

# 3

## **Correlation of six anthropogenic markers in waste water, surface water, bank filtrate, and soil aquifer treatment**

Six trace contaminants (acesulfame (ACE), sucralose (SUC), carbamazepine (CBZ), diatrizoic acid (DTA), 1*H*-Benzotriazole (BTZ) and its 4-methyl analogue (4-TTri)) were traced from wastewater treatment plants (WWTPs) to receiving waters and further to river bank filtration (RBF) wells to evaluate their prediction power as potential wastewater markers. Furthermore, the persistence of some compounds was investigated in advanced wastewater treatment by soil aquifer treatment (SAT). During wastewater treatment in four conventional activated sludge WWTPs ACE, SUC, and CBZ showed a pronounced stability expressed by stable concentration ratios in influent (in) and effluent (out) (ACE/CBZ: in45, out40; SUC/CBZ: in1.8, out1.7; ACE/SUC: in24, out24). In a fifth WWTP, additional treatment with powdered activated carbon led to a strong elimination of CBZ, BTZ, and 4-TTri of about 80 % and consequently to a distinctive shift of their ratios with unaffected compounds. Data from a seven month monitoring program at seven sampling locations at the rivers Rhine and Main in Germany revealed the best concentration correlation for ACE and CBZ ( $r^2=0.94$ ) and also a good correlation of ACE and CBZ concentrations to BTZ and 4-TTri levels ( $r^2 = 0.66$  to  $0.82$ ). The comparison of ratios at different sampling sites allowed for the identification of a CBZ point source. Furthermore, in Switzerland a higher consumption of SUC compared to Germany can be assumed, as a steadily increasing ACE/SUC ratio along the river Rhine was observed. In RBF wells a good correlation ( $r^2=0.85$ ) was again observed for ACE and CBZ. Both also showed the highest stability at a prolonged residence time in the subsurface of a SAT field. In the most peripheral wells ACE and CBZ were still detected with mean values higher than  $36 \mu\text{g/L}$  and  $1.3 \mu\text{g/L}$ , respectively. Although SUC concentrations in wastewater used for SAT decreased by more than 80 % from about  $18 \mu\text{g/L}$  to  $2.1 \mu\text{g/L}$  and  $3.5 \mu\text{g/L}$  in these outlying wells, the compound was still adequate to indicate a wastewater impact in a qualitative way.

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### 3.1 Introduction

One of the main objectives of drinking water suppliers is to be aware of the influences on their raw water sources used for drinking water production to minimize water treatment effort and to ensure potable water of high quality. This is of particular importance, if an anthropogenic impact on raw water sources by diluted wastewater is given to a certain extent, *e.g.* when river bank filtrate is used as raw water. Therefore, it is necessary to identify markers that are able to provide an early indication of contamination and which can be used for the quantification of the wastewater burden. Furthermore, wastewater tracers can also be used to assess the hydrological fate of biodegradable compounds. The differentiation between transformation and dilution allows for the evaluation of purification processes during river bank filtration (RBF) or soil aquifer treatment (SAT).

A conservative tracer for wastewater impact should neither tend to degrade nor to adsorb. It must be source-specific for wastewater treatment plant (WWTP) effluents and has to be unaffected by the treatment steps applied in the WWTP. Furthermore, it should be persistent in the receiving waters and production wells, respectively. Both, a high effluent concentration as well as a sensitive analytical method, which allows low limits of quantification (LOQ), are also advantageous. For wells unaffected by wastewater the capability of an early detection of traces of the compound can be used as an early warning system of a wastewater breakthrough, *e.g.* when the pumping regime is altered or a leakage in the sewer system occurs.

Microbial indicators for water contamination lack selectivity and are often time-consuming in analysis (Glassmeyer et al., 2005). The approach of using trace elements (Babcock et al., 2006; Schreiber and Mitch, 2006) or their ratios (Vengosh and Pankratov, 1998; Rabiet et al., 2005; Alcalá and Custodio, 2008) as conservative tracers raises the problem of distinguishing between their natural background concentrations and anthropogenic origin. A high reliability is only given when background levels are low and differences between their natural presence and wastewater borne concentrations are high.

Therefore, organic trace pollutants with anthropogenic origin have become more and more popular as markers for wastewater impact in the last decade. Caffeine was suggested by several authors (Seiler et al., 1999; Buerge et al., 2003b) due to high concentrations reported for WWTP influents, but its good biodegradability and sorption behaviour seem to limit its use as a potential marker for domestic wastewater. Schramm et al. (2006) reported about compost piles and the agricultural use of cesspool matter as another source of caffeine in addition to WWTP effluents.

Polycyclic musks and nicotine derivatives were also presented as possible markers for domestic activities. The latter showed a high biodegradability in WWTPs but a good correlation was observed between the expected anthropogenic burden and the measured concentrations of the nicotine derivatives in Swiss lakes (Buerge et al., 2008). Polycyclic musks showed the correlation only in winter time whereas a significant loss in the summer occurred in the epilimnion due to their photochemical lability (Buerge et al., 2003a).

Pharmaceuticals and personal care products (PPCP) were also extensively surveyed (Nakada et al., 2008; Kahle et al., 2009; Kasprzyk-Hordern et al., 2009) as their occurrence in water bodies is mostly evoked by human usage. Diatrizoic acid (DTA), an X-ray contrast medium, was one of the most promising candidates among these compounds as it was found to be stable during wastewater treatment and simulated biological sewage treatment (Ternes and Hirsch, 2000; Haiss and Kümmerer, 2006). Another PPCP, which already proved its suitability as a possible wastewater marker due to a pronounced persistence in the aquatic environment, is the antiepileptic drug carbamazepine (CBZ) (Clara et al., 2004). Gasser et al. (Gasser et al., 2010) successfully used carbamazepine to calculate and estimate the impact on drinking water wells by infiltrated wastewater. An approach using benzotriazoles as tracers for domestic wastewater also seems to be promising. They are widely used as corrosion inhibitors, *e.g.* in anti-freezing and deicing fluids as well as in dishwashing liquids (Hart et al., 2004). One of the main benzotriazole chemicals is 1*H*-benzotriazole (BTZ). Its elimination during wastewater treatment is limited and high concentrations of several tens of µg/L in WWTP effluents were reported (Voutsas et al., 2006; Reemtsma et al., 2010). Consequently, BTZ was found in the range from several hundred ng/L in the Danube basin (Loos et al., 2010) up to several µg/L in a Swiss river (Giger et al., 2006). BTZ shows only little sorption onto soils (Jia et al., 2007) and biodegradation in the subsurface is slow as the compound was found in RBF production wells after a travel time of several months (Weiss et al., 2006). The 4-methyl analogue (4-methylbenzotriazole, tolyltriazole, 4-TTri) is even more persistent in the aquatic environment (Weiss et al., 2006).

In recent publications artificial sweeteners (ASs) have emerged as a new class of compounds applicable as anthropogenic markers (Buerge et al., 2009; Scheurer et al., 2009). Especially acesulfame (ACE) and sucralose (SUC), two sweeteners, which are persistent during wastewater treatment, were suggested as tracers. High concentrations of ACE of several µg/L in surface water, groundwater, and wells at river bank filtration sites support this approach. SUC seems to be persistent to a similar extent, but its usage within Europe is rather

inhomogeneous based on concentrations measured in rivers and streams (Loos et al., 2009) and generally lower than in the USA.

The aim of this study was to prove the suitability of artificial sweeteners as wastewater markers and to assess their prediction power compared to other organic trace pollutants, in particular to CBZ and BTZ. For this reason we focused not only on one aquatic compartment but followed the ratio of several potential wastewater tracers from their source to receiving waters, RBF, and recovery wells of a SAT site, where treated wastewater is infiltrated.

## **3.2 Experimental**

### **3.2.1 Materials**

Chemical standards were purchased from the following suppliers: DTA (purity 100 %), CBZ, (analytical grade) and BTZ (99 %) from Sigma Aldrich (Steinheim, Germany), DTA-d<sub>6</sub> (98 %) from LGC (Luckenwalde, Germany); ACE potassium (99 %), SUC (>98 %) and CBZ-d<sub>10</sub> (>99 %) from Dr. Ehrenstorfer GmbH (Augsburg, Germany), ACE-d<sub>4</sub> (98 %), SUC-d<sub>6</sub> (98 %), BTZ-d<sub>4</sub> (98 %) and 4-TTri-d<sub>3</sub> (98 %) from Campro Scientific GmbH (Berlin, Germany), 4-TTri (98 %) from Chemos (Regenstauf, Germany).

### **3.2.2 Analytical methods**

All compounds were pre-concentrated using solid phase extraction (SPE) and analyzed by high-performance liquid chromatography (HPLC) coupled with tandem mass spectrometry (MS/MS). Samples were pH adjusted in the lab at the day of extraction. Corresponding internal standards for all analytes were spiked into the samples before SPE and used for quantification. Limits of quantification (LOQ) were 10 ng/L in all cases, except for SUC (50 ng/L). The sample preparation and analysis of ASs were described previously in detail by Scheurer et al. (2009).

For DTA analysis 200 mL of a water sample (pH 3) were pre-concentrated using Bakerbond SDB 1 cartridges (200 mg / 6 mL from J.T. Baker, Deventer, The Netherlands). After extraction the cartridges were eluted with 5 mL methanol and 5 mL acetonitrile. The eluate was evaporated to dryness and reconstituted with 500 µL ultra pure water. The compound was separated by a Finnigan Surveyor HPLC on a Hypersil Gold column (150 mm x 2.1 mm; 3 µm) (both Thermo Scientific, Waltham, USA). The HPLC system was

connected to a TSQ Quantum Ultra mass spectrometer (Thermo Scientific, Waltham, USA) operated in positive ionization mode.

For the pre-concentration of CBZ the same conditions as described above were applied, with the exception of a higher sample volume of 1 L. After extraction the cartridges were eluted with 10 mL acetone, the eluate was evaporated to dryness and reconstituted with 50  $\mu$ L methanol followed by 50  $\mu$ L ultra pure water. The compounds were separated by an Agilent 1100 HPLC (Agilent Technologies, Waldbronn, Germany) on a Luna C18 column (250 mm x 2 mm; 5  $\mu$ m) (Phenomenex, Aschaffenburg, Germany). An API 2000 mass spectrometer (AB Sciex, Foster City, USA) was used for detection and quantification of CBZ.

For BTZ and 4-TTri analyses a 10 mL water sample (pH 7) was extracted using Bond Elut PPL cartridges (200 mg / 3 mL from Varian, Palo Alto, USA). For elution of the analytes 6 mL methanol were used. After solvent exchange into 100  $\mu$ L ultra pure water the compounds were separated by an Agilent 1200 HPLC (Agilent Technologies, Waldbronn, Germany) on a Kinetex PFP column (100 mm x 2.1 mm; 2.6  $\mu$ m) (Phenomenex, Aschaffenburg, Germany). The HPLC system was connected to an API 4000 mass spectrometer (AB Sciex, Foster City, USA) operated in positive ionization mode.

For further details on chromatographic conditions see supplementary information in chapter 3.6.

### 3.2.3 Sampling sites and protocol

*Wastewater* Five WWTPs in Southwest Germany were sampled between May and July 2010. Samples for all WWTPs were corresponding 24 h composite samples. WWTP influents and effluents were stabilized by 100 mg/L  $\text{NaN}_3$ , stored at 4 °C and analyzed within two days after sampling.

WWTP 1 is applying conventional, *i.e.* mechanical and biological, treatment. It has a capacity of 20,000 population equivalents (PE) with about 15,000 inhabitants living in the catchment area and treats 2,500 to 3,500  $\text{m}^3/\text{d}$  of domestic wastewater. Hydraulic retention time is about 5 h with an average sludge retention time of 20 d. WWTP 2 treats 40 million  $\text{m}^3$  (capacity 875,000 PE) per year for about 350,000 inhabitants. It applies mechanical treatment with additional phosphate precipitation, followed by biological treatment with a denitrification/nitrification unit, equipped with a trickling filter. Hydraulic retention time is about one day for dry weather conditions. WWTP 3 has a rather high industrial impact and is designed for 75,000 PE with about 35,000 people living in the catchment area. The dry weather flow is approximately 12,000  $\text{m}^3/\text{d}$  but was 28,500  $\text{m}^3/\text{d}$  at the day of sampling due to

heavy rain. Serving 12,000 people, WWTP 4 was the smallest of all WWTPs under investigation. The residence time in WWTP 4 is about 48 h. Therefore, influent and effluent 24 h composite samples are non-corresponding samples. As WWTP 4 was also sampled after a night of heavy rain this effect is even more pronounced. In WWTP 5 a new approach for the effective removal of organic trace pollutants during wastewater treatment is applied. The conventional mechanical and biological treatment is followed by the addition of 5-20 mg/L powdered activated carbon (PAC). After a contact time of >30 min a flocculant agent is dosed and the wastewater is directed to a sedimentation pond, from where some of the PAC is pumped back to the reactor. After sedimentation aluminum salt is added as a second flocculant and the remaining PAC is removed in a two-layer sand filter. WWTP 5 treats 380,000 PE with 220,000 residents living in the catchment area.

*Surface water and river bank filtrate* Samples from the rivers Rhine and Main were taken as grab samples between January and July 2010. The water was filled into 1 L brown glass bottles and cooled at 4 °C until analysis. Sampling locations were the cities of Basel (km 163.9), Karlsruhe (km 359.3), Mainz (km 500.6), Cologne (km 685.8), Düsseldorf (km 732.1), and Lobith (km 865) along the Rhine. The sampling point at the river Main was 30.1 km upstream from the confluence of Rhine and Main (Figure 3-6). The sampled RBF wells were all located at the river Rhine. They yield exclusively bank filtrate or a mixture of bank filtrate and landside groundwater. Measurements of redox sensitive parameters by the water suppliers confirmed aerobic conditions.

Other data used for evaluation were taken from Bayerisches Landesamt für Umwelt (LfU Bavaria, 2010) which sampled surface and well water in September 2009. Only those samples whose values for all compounds were >LOQ were taken into account. An evaluation of 4-TTri was not possible here, as a mixture of the methyl isomers was measured.

*Soil aquifer treatment* The SAT site is located in Israel and treats the secondary effluent from a WWTP that processes over 100 million m<sup>3</sup>/a of wastewater. Treatment includes mechanical treatment, followed by conventional activated sludge treatment including nitrification/denitrification and a limited biological phosphorous removal. The WWTP effluent is spread intermittently in percolation basins, where it infiltrates through an unsaturated zone, up to 40 m in depth. The effluent flows lateral in the saturated zone to observation and recovery wells located in a circle of up to 1,000 m in the periphery of the percolation basins. Samples from the SAT site were taken in the years 2009 to 2010 and

comprised the WWTP effluent used for aquifer recharge, a sampling point located vertically below the percolation basin (V1) and four sampling points in the periphery, two in the west (W1 and W2) and two in the east (E1 and E2) of the percolation basin sampling site. Right after sampling the samples were cooled and shipped to Germany for analysis.

### **3.3 Results and discussion**

#### **3.3.1 Wastewater**

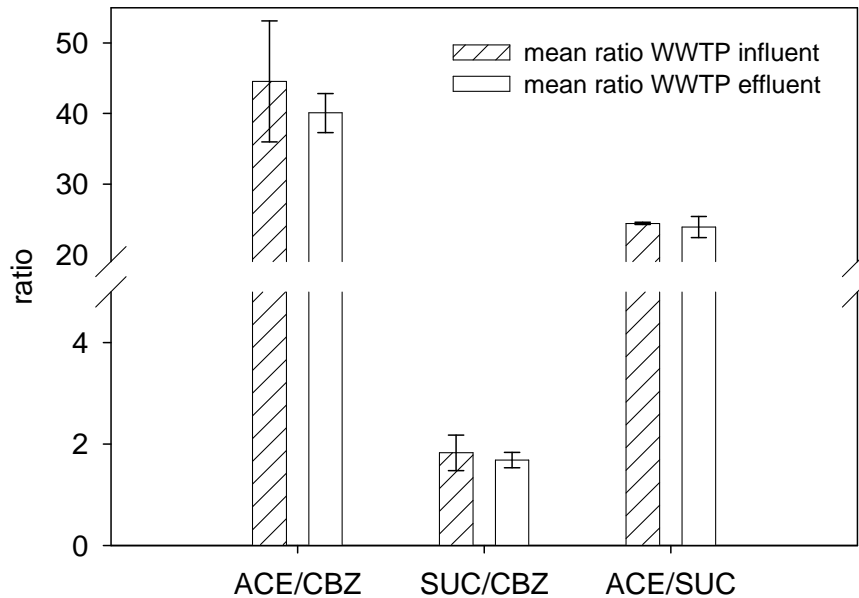
Six organic trace contaminants CBZ, DTA, ACE, SUC, BTZ, and 4-TTri were analyzed in the sampled wastewater to investigate their suitability as wastewater tracers (Table 3-1). WWTP 1 and 2 were sampled during dry weather conditions in contrast to WWTP 3 and 4 where samples were collected after a night of heavy rain resulting in an almost doubled throughput. This is reflected by the influent concentrations of CBZ, ACE, and SUC in WWTP 3 and 4 which are only about 50% of the levels in the first two WWTPs.

Our results show that the concentration ratios of these three compounds are independent within the investigated German WWTPs. Due to constant concentrations during wastewater treatment, each individual ratio among two of those three compounds in WWTPs 1–4 was neither affected by the applied treatment technologies nor by any other boundary condition (Figure 3-1). A concentration change along the treatment train resulting in lower (or even higher) effluent concentrations is due to changing influent concentrations, and other uncertainties rather than to degradation.



**Table 3-1** Influent and effluent data of six potential wastewater tracers in five German municipal WWTPs (concentrations rounded to two significant digits)

WWTP (population served)	throughput m <sup>3</sup> /d	influent concentration µg/L	calculated consumption t/a in Germany	effluent concentration µg/L	elimination %	input in receiving rivers t/a in Germany
WWTP 1 (350,000)	70,000					
acesulfame		31	190	24	25	140
sucralose		1.3	7.8	1.1	15	6.6
carbamazepine		0.71	4.3	0.60	16	3.6
diatrizoic acid		4.4	26	2.3	48	14
1 <i>H</i> -benzotriazole		10	60	5.1	49	30
4-methylbenzotriazole		4.2	25	2.8	33	17
WWTP 2 (15,000)	3,300					
acesulfame		37	240	39	-5	250
sucralose		1.5	9.9	1.53	-1	10
carbamazepine		1.2	7.9	1.0	17	6.6
diatrizoic acid		0.71	4.7	0.40	44	2.6
1 <i>H</i> -benzotriazole		12	79	1.8	85	12
4-methylbenzotriazole		5.3	35	2.9	45	19
WWTP 3 (40,000)	28,500					
acesulfame		18	370	19	-9	400
sucralose		0.71	15	0.77	-9	16
carbamazepine		0.35	7.5	0.42	-20	9
diatrizoic acid		4.3	92	2.8	35	60
1 <i>H</i> -benzotriazole		4.8	103	3.7	23	80
4-methylbenzotriazole		2.2	47	2.3	-5	49
WWTP 4 (12,000)	6,250					
acesulfame		17	270	19	-9	290
sucralose		0.7	11	0.77	-10	12
carbamazepine		0.32	5.0	0.50	-56	7.8
diatrizoic acid		<LOQ	-	0.06	-	0.9
1 <i>H</i> -benzotriazole		4.5	70	2.0	56	31
4-methylbenzotriazole		1.8	28	2.2	-22	34
WWTP 5 (220,000)	151,000					
acesulfame		8.2	170	11	-34	230
sucralose		0.44	9	0.44	0	9.1
carbamazepine		0.33	6.8	0.053	84	1.1
diatrizoic acid		2.2	45	2.9	-32	60
1 <i>H</i> -benzotriazole		3.9	80	0.87	78	18
4-methylbenzotriazole		2.4	49	0.33	86	7



**Figure 3-1** Mean ratios of ACE/CBZ, SUC/CBZ, and ACE/SUC based on the influent and effluent concentrations in WWTPs 1-4. Error bars indicate standard deviations

The highest DTA influent concentrations ( $4.4 \mu\text{g/L}$  and  $4.3 \mu\text{g/L}$ ) were measured in WWTP 1 and WWTP 3 which serve urbanized areas with several hospitals in the case of WWTP 1 and one in the case of WWTP 3 in the catchment area. X-ray contrast media are expected to be used to a much higher extent in hospitals than by physicians in private practice which can be an explanation for the higher values. Even if DTA is source specific to WWTPs and only partly degraded during wastewater treatment, its general use as a tracer seems to be limited and it cannot be used to predict the concentration of persistent trace pollutants. In fact, DTA is suitable to indicate a wastewater impact but the ratio to other organic trace pollutants will vary to a great extent as the usage of DTA depends on its application in the catchment area.

In WWTP 5 a significant shift of the ACE/CBZ and SUC/CBZ ratios from the influent to the effluent was observed. After the secondary sedimentation basin CBZ was still detected with a concentration of  $0.38 \mu\text{g/L}$  (Table 3-5), indicating no biological degradation but a removal (84%) by activated carbon alone (Table 3-1). The input per year of CBZ in the receiving river, calculated by specific throughput conditions at the sampling day, is reduced from 21 kg to less than 3 kg in the case of WWTP 5. PAC seems to be a promising tool not only for minimizing the input of dissolved organic carbon into rivers and streams, but in the case of CBZ, also for organic trace contaminants which proved to be persistent in the aquatic environment. ASs were not affected by the PAC application and the ACE/SUC effluent ratio of 25 is in good correlation to the mean ratio for WWTPs1-4 effluents ( $23.9 \pm 1.5$ ). A good SUC removal by activated carbon during drinking water production has been reported

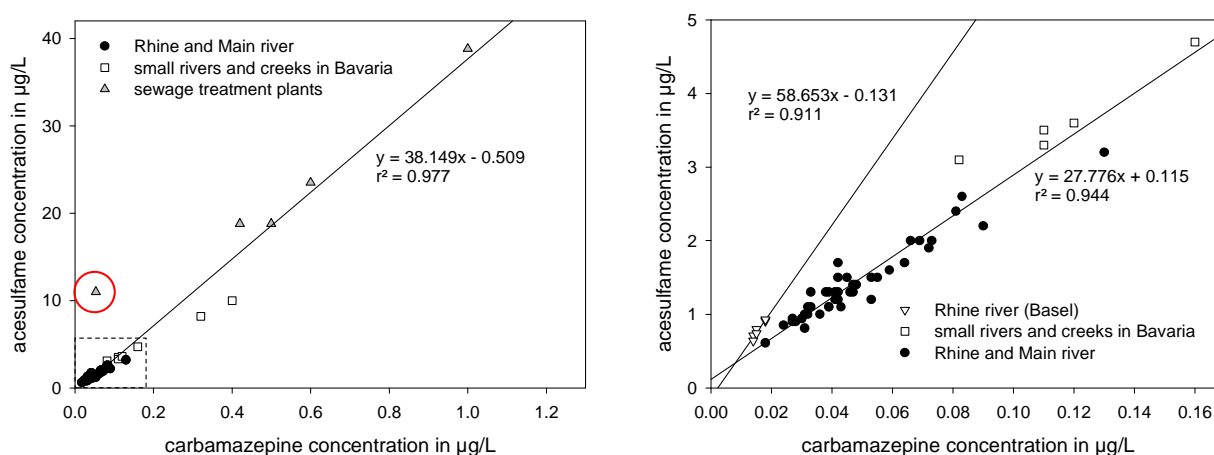
(Scheurer et al., 2010). It appears that in wastewater treatment a pronounced competition for activated carbon sorption sites occurs and SUC tends to adsorb less strongly than many other wastewater constituents based on its physical-chemical properties.

It has to be noted that presently the application of activated carbon for wastewater treatment is not very common but steadily increasing (*e.g.* WWTP 2 decided to implement this approach in the near future). A widespread usage of activated carbon in WWTPs will consequently lead to a shift in the ratio of some organic trace pollutants which has to be considered in data interpretation in the future. Other treatments like ozonation discussed for the application in WWTPs can also lead to a distinctive transformation of certain compounds, *e.g.* ACE was transformed to a great extent during ozonation in drinking water treatment plants (Scheurer et al., 2010).

### 3.3.2 Surface water and river bank filtrate

The linear regression displayed in Figure 3-2 is based on all sampling sites along the river Rhine and Main (except for the one in Switzerland, see explanatory statement below), surface water data from LfU Bavaria and wastewater effluent samples. The data for CBZ and ACE correlate well ( $r^2 = 0.977$ ) over the whole sampling period, although we measured values within a broad concentration range from 0.61 µg/L to 3.2 µg/L for ACE along the Rhine and Main rivers (image section of Figure 3-2). The slope of the linear regression indicates that about 28 times more ACE than CBZ was found on average in rivers. The concentrations of the data taken from LfU Bavaria are generally higher than the ones obtained for the river Rhine, where a reasonable dilution factor of the WWTP effluent concentrations of more than ten was observed. The small creeks in Bavaria have a higher wastewater burden due to a lower dilution in comparison to the Rhine, but the data fit to the regression is excellent. The slope of the linear regression when including data of the effluents of WWTPs 1-4 is about 38 which represents a higher ACE/CBZ ratio, although the same ratio in WWTP effluents and receiving rivers can be expected assuming a pronounced stability of ACE and CBZ, respectively. A possible explanation for this phenomenon is a higher consumption of ACE in the summer months as it is one of the main sweeteners in soft drinks. All WWTPs were sampled between May and July. However, data for river water comprise the first seven months in 2010 including rather cold periods when hot beverages are preferred, which are sweetened (when sweetened at all with ASs) more likely with cyclamate and saccharin, which are typical ASs in tabletop sweeteners. To confirm this assumption we used official stream flow values for the sampling point in Basel (values for Germany will not be published before

2011) to calculate the loads of ACE (see supplementary information Table 3-6). Comparing the daily load of the particular days of sampling an increase of more than 30 % to 70 kg/d in June and July compared to the winter months was observed for this sampling point. When using the ratios of two compounds as prediction tool for one of them, it seems that a seasonal variation has to be considered for certain compounds like ASs. The results should be confirmed by long term statistical series or by systematic seasonal sampling of wastewater to prove that the load of ACE is linked to the consumption of certain products based on the ambient temperature.

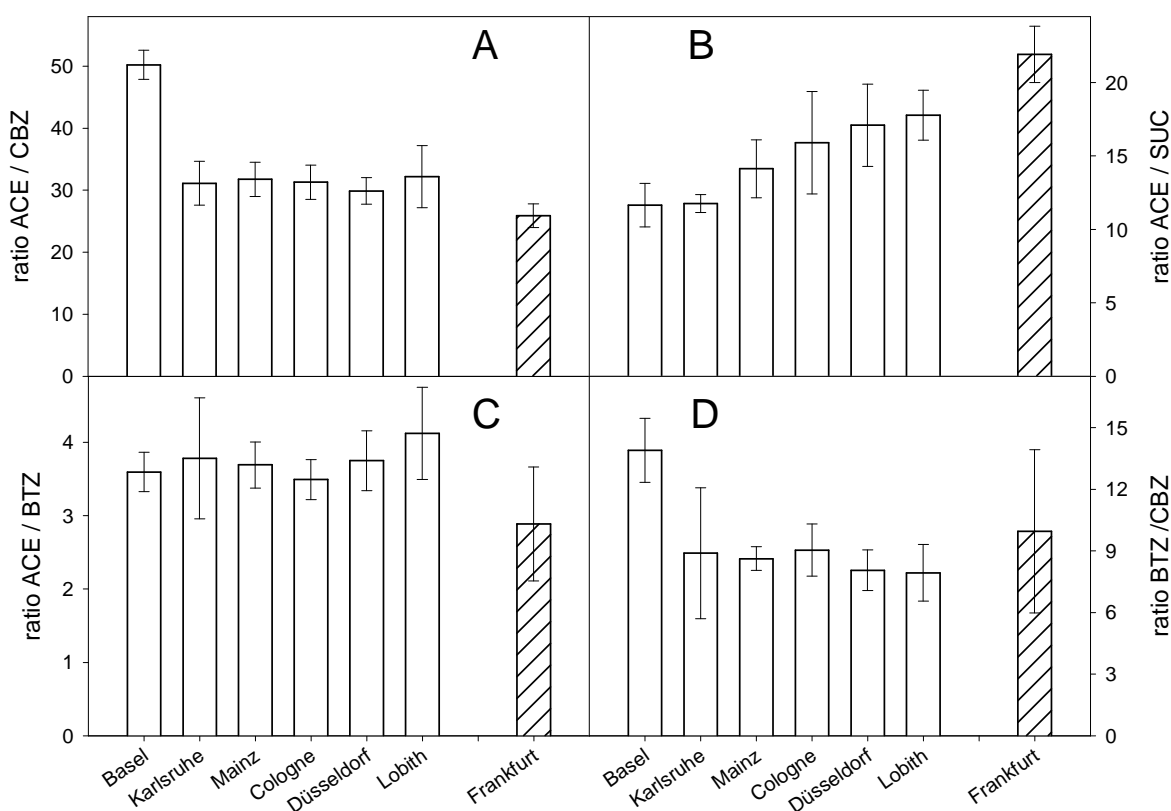


**Figure 3-2** ACE over CBZ concentrations of six surface water sampling sites along the rivers Rhine and Main (each  $n=7$ ), comparative surface water data obtained by LfU Bavaria and effluent concentrations of five WWTP. Circle indicates the application of powdered activated carbon in WWTP 5 (data point not included in linear regression (left)). Image section with corresponding linear regression including the additional sampling point in Basel, Switzerland (right)

The sampling point in Switzerland was not included in the linear regression displayed in Figure 3-2 because the ACE/CBZ ratio was about 59 and thus significantly different from the ratio of 28 along the river Rhine (Figure 3-3 A). Close upstream of the sampling point in Basel a leading producer and supplier of vitamins, personal care products, and pharmaceuticals is located, which we first suspected to be a point source of ACE. It was noticeable that the shifted ratio evened out completely already at the next sampling point downstream (Karlsruhe, distance about 200 km), although the increase in river flow is negligible. Therefore, we examined the shift of these potential tracers *versus* another rather persistent trace pollutant, BTZ (for a graphical presentation of the linear regression of ACE vs BTZ and CBZ vs BTZ see Figure 3-7 supporting information). The results showed almost no change in the ACE/BTZ ratio along the river Rhine sampling locations, including Basel (Figure 3-3 C). However, for the BTZ/CBZ ratio a sharp drop between the sampling points Basel and

Karlsruhe was evident (Figure 3-3 D), indicating a point source for CBZ in between as the only reason for the decrease of the ACE/CBZ ratio. The data set for the three ratios (ACE/CBZ, ACE/BTZ, and BTZ/CBZ) was statistically analyzed with the Tukey-Test ( $p < 0.05$ ) and revealed also a significant difference for the sampling point in Basel to all other sampling sites along the river Rhine for the ACE/CBZ and BTZ/CBZ ratios (Figure 3-8). In contrast, the Rhine sampling stations did not differ in the case of the ACE/BTZ ratio based on the same test.

The area of the CBZ input could be further localized by evaluating data from the Rhine monitoring station near the city of Basel, which is located downstream of our sampling point in Basel. At that station, CBZ was measured two to eight times per month between January and July 2010 with a mean value of  $0.0281 \pm 0.0082 \mu\text{g/L}$  (Basel-Stadt, 2010). This value is very similar to the mean concentration of our next sampling location (Karlsruhe;  $0.0287 \pm 0.0071 \mu\text{g/L}$ ) and significantly higher than the one in Basel ( $0.0157 \pm 0.0017 \mu\text{g/L}$ ) narrowing the point source of CBZ down to the area of Basel.

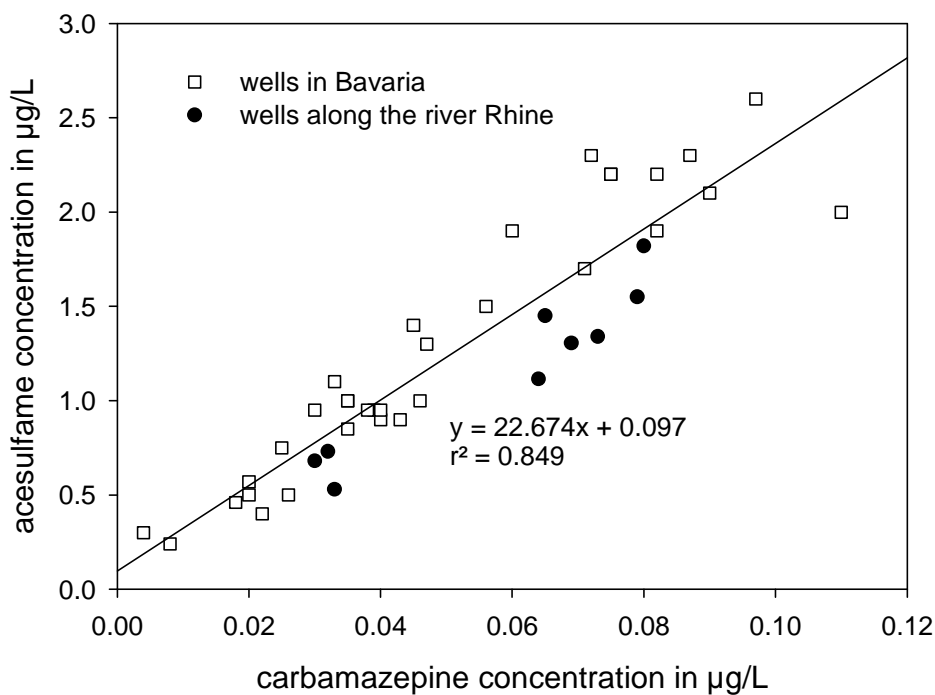


**Figure 3-3** Ratios of ACE/CBZ (A), ACE/SUC (B), ACE/BTZ (C), and BTZ/CBZ (D) of six sampling points along the river Rhine (white bars) and at the river Main (striped bars), error bars indicating standard deviation. All sampling points  $n=7$

Contemplating the ACE/SUC ratio (Figure 3-3 B), we observed that SUC seems to have a higher per capita consumption in Switzerland than in Germany based on the lower ratio. This effect was clearly leveling along the Rhine, most obviously at the sampling point Frankfurt/Main where no Swiss influence exists. Generally speaking, values from one sampling site to another directly downstream or upstream did not differ significantly, but from all others located further away (Figure 3-8). The observations are supported by Loos and co-authors (2009), who found SUC in every Swiss river water sample ( $n=7$ ) with a maximum value of  $0.45 \mu\text{g/L}$ , whereas only in seven out of 19 German river water samples the compound was detected above the LOQ with a maximum of  $0.08 \mu\text{g/L}$ . As described above, the dilution factor of the WWTP effluent concentration in the receiving rivers was at least ten. For SUC this gives a concentration range between  $0.05 \mu\text{g/L}$  and  $0.17 \mu\text{g/L}$ , making ACE a much better tracer regarding detectable concentrations. A recent study about consumption related SUC emissions in Linköping/Sweden showed that daily household emissions decreased dramatically from  $225 \text{ g/d}$  in 2007 to  $88 \text{ g/d}$  in 2009 (Schmid-Neset et al., 2010). This was mainly attributed to the removal of the SUC containing Coca Cola light from the Swedish market in 2008. A further reduction, due to the announcement of local producers that the compound will also be removed from other consumer products, is expected. This example shows that a sudden pronounced ratio shift has to be investigated carefully as it can be not only a hint for a point source, but also an indication for modified usage patterns or the replacement and ban of certain compounds.

We followed several tracer concentration ratios further to river bank filtration wells to evaluate which compound ratio is most applicable to be used as a prediction tool. Both, ACE as well as CBZ concentrations, correlated well with BTZ in river water samples with  $r^2$  of around 0.8 (Figure 3-7). In samples derived from the river bank filtration wells  $r^2$  decreased dramatically (Figure 3-9), most likely due to a variable degradation of BTZ based on different residence time and environmental conditions in the subsurface. In contrary to BTZ, 4-TTri correlations to ACE and CBZ remained stable when comparing surface water and river bank filtration wells (Figure 3-10 and Figure 3-11) with a better fit for ACE ( $r^2 = 0.883$ ). For both ratios including 4-TTri the slope of the linear regression shifted only slightly by about 15 %. The higher persistence of 4-TTri in comparison to BTZ in the environment, namely in a partially closed water cycle in Berlin, is supported by a previous study (Weiss et al., 2006). The authors observed a decreasing BTZ/4-TTri ratio from a WWTP effluent containing trench to a receiving lake and further to a transect used for bank filtration. A good correlation in both, river water samples ( $r^2 = 0.94$ ) and bank filtrate ( $r^2 = 0.85$ ), was achieved when

comparing the two compounds most stable in wastewater treatment, ACE and CBZ (Figure 3-4). The slope of the best fit straight line of ACE and CBZ shifted slightly and simultaneously as observed for the ratios of these two compounds compared to BTZ and 4-TTri. Since ACE was measured in more than 22 times higher concentrations than CBZ, it seems to be a slightly better wastewater tracer, as the detection limits of both compounds are comparable. However, ACE findings should be evaluated carefully as it is widely used in sugar free soft drinks. A spill of 1 L soft drink, containing the maximum ACE level, valid for the European Union, of 350 mg/L (EU, 1994), would “contaminate” 35,000 m<sup>3</sup> of water to the level of our LOQ (10 ng/L).

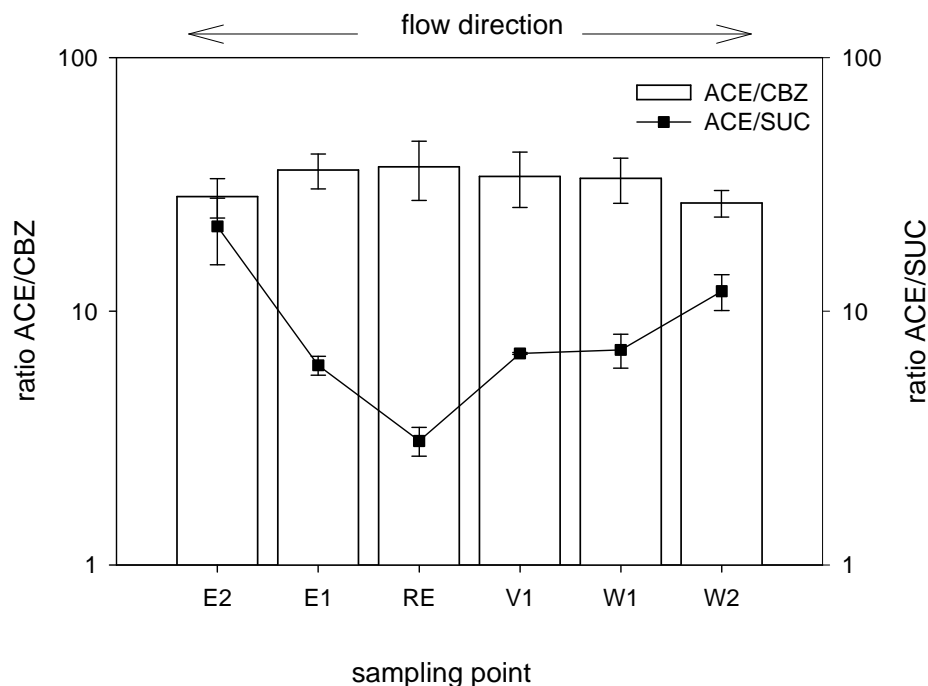


**Figure 3-4** ACE over CBZ concentrations of RBF wells along the river Rhine (data from this study) and wells in Bavaria (data from LfU Bavaria, 2010)

### 3.3.3 Soil aquifer treatment

For the SAT site it became worthwhile to evaluate SUC as a possible tracer for wastewater, as in Israel a remarkably higher consumption than in Germany of this sweetener seems to exist. Whereas in Germany in four out of five WWTPs ACE was detected in about 25-fold higher concentrations (WWTP 5 19-fold) than SUC the ACE/SUC ratio was only about 3 in the WWTP effluent in Israel. Here, average concentrations of ACE and SUC were  $55 \pm 7.1 \mu\text{g/L}$  and  $18 \pm 2.4 \mu\text{g/L}$ , respectively, whereas CBZ concentrations were in both countries in the same range ( $1\text{--}2 \mu\text{g/L}$ ) (Gasser et al., 2010; Gasser et al., 2011). For the ACE/CBZ ratio a pronounced stability in the variably saturated vadose zone (V1) and for the first recovery wells in the east and west (E1 and W1) was observed (Figure 3-5). The detention time to V1 is about 1.5 months and thus in the same range of riverbank filtrate retention times in Germany where the same persistence of both compounds was observed. Even in E1, which is about 250 m away from the closest recharge basin, no significant decrease of the ACE/CBZ ratio within more than one year of residence time in the subsurface occurred. In wells located further away from the recharge area, with residence times of about two years, a slight decrease of this ratio was monitored due to decreasing ACE concentrations in the periphery. However, in both peripheral wells ACE was still detected with several tens of  $\mu\text{g/L}$ . Thus this compound demonstrated its prediction power even at prolonged residence times as a breakthrough of wastewater in unaffected drinking water wells can be observed earlier with ACE rather than with CBZ based on comparable LOQs. For SUC a considerable concentration decrease of more than 50 % was observed in well V1, which is also displayed in a significant change of the ACE/SUC ratio. In laboratory batch experiments with three different soils with different percentages of organic carbon (0.74 %–2.99 %) sorption was neither observed for ACE nor for SUC when a water/soil ratio of 1 : 1 was applied (data not shown). These results support that in the case of SUC biodegradation or slow hydrolysis in the upper layer of the recharge basins is more likely than its removal by sorption processes. Further elimination on the way to the first observation wells (W1 and E1) was minor which revealed the mobility of the compound in the subsurface. Average SUC concentrations in the two outermost wells E2 and W2 were  $2.1 \mu\text{g/L}$  and  $3.5 \mu\text{g/L}$ , respectively, and therefore still higher than the findings for CBZ ( $1.3 \mu\text{g/L}$  and  $1.6 \mu\text{g/L}$ ).





**Figure 3-5** Ratios of ACE/CBZ and ACE/SUC in the recharged WWTP effluent (RE), an observation well below (V1), two wells east (E1 and E2) and two wells west (W1 and W2) of the percolation basin of a SAT site in Israel

### 3.4 Conclusions

Compounds with a pronounced stability during wastewater treatment were ACE, SUC, and CBZ which was consequently shown by their stable ratios within four WWTPs. However, already during wastewater treatment a shift of the ratios is possible when applying further treatment steps like activated carbon or ozonation. Ratios can also be affected by a country specific usage of certain compounds, like ASs. Other possible interferences are point sources deriving from production or application, *e.g.* deicing fluids at nearby airports in the case of BTZ. Even seasonal variations in consumption reflected in different ratios during summer and winter time seem possible in the case of ACE, which is one of the main sweeteners used in soft drinks. All the investigated compounds can give hints for a wastewater impact even for SUC, where a concentration decrease at prolonged residence time in the subsurface was found. The compound is still useful to indicate wastewater impact in a qualitative way for countries like Israel, where it is used in large amounts. The prediction power increases with increasing concentrations and decreasing LOQ. CBZ and ACE seem to have the strongest prediction power if the influence of point sources and regional differences are limited. In any case ratios of organic trace pollutants should not just be transferred from one sampling point to another for the calculation of predicted concentrations as they might vary. Once

transferability is checked concentrations of one stable and mobile organic trace pollutant can be estimated with good accuracy based on measurements of another.

### 3.5 Acknowledgements

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### 3.6 Supplementary information

**Table 3-2** Gradient program for the analysis of DTA

step	time in min	flow rate in $\mu\text{L}/\text{min}$	eluent A <sup>a</sup> in %	eluent B <sup>b</sup> in %	eluent C <sup>c</sup> in %
1	0.0	200	80	10	10
2	1.0	200	80	10	10
3	13.0	200	0	90	10
4	17.0	200	0	90	10
5	17.1	200	80	10	10
6	21.0	200	80	10	10

<sup>a</sup> water with 0.1% formic acid, <sup>b</sup> methanol with 0.1% formic acid, <sup>c</sup> 50 mM ammonium acetate

**Table 3-3** Gradient program for the analysis of CBZ

step	time in min	flow rate in $\mu\text{L}/\text{min}$	eluent A <sup>a</sup> in %	eluent B <sup>b</sup> in %
1	0.0	200	80	20
2	20.0	200	0	100
3	28.0	200	0	100
4	29.0	200	80	20

<sup>a</sup> water with 20 mM ammonium formiate, <sup>b</sup> 2/3 acetonitrile 1/3 methanol with 20 mM ammonium acetate

**Table 3-4** Gradient program for the analysis of BTZ and 4-Ttri

step	time in min	flow rate in $\mu\text{L}/\text{min}$	eluent A <sup>a</sup> in %	eluent B <sup>b</sup> in %
1	0.0	100	70	30
2	2.0	100	70	30
3	4.0	100	20	80
4	10.0	100	20	80
5	11.0	100	70	30

<sup>a</sup> water with 2 mM ammonium carbonate, <sup>b</sup> methanol with 2 mM ammonium carbonate

**Table 3-5** Effluent concentrations of the secondary sedimentation basin in WWTP 5

WWTP (population served)	throughput $\text{m}^3 \text{d}^{-1}$	effluent concentration secondary sedimentation basin $\mu\text{g L}^{-1}$
WWTP 5 (220,000)		
acesulfame		9.0
sucralose		0.51
carbamazepine	151,200	0.38
diatrizoic acid		2.4
benzotriazole		3.2
4-methylbenzotriazole		2.4

**Table 3-6** Daily loads of acesulfame in kg/d at the sampling point Basel/Switzerland based on the measured stream flow and detected concentrations at different sampling dates

sampling date	stream flow (Q) in $\text{m}^3/\text{s}$	acesulfame in $\mu\text{g}/\text{L}$	load in $\text{kg}/\text{d}$
12.01.2010	781	0.80	54
11.02.2010	643	0.91	51
08.03.2010	701	0.93	56
07.05.2010	1074	0.71	66
07.04.2010	814	0.74	52
01.06.2010	1688	0.48	70
01.07.2010	1282	0.64	71

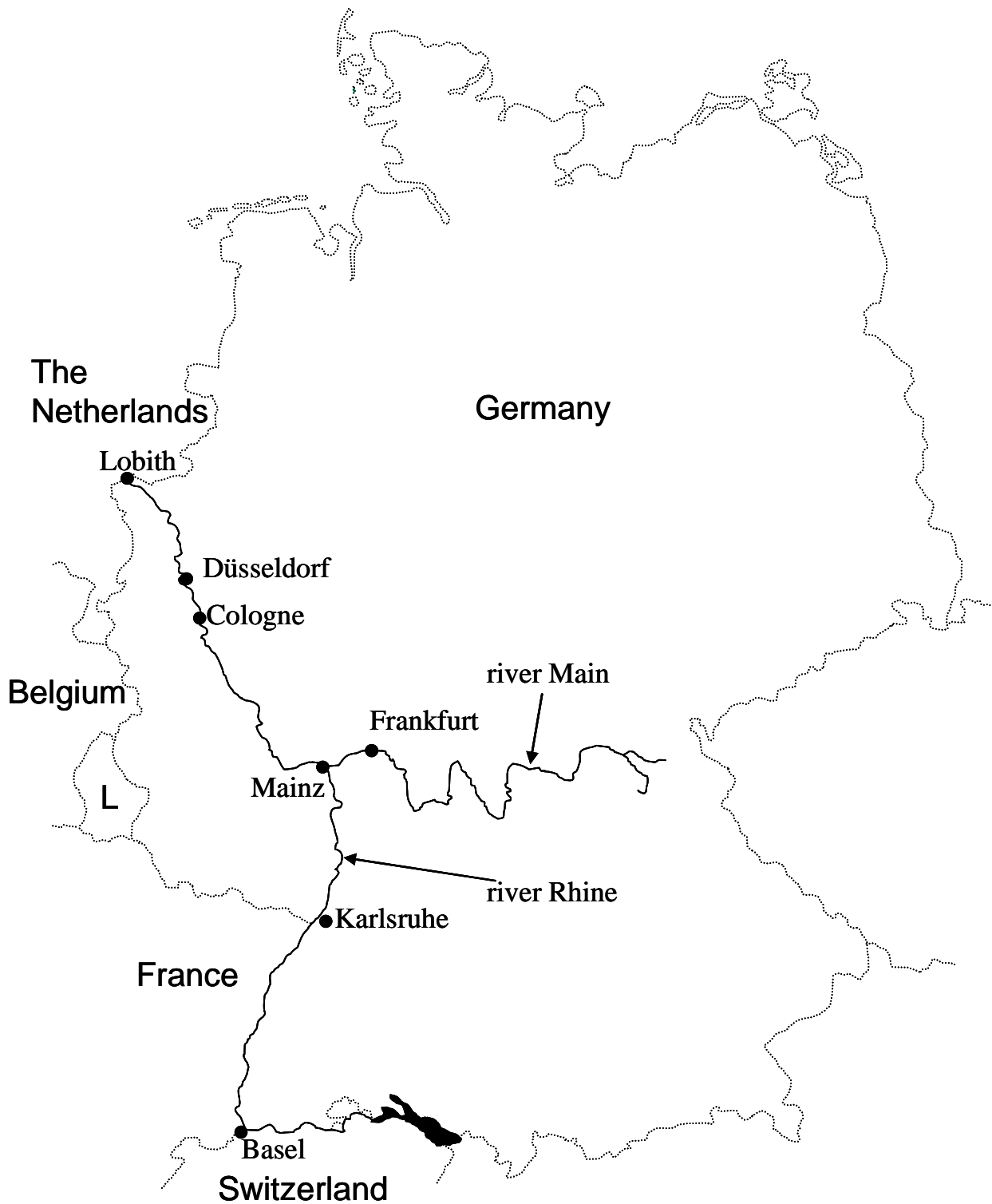
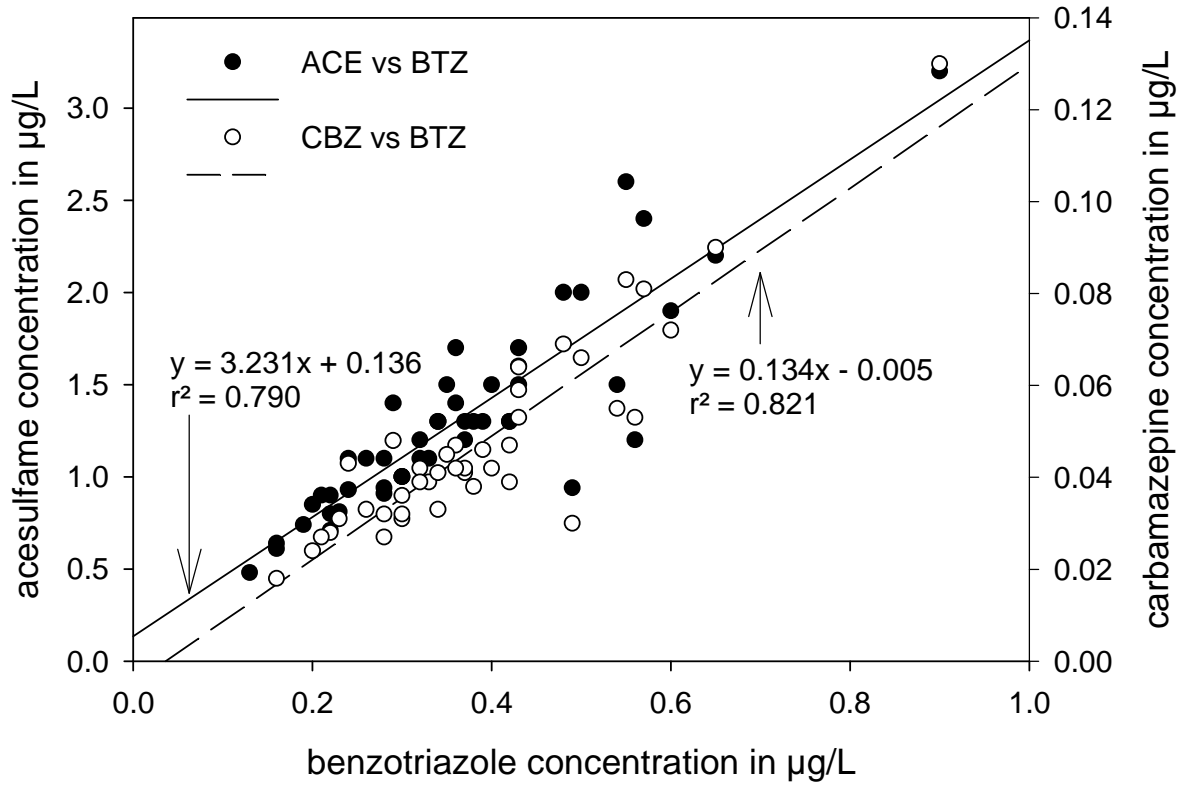


Figure 3-6 Sampling points at the rivers Rhine and Main



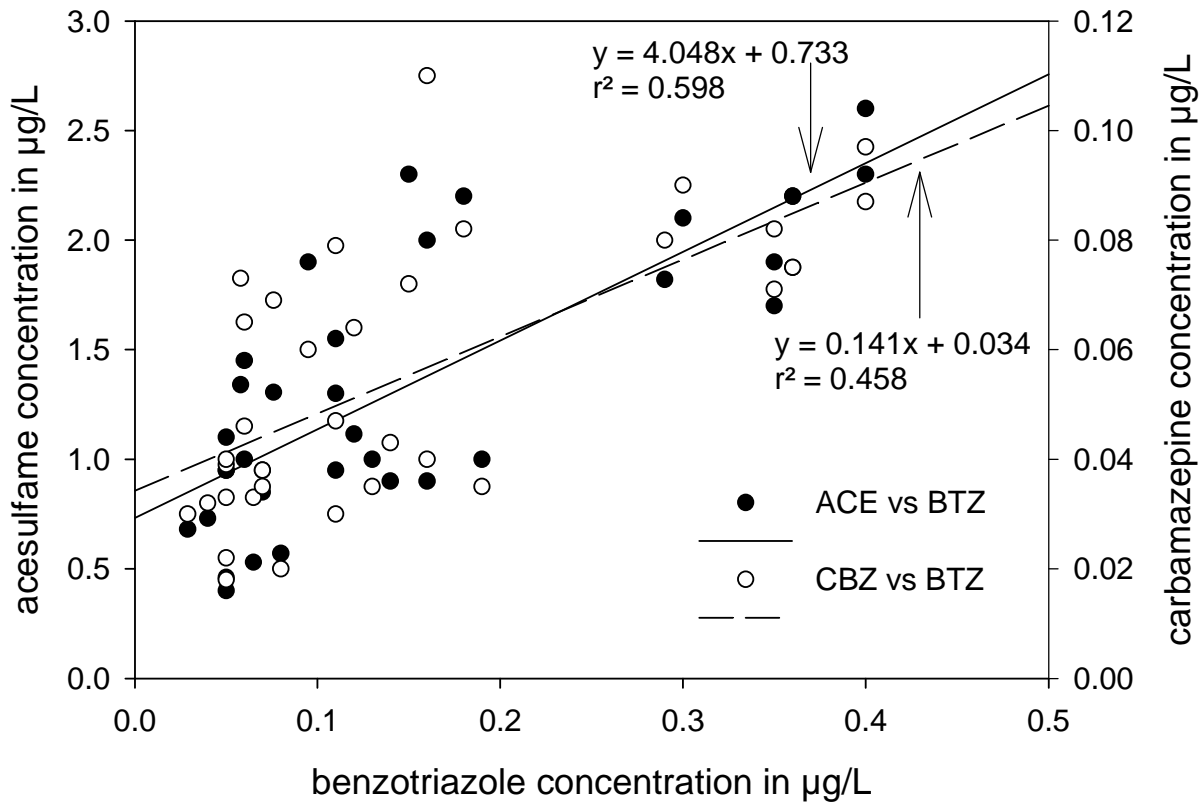
**Figure 3-7** Linear regression of ACE vs BTZ and CBZ vs BTZ for all sampling points (except sampling point Basel for CBZ vs BTZ) at the rivers Rhine and Main

ratio ACE / CBZ		ratio ACE / SUC													
	Basel	Karlsruhe	Mainz	Cologne	Düsseldorf	Lobith	Frankfurt		Basel	Karlsruhe	Mainz	Cologne	Düsseldorf	Lobith	Frankfurt
Basel	■	X	X	X	X	X	X	Basel	■	O	O	X	X	X	X
Karlsruhe	X	■	O	O	O	O	O	Karlsruhe	O	■	O	O	X	X	X
Mainz	X	O	■	O	O	O	X	Mainz	O	O	■	O	O	O	X
Cologne	X	O	O	■	O	O	O	Cologne	X	O	O	■	O	O	X
Düsseldorf	X	O	O	O	■	O	O	Düsseldorf	X	X	O	O	■	O	X
Lobith	X	O	O	O	O	■	X	Lobith	X	X	O	O	O	■	O
Frankfurt	X	O	X	O	O	X	■	Frankfurt	X	X	X	X	X	O	■

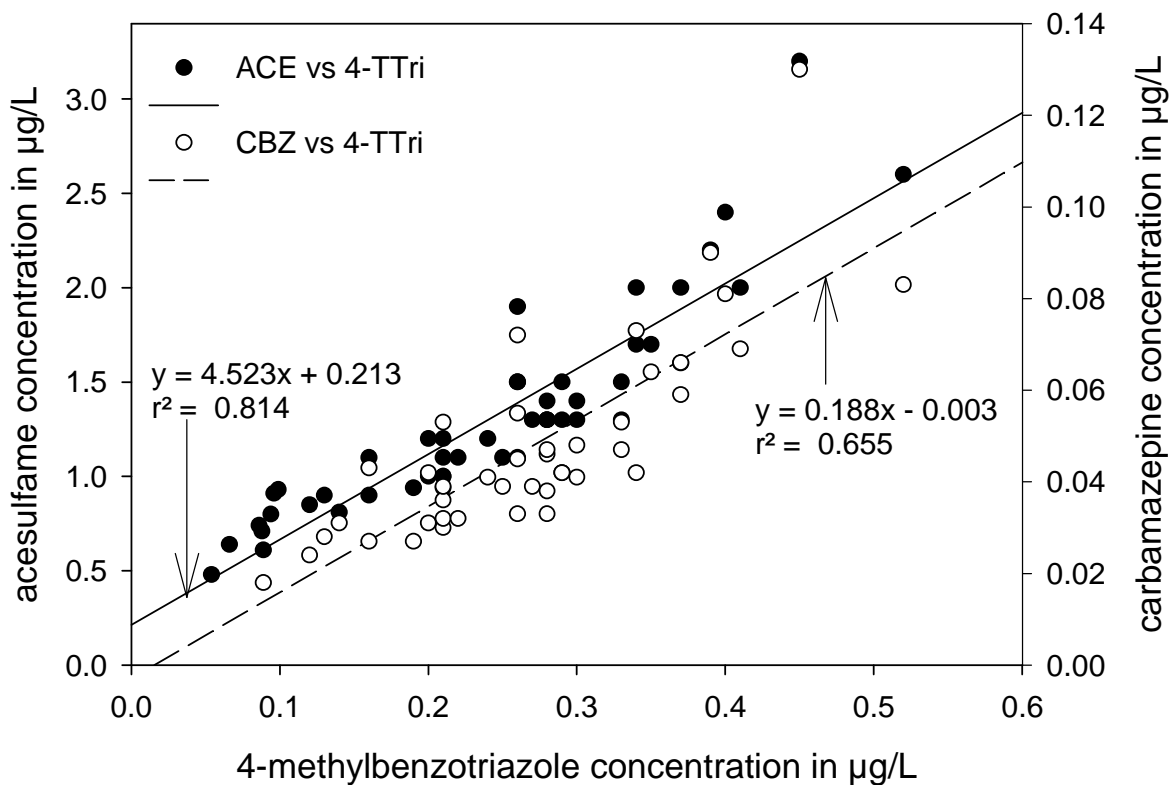
  

ratio ACE / BTZ		ratio BTZ / CBZ													
	Basel	Karlsruhe	Mainz	Cologne	Düsseldorf	Lobith	Frankfurt		Basel	Karlsruhe	Mainz	Cologne	Düsseldorf	Lobith	Frankfurt
Basel	■	O	O	O	O	O	O	Basel	■	X	X	X	X	X	O
Karlsruhe	O	■	O	O	O	O	O	Karlsruhe	X	■	O	O	O	O	O
Mainz	O	O	■	O	O	O	O	Mainz	X	O	■	O	O	O	O
Cologne	O	O	O	■	O	O	O	Cologne	X	O	O	■	O	O	O
Düsseldorf	O	O	O	O	■	O	O	Düsseldorf	X	O	O	O	■	O	O
Lobith	O	O	O	O	O	■	X	Lobith	X	O	O	O	O	■	O
Frankfurt	O	O	O	O	O	X	■	Frankfurt	O	O	O	O	O	O	■

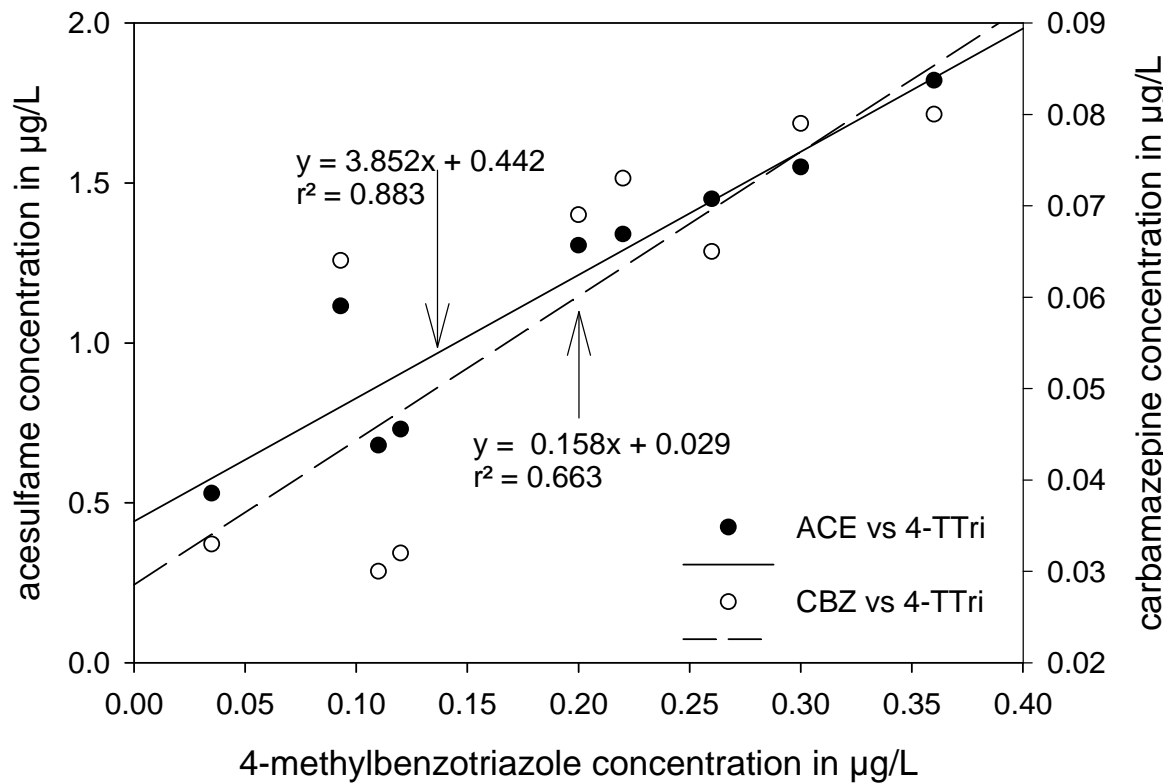
**Figure 3-8** Statistical analysis of the calculated ratios by one-factor variance analyses and Tukey-Test with  $p < 0.05$ . X = significantly different, O = not significantly different



**Figure 3-9** Linear regression of ACE vs BTZ and CBZ vs BTZ for RBF sampling points along the Rhine river and wells in Bavaria taken from Bayerisches Landesamt für Umwelt



**Figure 3-10** Linear regression of ACE vs 4-TTri and CBZ vs 4-TTri for all sampling points (except sampling point Basel for CBZ vs 4-TTri) at the rivers Rhine and Main



**Figure 3-11** Linear regression of ACE vs 4-TTri and CBZ vs 4-TTri for all RBF sampling points at the Rhine river

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