imply no need for direct action according to EU legislation. Nevertheless, the discharge of untreated wastewater poses a considerable risk for the environment as numerous substances with known (and unknown) toxicity along with microbial contamination enter the river.

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