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Assessing the urban wastewater pollution in small agricultural water bodies via marker substances

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ABSTRACT

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Humanity is facing the challenge of increasing drinking water scarcity. One reason for the lack of clean water is its contamination with anthropogenic pollutants. In order to protect drinking water, prevent further pollution and improve global water management, the contaminants, their sources, distribution, properties and fate in nature must be determined. The German project “Small water body monitoring” (KgM) at the Helmholtz-Centre for Environmental Research examines the input of pesticides into receiving agricultural streams (catchment area<30km², agricultural land use>40%). To measure peak concentrations and thereby peak toxicity of the emitted pesticides, measurements at 69 sites with limited urban influence were conducted nationwide. In this work, the collected data of two different sampling methods (grab bottle and event sampling) were analysed for urban wastewater marker to assess the influence of municipal and industrial wastewater at the sites. In doing so, 13 marker substances were chosen to indicate urban wastewater impact. All selected wastewater indicators were present in widely varying frequency and magnitude. Analysing the samples, a significant difference in the measured concentrations depending on the sampling method was shown. After precipitation events with an increased water level of at least 5cm, the overall marker concentration rose for an averaged 0.39µg/l. With the help of reference sites and measuring points under the known influence of wastewater treatment plants (WWTP) a ratio was established to trace back potential polluting sources. Although the measured concentrations differ greatly between grab and event samples, the ratio of Caffeine(CF)/Acesulfame(ACE) resulted in a successful evaluation of both datasets. Additionally, the ratio of summed concentrations of non-persistent versus persistent indicators emphasized the results. This work shows the undeniable influence of urban wastewater in agricultural small water bodies. The concentrations of wastewater marker change significantly during precipitation and should be considered when conducting measurements and analyses. In case of the examined agricultural small water bodies, the wastewater sources can be distinguished in untreated or treated origin with the help of the ratios CF/ACE and WWTP- degradable/persistent marker substances.

Key words: wastewater indicators, agricultural water bodies, event sampling

CONTENTS

ABBREVIATIONS AND TERMS

1H-BTZ	1H-benzotriazole
5H-1H-BTZ	5-Methyl-1H-benzotriazole
AC	Acetaminophen
ACE	Acesulfame
AS	Artificial Sweetener
ASMX	Acetyl-sulfamethoxazole
CMZP	Carbamazepine
CYC	Cyclamate
CF	Caffeine
DCF	Diclofenac
eds	Event-driven composite sampling
KgM	Kleingewässermonitoring (Small water body monitoring project)
LC-HRMS/MS	Liquid Chromatography – High Resolution Mass Spectrometer
PNEC	Predicted no effect concentration
PPCP	Pharmaceutical and Personal Care Products
SAC	Saccharin
SUC	Sucralose
SMX	Sulfamethoxazole
THEO	Theophylline
UFZ	Helmholtz-Zentrum für Umweltforschung (Helmholtz-Centre for Environmental Research)
WWTP	Wastewater Treatment Plant

1 INTRODUCTION

Water is a source of life. Just like air, soil and sunshine, terrestrial life cannot sustain without it (NASA 2019). Looking at the amount of water we are surrounded by, it seems like an infinite resource. But this appearance is deceiving (Groundwater Foundation 2019). Most of the available water is salty sea water and plenty of sweet water sources are polluted by humans. Therefore, clean, harmless water is becoming a scarce resource. (UN-Water 2018)

A major source of pollutants is anthropogenic wastewater. Only about 20% of the global man-made wastewater is treated (Bokova 2017) before its release into nature, negatively effecting human health, global environment and the economy. (WHO 2019; Mema 2010; UN-Water 2018; UNESCO WWAP 2017)

In order to improve water quality, we need to improve WWTPs effectiveness by researching their effluents and impact on the environment. Many scientists all over the world study WWTP effluents - their content, potential hazards and overall effects (Fang et al. 2017). The better the polluting chemicals are understood, the better negative effects can be prevented (UNEP 2016). A recent approach in researching anthropogenic water pollutants are wastewater marker.

Wastewater marker are substances of anthropogenic origin, which are ubiquitously present and detected in high concentration in raw wastewater, but not necessarily harmful. With the help of such wastewater marker, pollution sources, distribution and fate can be identified, the effectiveness of WWTPs stated, the influence of wastewater estimated, and contaminations determined. (Jekel & Dott 2013)

Sorted by their original function, the most common indicators are classified as Artificial Sweetener (AS), Pharmaceuticals and personal care products (PPCP), Fluorescent whitening agents (FWA) and Sterols/Stanols (SS). Knowing their specific utilisations, the marker substances can be traced back to their source. For instance, caffeine is mainly used as stimulant in form of drinks. It occurs frequently in water bodies and is extracted very efficiently in conventional WWTPs. (Buerge et al. 2003, 2006) If an industrial utilisation at a measurement site can be excluded, the occurrence of high concentrations of caffeine in a water body implies the influence of untreated, domestic wastewater. In

this manner, a variety of substances was found to potentially serve as tracer of different wastewater sources. (Fang et al. 2017; Jekel & Dott 2013)

In order to study the marker substances properties during wastewater treatment and link them to anthropogenic sources, most studies take place in, around and close-by WWTPs, e.g. Zirlewagen 2016; Buerge 2003, 2006; Tran et al. 2013a, 2014; Glassmeyer et al. 2005; Nakada et al. 2017; Poopitatta et al. 2018; Dickenson et al. 2010; Van Stempvoort et al. 2011; Kiguchi et al. 2016; Kahle et al. 2009; James et al. 2016; Oppenheimer et al. 2011, 2012; Madoux-Humery et al. 2013 or Daneshvar et al. 2012. This is not the case in this work. The data was collected in agricultural small water bodies all over Germany within the "Monitoring of small water bodies"-project (KgM). The project was established to monitor and assess the influence of pesticides in small water bodies by excluding urban (municipal and industrial) influences like WWTPs. An assessment of the collected data concerning non-agricultural wastewater pollution was not planned.

Prior to the KgM-project, a long-term research was conducted and the criteria for sampling methods and measurement sites set. The results and thereby the framework for the KgM-project are to find in "Implementation of the National Action Plan on sustainable use of pesticides – survey on the state of data on the pollution of small water bodies in the agricultural landscape" by Brink et al. 2017. In doing so, an agricultural small water body was defined as water body with a maximum catchment area of 30km² and at least 40% agricultural land use of the catchment. This way, urban influence was meant to be limited, but agricultural influence maximised.

1.1 Research Questions

In this thesis, three research questions concerning the influence of urban wastewater at 69 measurement sites in Germany will be answered. The sites were selected and analysed for the “Small water body monitoring”-project at the Helmholtz-Centre for Environmental Research (UFZ) in Leipzig. The project aims on the improvement of the current pesticide toxicity assessment by examining small water streams, mainly affected by agriculture. Based on the project-specific site characteristics and gathered data, the following research questions were phrased:

i) Are there any urban wastewater marker substances in agricultural small water bodies?
ii) How do concentrations of marker substances change during precipitation?
iii) Which ratio can be used to assess the wastewater sources at the tested sites?

1.2 Aim

This study aims on identifying municipal and industrial wastewater sources in agricultural small water bodies with the help of marker substances. According to the site requirements (Brink et al. 2017) the water bodies should be free of urban wastewater. If wastewater marker substances are found, their concentration during precipitation events and dry weather periods will be compared and the appropriate ratio for assessing their source determined. As a result, distribution patterns, anthropogenic influence on agricultural water bodies and potential ratios for further analyses should be established.

2 CURRENT STATE OF RESEARCH

In 2017, the MDPI-journal “water” published an article by Fang et al. reviewing 191 studies concerning wastewater, its pollutants, their sources and based on that, the development of indicator ratios. The review depicts the global interest in wastewater assessment and shows the potential of wastewater marker. But the results of the studies vary and thereby state the complexity of the subject.

For instance, caffeine is one of the most researched indicators for raw wastewater. While it was considered useful for domestic wastewater indication thanks to its negative correlation to coliform and positive correlation to the amount of population in a Canadian study (Kurissery et al. 2012), Foolad et al. (2015) do not recommend the substance due to its biodegradability and efficient extraction in treatment plants.

Researches using the AS Saccharin resulted in likewise inconsistent outcomes. The sweetener was successfully used as wastewater marker in several studies and even showed correlations to the population of the examined catchment area. (Lange et al. 2012; Perkola & Sainio 2014) But in case of the study by Ekklesia et al. 2015 in Singapore, Saccharin was not recommended as indicator due to its uncontrolled discharge by open-air food courts. Similar contradictory studies are to find for 1H-benzotriazole (Funke et al. 2015; Kahle et al. 2009) or Carbamazepine (Oppenheimer et al. 2011, 2012).

Thus, the indicator substances need to be set into perspective, which can be done with the help of ratios and correlations. (Kiguchi et al. 2016; Scheurer et al. 2011; Zirlewagen et al. 2016; Tran et al. 2013a; Peeler et al. 2006; Sun et al. 2016; Ekklesia et al. 2015; Daneshvar et al. 2012; Madoux-Humery et al. 2013; Nakada et al. 2016; Perkola&Sainio 2014; Oppenheimer et al. 2012; James et al. 2016¸ Devane et al. 2006; Furtula et al. 2012; Gourmelon et al. 2010)

2.1 Sources of wastewater

2.1.1 Industry

Industrial wastewater is considered one of the most toxic discharges (Bokova 2017). The pollutants vary with the type of industry they are emitted from. For instance, nutrients like nitrogen and phosphorus, proteins, sugar, hormones, salts, fats, acids, stabilizers, colouring or lubricants are typical wastewater compounds of manufacturers. Mines and metal production often release greater numbers of hydraulic oils, gases and gasification products or heavy metals. The paper industry on the other hand, stands out due its high amounts of emitted chlorinated organic compounds and organic halogens. (Palaniappan et al. 2010) Hence, the emitted pollutants depend on the industry branch present at the measurement site.

2.1.2 Municipalities

Domestic discharges are not considered hazardous, when treated properly in WWTPs (Falconer 2006). But run-offs, leakage, technical inefficiency of WWTPs or sewage overflow can lead to emissions of PPCP remains, fats, nutrients, micropollutants, pathogens and even heavy metals. (Blaettler 2018; Von Sperling 2007) Astaraie-Imami et al. (2002) showed in a study that the concentration of ammonium and dissolved oxygen in the examined rivers increases with population, effecting the aquatic environment negatively. Furthermore, the impact of released pharmaceuticals and endocrine disruptors is a rising concern and became the object of interest in more and more studies with complex results. (Bolong et al. 2009; Deblonde 2011; Falconer 2006) The probably most notorious pollutants released by domestic wastewater are pathogens. Their impact on human health is well-known and the supply of pathogen-free drinking water, especially in developing countries, is the aim of many globally operating organisations likes WHO, Wateraid, UNEP, UN or EWP (WSP 2018).

2.1.3 Agriculture

The pollutants emitted by agricultural activity are not only various, but in many countries the biggest contributor to local water impurification. (Mateo-Sagasta et al. 2017) The substances can be classified according to their utilisation. Crop production tends to release high amounts of nutrients, pesticides, salts and washes sediment into water bodies. Livestock farming, including aquacultures, supports the distribution of nutrients and sediments, but stands out with its emission of organic matter, pathogens, antibiotics and hormones. (-) The overall contribution and final effects of contaminants introduced by farming are considered not well researched yet (UNESCO 2017). With that in mind, small agricultural water bodies present an excellent research basis for aquatic pollutants.

2.2 Effects of untreated wastewater

Polluted water can result in numerous negative effects for human health, the environment and even the economy (UNESCO 2017). The World Health Organisation (2019) states, that around 842.000 people die from diarrhoeal diseases due to the consumption of pathogen-infected water annually worldwide. Additionally, the destructive effects of contaminants on natural processes and ecosystems leads to a decrease of natural resources like fish and the related earnings (UNEP 2016). The different biological and chemical processes leading to the final effects are various and substance-depending.

Von Sperling (2007) summarized the effects of water pollutants in his book "Wastewater characteristics, treatment and disposal" as follows (table 1):

TABLE 1. Extract of wastewater pollution effects according to Von Sperling (2007)

Pollutant	Effect
Suspended Soils	Aesthetic problems, Sludge deposits, Pollutant adsorption, Protection of pathogens, Biodegradable organic matter, Biochemical oxygen demand
Biodegradable organic matter	Oxygen consumption, Death of fish, Septic conditions
Nutrients	Excessive algae growth, Toxicity to fish (ammonia), Illness in new-born infants (nitrate), Pollution of groundwater
Pathogens	Water-borne diseases
Non-biodegradable organic matter	Toxicity (various), Foam, Reduction of oxygen transfer, Non-biodegradability, Bad odour
Metals	Toxicity, Inhibition of biological sewage treatment, Problems in agriculture use of sludge, Contamination of groundwater
Inorganic dissolved solids	Excessive salinity – harm to plantations (irrigation), Toxicity to plants (some ions), Problems with soil permeability (sodium)

2.3 Indicator properties

2.3.1 Identification of marker substances

Wastewater marker are a recently developed tool to trace back water polluting sources. With advanced knowledge about the contaminating substances, their degradability, fate and distinctive sources, the approach of pollutant source identification via wastewater indicators became more and more prominent in recent years. (Fang et al. 2017)

Kreitler (1979) was one of the first scientist to use ^{15}N-isotopes as wastewater marker for groundwater and soil analyses. He identified the polluting sources via ratios, aware of the relation between different ^{15}N-isotopes, their occurrence in soil and the local land use. He thereby relied on the studies of Bremner et al. (1966, 1973), who analysed the occurrence of nitrogen isotopes and its compounds in soil. Due to the increasing challenges of global water management, the interest in such markers increased within the last 20years, resulting in many current researches. (Fang et al. 2017)

The selection of appropriate indicators depends on a range of parameters. As defined by Jekel & Dott (2013), the potential marker should meet most of the requirements mentioned in their guideline. Especially the frequent occurrence above the detection limit should be given and thereby deliver a reasonable base for the analysis. Furthermore, a good knowledge of the different indicator sources, properties and the measurement site's surroundings supports accurate examination. Ekklesia et al. (2015) and Madoux-Humery et al. (2013) especially emphasize the importance of local land use analysis when evaluating wastewater contribution with the help of markers. Additionally, knowledge about the persistency in WWTP as well as the biodegradability of the potential marker substance are indispensable for its correct utilisation (Jekel & Dott 2013).

For this study, 13 indicators were chosen. The selected substances comply with the suggested guideline by Jekel & Dott (2013) and in doing so, were measured frequently in the collected samples, are easily detectable by the means of the available laboratory equipment and finally enable the establishment of evaluating ratios due their well-known, differing properties (table 2). The compounds were subject of similar studies, researching their characteristics and confirming their potential as wastewater marker. (Scheurer et al. 2011; Kahle et al. 2009; Nödler et al. 2014; Van Stempvoort et al. 2011,

2013; Yang et al. 2017; Tran et al. 2013a, 2014; Jekel&Dott, 2013; Glassmeyer et al. 2005; Yu et al. 2009; Poopipattana et al. 2018; Harwood 2013)

TABLE 2. Properties of selected wastewater indicator substances

Compound	**PNEC (in ng/l)**	**Most common source**	**Persistent in WWTP**
Acesulfame	-	Domestic, Food processing[7, 8, 9, 10]	Yes[7, 9, 10,14, 16]
Sucralose	930.000 [1]	Domestic, Food processing[7, 10]	Yes[10,14, 16]
Saccharin	5.000.000[2]	Domestic, Food processing[7, 10]	No[10, 14, 16]
Cyclamate	-	Domestic, Food processing[7, 8, 10]	No[10, 14]
Caffeine	870.000 [3]	Domestic, Food processing[8, 9]	No[9, 14, 17]
Theophylline	166.000 [4]	Human faecal[11]	No[17]
Acetaminophen	134.000 [2]	Domestic, untreated[11]	No[17, 18]
Diclofenac	50 [5]	Human faecal[11]	Yes[14, 18]
Acetyl-sulfamethoxazole	100[4]	Domestic [12]	No[14]
Sulfamethoxazole	100[4]	Domestic[12]	Yes[14, 18]
Carbamazepine	2.500[4]	Domestic[13]	Yes[14, 18]
1H-Benzotriazole	30.000[6]	Industrial[14, 15]	Yes[14]
5-Methyl-1H-benzotriazole	30.000[6]	Industrial[14, 15]	Yes[14]

Most of the designated indicators are considered trace compounds. Thus, when analysing water samples of any kind, the marker substances are usually not considered. (WWTP Haseldorfer Marsch 2019) Unfortunately, this leads to a lack of comparable data. As mentioned before, wastewater marker gained scientific recognition rather recently. This means, their influence on the environment is not well studied yet and references concerning their ecotoxicology are rare. But with the help of recent studies, characteristics of the potential indicators can be determined, and specific properties estimated.

[1] Tollefsen et al. (2012); [2] ECHA (2019); [3] Carl Roth (2019); [4] LANUV (2007); [5] UBA (2018); [6] LAWA (2016); [7] Scheurer et al. (2011); [8] Zirlewagen et al. (2016); [9] Buerge et al. (2003, 2006); [10] Tran et al. (2013a); [11] Hajj-Mohamad et al. (2014); [12] Radke (2009); [13] Kiguchi et al. (2016); [14] Yang et al. (2017); [15] Kahle et al. (2009); [16] Subedi&Kannan (2014); [17] Kim et al. (2012); [18] Kasprzyk-Hordern et al. (2008)

2.3.2 Application of ratios to detect wastewater sources

The variety of compounds and the complexity of aquatic ecosystems complicates the determination of pollutants and identification of their sources. Usually, water samples contain a set of marker substances in varying concentrations, as to see in the results of this study. Considering the varying marker properties, ratios help to evaluate the measured quantities and estimate the contribution from different sources. (Glassmeyer et al. 2005; Peeler et al. 2006; Zirlewagen 2016, Tran et al. 2015)

Since the selection of useful marker substances depends on a range of parameters, the assessing ratios do as well. The ratio must be chosen according to the available indicators, their concentrations and the measurement site surrounding. Depending on a study's framework, ratios may have to withstand statistical parameters as well.

In case of this work, the data evaluation resulted in the ratio of formula (1) to determine whether the small water stream was primarily influenced by treated or untreated wastewater.

$$\frac{c(degradable\ substance)}{c(persistent\ substance)} \qquad (1)$$

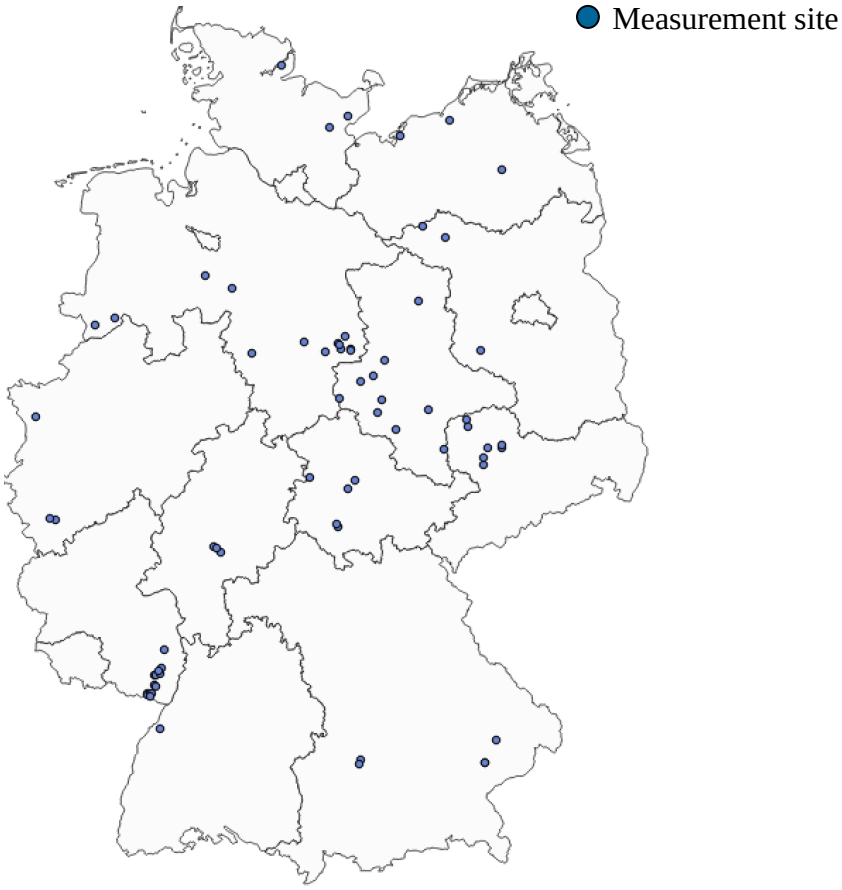
The comparison of degradable marker substances with WWTP-persistent indicators in form of a ratio, was already tested in prior studies. (Glassmeyer et al. 2005; Tran et al. 2013a, 2014; Sun et al. 2016) Simply dividing the according marker concentrations, results in a value of >1 for untreated water, due to the great amount of WWTP-degradable substance. A ratio <1 implies an increased impact of treated water. According to this ratio, the measurement sites close to a WWTP should result in a ratio below 1. Using the Log_{10} of the measured concentrations instead of the original values, treated wastewater is indicated by <0 and untreated wastewater by >0.

3 METHODS

3.1 Data collection on site

3.1.1 Measurement site criteria

The examined sites (picture 1) were initially chosen for the KgM-project as part of the "National Action Plan on sustainable use of pesticides". The KgM-project was established to develop a Germany-wide pesticide monitoring system in order to utilise biocides more sustainably and thereby protect the environment from their toxic effects. In doing so, the sampling aimed on the observation of peak concentrations and thereby peak toxicity of pesticides.



PICTURE 1. Measurement sites 2018[19] (KgM 2018)

To enable such measurements, several site requirements were established. For one, the small streams should be free of municipal and industrial influence. Hence, a small agricultural water body is considered a standing or flowing water body with "…an upper limit of the catchment area size < 30km²..." and"...a lower limit of 40% agricultural land use in the catchment". The surface area of the water body was not defined. Furthermore,

[19] The measurement sites are listed in Appendix 1. For the purpose of data protection, the sites are not assigned to their location on the map.

the sampling time was limited to 3,5 months, from April to mid-July (fertilizing season) and the distribution of the measurement sites established in accordance to the percental contribution of agricultural land to the German total. (Brink et al. 2017)

Finally, 10 reference sites were determined. These water bodies are ought to be free of agricultural influence due to specific land use characteristics (agriculture ≤ 5%, urban = 0%, grassed area ≤ 10%, nature protected area ≤ 10%, unquantifiable area ≤ 20%), must contain at least 10 different taxa as indication of biodiversity and originate within 100km of the measurement point. (Wick et al. 2019) Thereby, the reference sites are subject to much stricter and more detailed requirements than non-reference sites.

3.1.2 Grab bottle sampling

A grab bottle sample is a standard method for water and air sampling, representing the current state of the material of interest at the location. Therefore, grab samples provide time- and location bound insights, not absolute results. (Duncan et al. 2007)

PICTURE 2. 250ml - grab bottle for sampling in the KgM-project (Eglinski 2019)

The method is used globally and for different kinds of water. Grab bottle sampling is applied by the World Health Organisation (WHO 2011), the U.S. National Association of Wastewater Technicians (Lee 2010), the German Environmental Agency (UBA

2017) or the Australian Environmental Protection Agency (Duncan et al. 2007) - for groundwater just as well as wastewater, industrial or surface water. The global guideline for the sampling method is set by a variety of guidelines, based on ISO 5667-14:2014.

In water sampling, the grab bottle method is preferably used for the determination of unstable properties like pH, nitrite or temperature. Unfortunately, examining water quality parameters like specific contaminants can be unreliable due to water flow or the unequal distribution of pollutants. Hence, the method is recommended for analyses in low-velocity, shallow water bodies. Due to the fluctuations in wastewater streams, single grab bottle sampling is not considered a reliable method for wastewater analyses. (Norweco 2008)

The sampling should be carried out with a disinfected glass bottle and exclude any kind of sediments, plants or microorganisms. The location for dunking the bottle should be not too close to the edges or any other potential sources of pollutants.

In case of this research, the bottles were cleaned with methanol and held a volume of 250ml (picture 2). After determining an appropriate dunking location, the bottle was firstly rinsed with sample water. Then, the bottle was filled with water by dunking it about 10cm to 30cm below the water surface until no air was left in the container. The extracted water was then stored coolly until laboratory analysis. (DIN EN ISO 19458)

3.1.3 Event-driven composite sampling (eds)

Composite sampling refers to an integrated collection of multiple samples, taken at the same location but in specific time intervals. (Lee 2010; Norweco 2008) Event means here precipitation, which leads to an increased water level of at least 5cm.

Unlike grab samples, composite samples compensate concentration fluctuations and differing flow velocity, resulting in average concentrations for the required period of time or volume. The sampling is usually done mechanically, preventing mistakes by manual sampling. Nonetheless, the composite sampling equipment is normally more costly than grab sample material and produces unreliable results for unstable parameters. (Norweco 2008)

In case of the KgM-project, eds enables the measurement of compounds washed into the water bodies during precipitation. Many researchers observed an increased concentration of pollutants in surface water during rain events. (Zirlewagen et al. 2016; Madoux-Humery et al. 2013; Rio et al. 2013; Buerge et al. 2003; Priegnitz, 2007; Poopipattana et al. 2018; Ekklesia et al. 2015; Tran et al. 2013a; Benotti & Brownawell 2007) Since the project's aim was to determine peak concentrations and grab samples do not provide sufficient accuracy during events (Liess et al. 1999, 2008; Liess & von der Ohe 2005; Schäfer et al. 2007, 2012), the automatic event-driven composite sampling was chosen.

PICTURE 3. Installed automatic event sampler "Maxx" on site (Eglinski 2019)

PICTURE 4. Interior of the Eds-MAXX (KgM 2018)

The sampling was conducted with the help of an automatic sampler called "Eds-MAXX" (picture 3; picture 4). The sampler was especially constructed for the project and programmed to take a water sample of 4ml every 5minutes over a period of 3,2hours as soon as the water level rose for 5cm. The sampler was activated by a floater, installed in the stream and the sample bottle integrated in a thermos container for constant refrigeration. In case of an event, a message was sent via email and SMS to the executive staff. This way the sample was picked up and brought to the laboratory for analysis within 48 hours.

3.2 Instrumental analysis

This work's data is based on the instrumental results of a LC-HRMS/MS via target and non-target analysis of the gathered samples (KgM 2019).

LC-HRMS/MS is the short term for Liquid Chromatography – High Resolution Mass spectrometry/Mass Spectrometer. Mass spectrometer are analytical instruments, identifying and quantifying the structure of molecules, based on their mass-to-charge ratio. This allows a sensitive examination of complex test material and thereby a broad range of applications. Typically, the instrument consists of an intake system, an ion source, a mass analyser and a detector. In case of the LC-HRMS/MS the intake system is charged with the test liquid, separated in a chromatographic column and then analysed by a tandem (two) mass spectrometer, subjected by both, a magnetic and an electrostatic field for high resolution. The result is a spectrum of peaks, which assign specific compounds. (Thermo Fisher Scientific 2018) The analysis can be conducted target or non-target specific.

Target analyses aim on the detection and quantification of expected compounds with the help of existing references. This LC-HRMS/MS analysis is comparably simple. A small sample extract is injected, tested and compounds easily identified, thanks to references. Unfortunately, the number of detectable compounds is limited to the available references and the instrumental detection range. (Lucke et al. 2015)

A non-target analysis on the other hand, is the examination of a broad range of substances in samples "…without any a priori information on the compounds to be detected" (Krauss et al. 2010). This method allows to research a wider spectrum of substances and recognition of patterns or frequent substance combinations. Due to the lack of references, the identification of the thousands of peaks in the mass spectrum is time consuming and needs improvement in terms of the LC-HRMS software. (-) The combination of both analysis methods enables the identification of unknown substances in complex sample matrices.

3.3 Data Processing

The samples, gathered in form of grab and event samples within the KgM-project 2018, were instrumentally analysed by the Department of Effect-directed Analysis at UFZ Leipzig. 513 samples were collected during the measurement campaign in 2018, 412 withstood the set statistical requirements and were used for the wastewater analysis.
In order to select reliable data, exclusive factors were determined, based on several wastewater marker studies (Dickenson et al. 2010; Ekklesia et al. 2015; James et al. 2016; Oppenheimer et al. 2011; Van Stempvoort et al. 2011, 2013) as well as statistical experience by the UFZ staff. The parameters were set as follows:

- only measurements of the 13 determined indicators were considered
- grab and eds samples were analysed individually due to the significant difference in measured concentrations
- a marker substance had to be measured at least twice per site and method to minimise sampling mistakes
- multiple values of one day, one site and one sampling method were entirely excluded
- concentrations were converted into Log_{10}-values for better depiction

The computation for such high amounts of data is usually realized with the help of specific computer programs or programming languages. In case of the UFZ, the programming language “R” is used. The R Foundation defines the data analysis tool as “...a language and environment for statistical computing and graphics.” The software is a popular tool, especially in the scientific and economic sphere and free of charge. (TIOBE 2019; O’Grady 2019; Carbonnelle 2019; Muenchen 2019; Tippmann 2015)

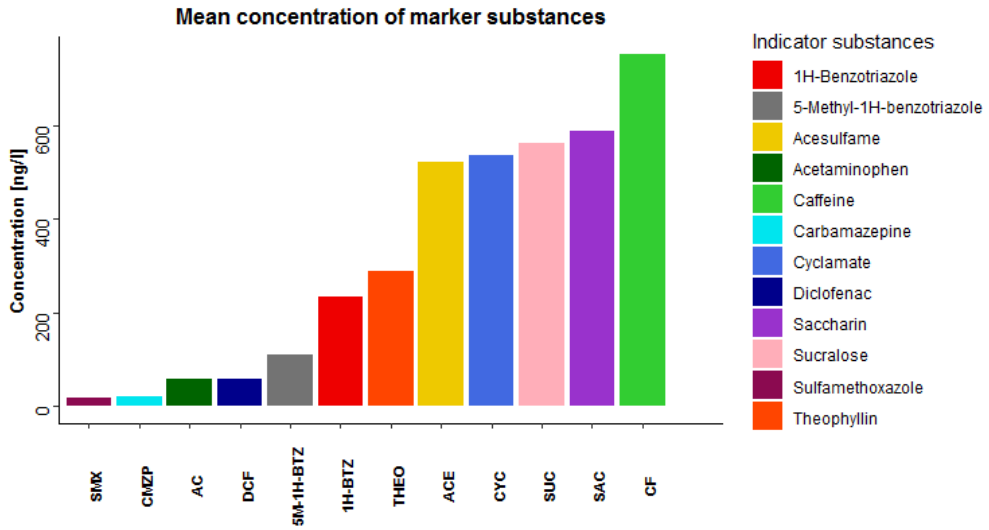
After the statistical data selection, the data distribution was analysed to ensure reliability. Furthermore, the difference of concentrations between the two sampling methods was tested for significance with the help of a paired t-test. Every stage of the analysis, from feeding the original data set into the program up to the final results can be checked and reproduced with the developed “R”-script (Appendix 2).

4 RESULTS

4.1 Detected wastewater marker substances

412 of the 513 samples contained a wastewater marker. In 51 of 69 sites marker substances were found frequently, meaning at least twice per sampling method and site. Picture 5 depicts the average indicator concentrations of all sites and both methods, picture 6 their frequency.

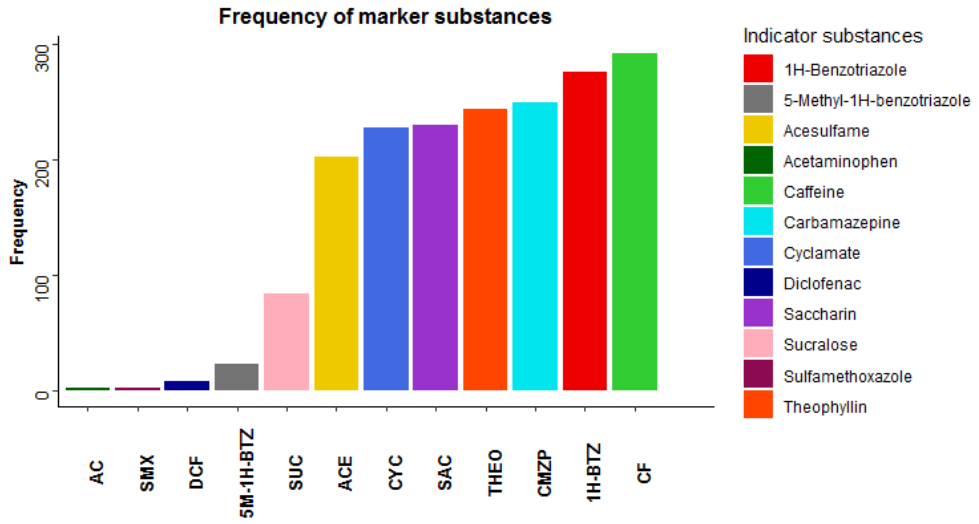
12 of the 13 chosen indicators occurred in a frequent, considerable amount above detection limit. According to the idealized measurement site requirements of the KgM-project no site was supposed to show any wastewater marker concentration, yet 12 of 13 indicators were present.



PICTURE 5. Mean concentrations of wastewater marker substances, site- and method-independent

CF, SAC and SUC were measured in the highest concentrations, while SMX, CMZP and AC were detected in comparably small magnitudes. The high concentrations of CF and AS indicate a general influence of domestic, untreated wastewater.

The 5 most prominent substances are also the least toxic once, according to their predicted no effect concentration (PNEC). The only substance exceeding its PNEC-value by the means of its average concentration is DCF.



PICTURE 6. Marker occurrences in selected 412 samples

CF, 1H-BTZ and CMZP were detected the most frequently. Thus, CF is not only emitted in the highest concentration, but also the most often. Occurring only twice, the AC and SMX occurrences are statistically neglectable. The high frequency of 1H-BTZ points at a common industrial wastewater influence.

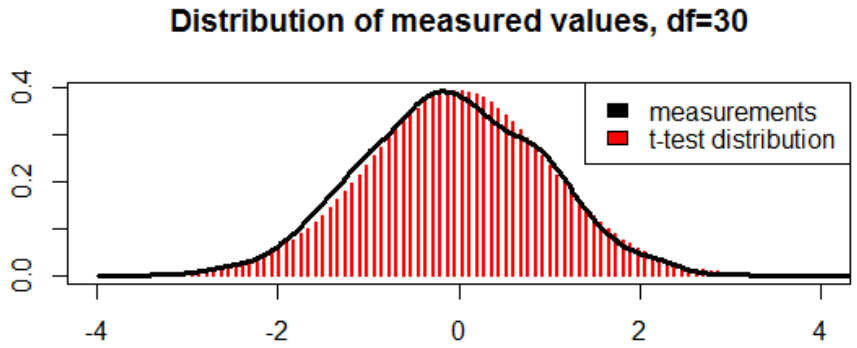
Table 3 summarizes the measured mean concentrations, PNEC values and frequencies of detections. The substances with a mean concentration above their PNEC are highlighted.

TABLE 3. Overview of mean concentration, PNEC and frequency

Compound	**Ø concentration (in ng/l)**	**PNEC (in ng/l)**	**Frequency**
Acesulfame	422	-	202
Sucralose	400	930.000	83
Saccharin	803	5.000.000	230
Cyclamate	463	-	227
Caffeine	668	870.000	292
Theophylline	308	166.000	244
Acetaminophen	139	134.000	2
Diclofenac	**129**	**50**	**7**
Sulfamethoxazole	15	100	2
Carbamazepine	24	2500	249
1H-Benzotriazole	208	30000	276
5-Methyl-1H-benzotriazole	80	30000	23

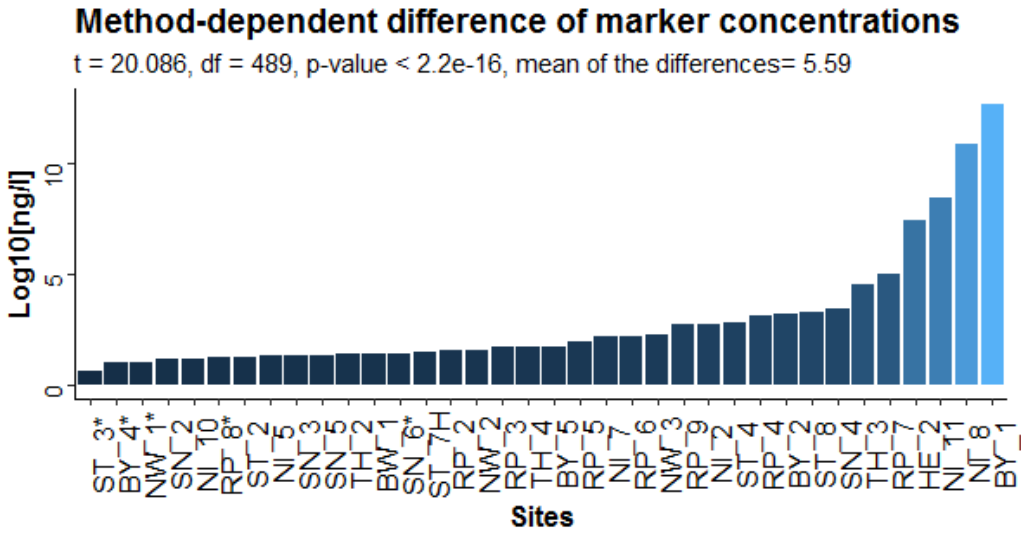
4.2 Concentration changes during precipitation

In order to determine a significant difference in concentrations during precipitation and dry weather, the distribution of the collected data was analysed first. The result is shown in picture 7.



PICTURE 7. Distribution of measured values, compared to statistical normal distribution

At a degreed freedom of 30, the measured values are distributed normally and thereby can be tested for significant difference with the help of a paired t-test. The test resulted in picture 8.

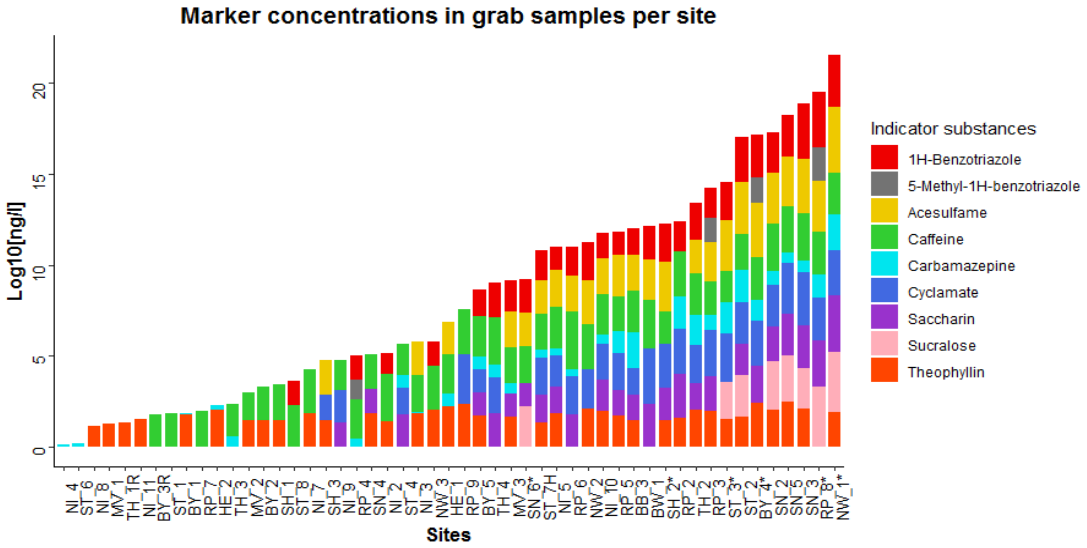


PICTURE 8. Difference of marker concentrations per site, regarding sampling methods

36 sites provided sufficient data in their grab and eds samples for comparison. The mean difference is stated with the Log_{10}-value of 5.59, representing a concentration of 389.045ng/l. Site names with the ending “R” indicate references sites, an “*” designate sites with a close-by WWTP. According to the test, the difference in marker concentrations between the two methods is indeed significant, suggesting further data analyses method dependent.

4.2.1 Grab bottle sampling

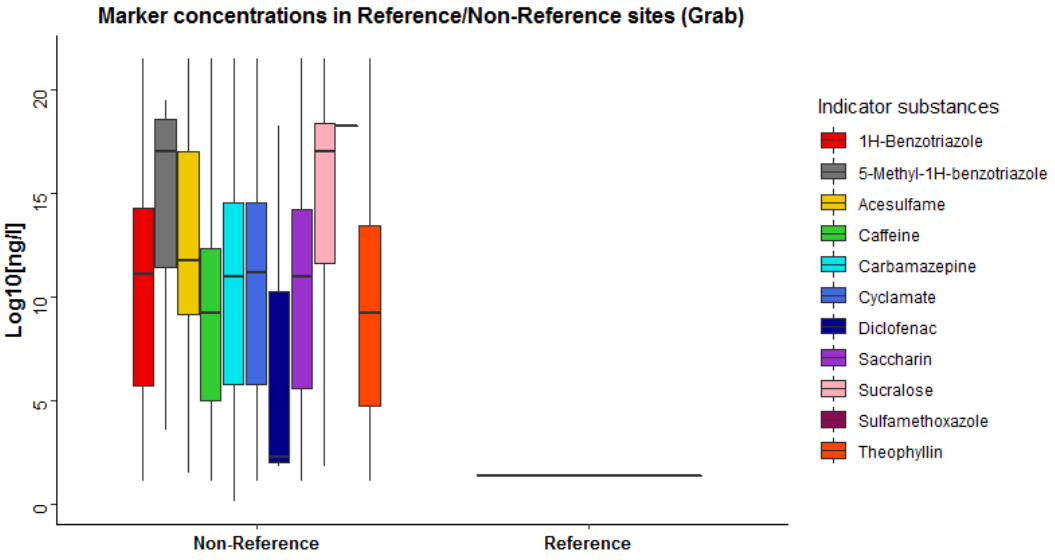
The result of the grab bottle analysis is shown in picture 9. The diagram depicts the average indicator concentrations per site. At 51 of 69 sites, 11 indicators were measured frequently, 9 of them in considerable amounts. As to see, with rising concentration, the variety of substances increases as well.



PICTURE 9. Mean concentrations of indicator substances in grab bottle

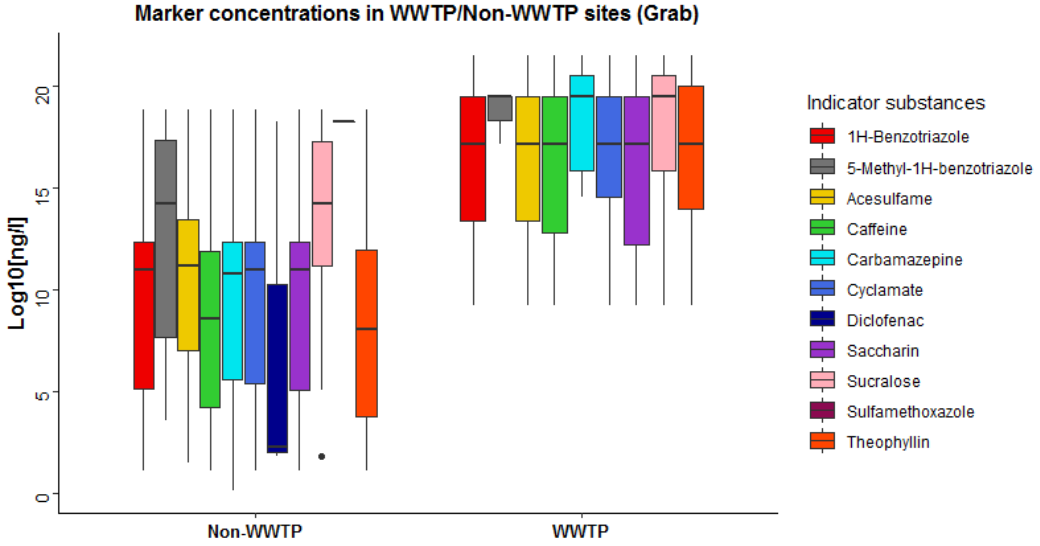
Two reference sites were affected by wastewater: BY_3R carried caffeine and TH_1R Theophylline at least twice. All 6 sites close to a WWTP show a high concentration of wastewater marker substances. Three of the six most polluted sites are WWTP-sites.

The presence of CF at BY_3R and THEO at TH_1R leads to picture 10. As expected, the reference sites show a much lower concentration of wastewater marker than the others. Overall, two of 10 reference sites were contaminated by a low amount of wastewater marker.



PICTURE 10. Comparison of indicator concentrations in grab samples between reference and non-reference sites

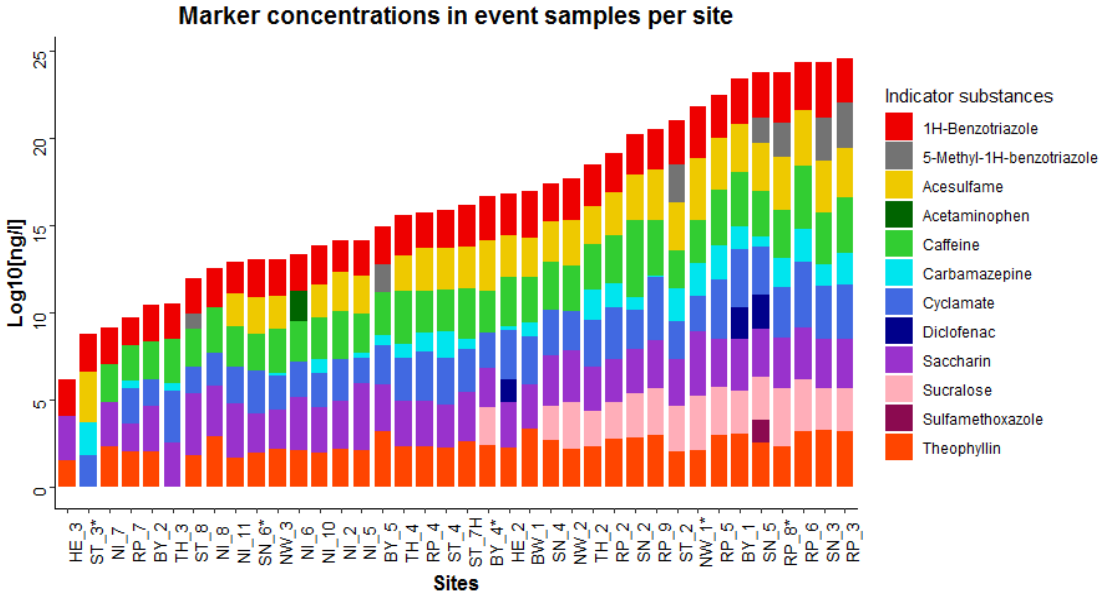
The influence of WWTPs is seen clearly in picture 11. The average concentration of every marker substances is higher on WWTP sites than on others and fluctuates less. This result suggests a constant influence of marker indicators at WWTP sites. Additionally, SMX and DCF were detected at non-WWTP. Both compounds are considered WWTP persistent and could occur just as well close-by WWTPs.



PICTURE 11. Comparison of indicator concentrations in grab samples depending on WWTP presence

4.2.2 Event-driven sampling

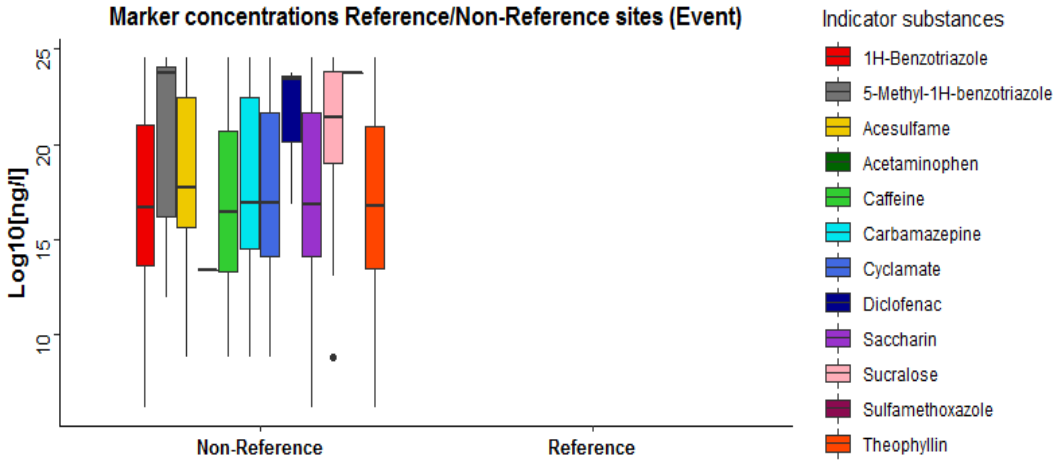
During precipitation, the marker concentrations vary significantly from those of grab samples. Picture 12 depicts the average marker concentrations during precipitation events. 38 sites met the statistical requirements of the analysis. Overall, the average amount and variety of measured substances increased. 12 indicator compounds were detected frequently, not affecting any reference site. The WWTP-site SH_2* is excluded from the analysis due to the lack of rain events.



PICTURE 12. Mean concentrations of indicator substances in event samples

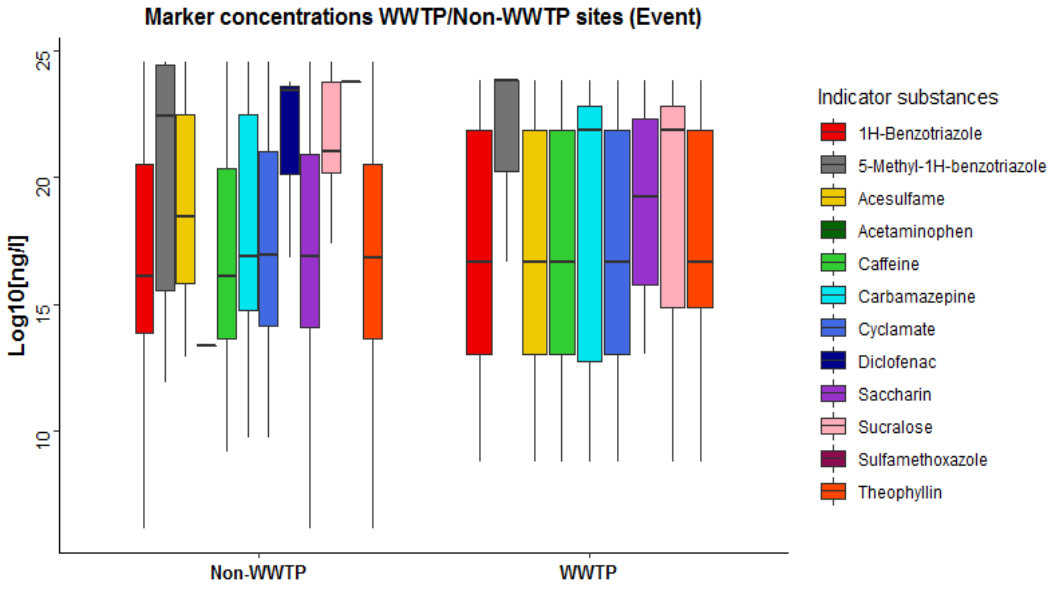
Furthermore, the WWTP sites do not show a tendency to high marker concentrations like in grab samples. So, the concentration of indicators as well as their variety increased, but not proportionally to the grab sample values.

Since there were no indicators found at reference sites, picture 13 depicts the mixture of indicators in high concentrations of regular measurement sites, while the reference sites were not affected at all.



PICTURE 13. Comparison of indicator concentrations in eds samples between reference and non-reference sites

The difference between WWTP-affected sites and others is to see in picture 14. The concentrations do not vary significantly, but sites without WWTP influence show a greater variety of indicators in form of the three additional pharmaceutical marker AC, DCF and SMX. DCF and SMX are considered WWTP-persistent compounds.



PICTURE 14. Comparison of indicator concentrations in event samples depending on WWTP presence on site

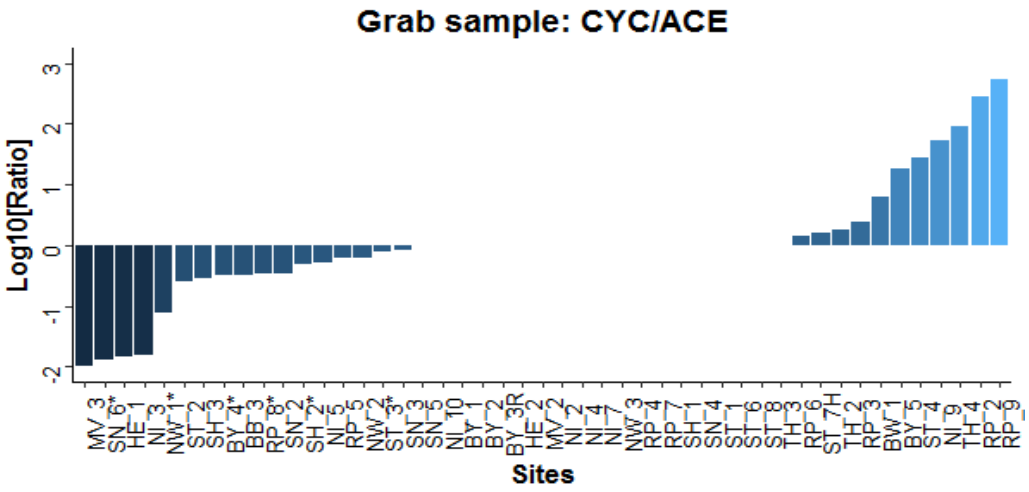
4.3 Determination of applicable ratios

In order to find a suitable ratio, the 13 marker substances, their properties and occurrence in the collected data was compared to literature-based ratios. (Kiguchi et al. 2016; Glassmeyer et al. 2005; Tran et al. 2013a, 2014; Sun et al. 2016; Danshevar et al. 2012, Oppenheimer et al. 2012; Dickenson et al. 2010) It became clear, that the available data supports the indication of treated and untreated wastewater.

The following figures show the results of successfully applied ratios. The collected data was assessed with ratios of a degradable versus a persistent indicator and thereby showing the primarily influence of treated or untreated wastewater. In doing so, sites with WWTP presence should result in a ratio below 0.

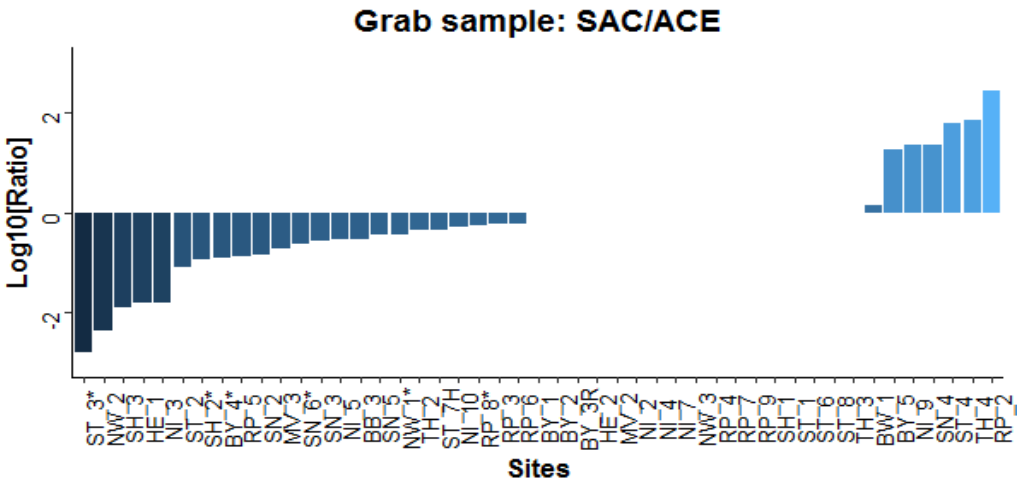
In case of the grab samples, 47 sites fulfilled the requirement for the ratio analysis. Due to a general lower amount of eds samples, 38 sites were analysed, whereby 1 of the initial 6 WWTP-sites had to be excluded.

4.3.1 Grab bottle samples



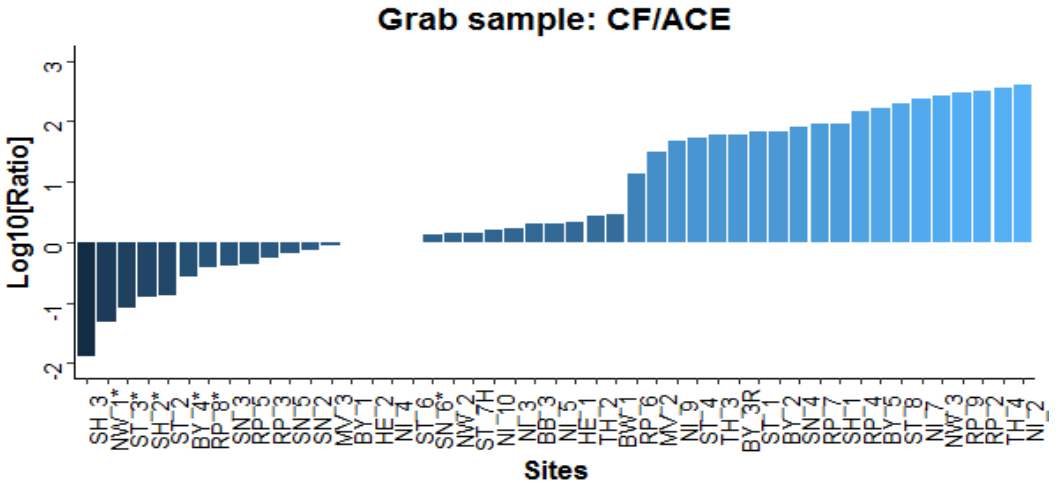
PICTURE 15. Results of the ratio Cyclamate/Acesulfame

The diagram in picture 15 shows the ratio distribution of Cyclamate versus Acesulfame. All WWTP-sites show the influence of treated wastewater with a ratio <0. According to this ratio, 11 other sites show similar patterns, while 17 of 47 sites are clearly influenced by untreated wastewater. The ranking of the WWTP-sites is a very positive outcome, but the number of sites with a ratio <0 is generally high.



PICTURE 16. Results of the ratio Saccharin/Acesulfame

In the results of the Saccharin versus Acesulfame ratio (picture 16) all 6 WWTP-sites are considered under treated wastewater influence, along with 17 others. 7 sites distinctly point at untreated wastewater impact. The high number of sites affected by treated wastewater is a suspicious outcome.

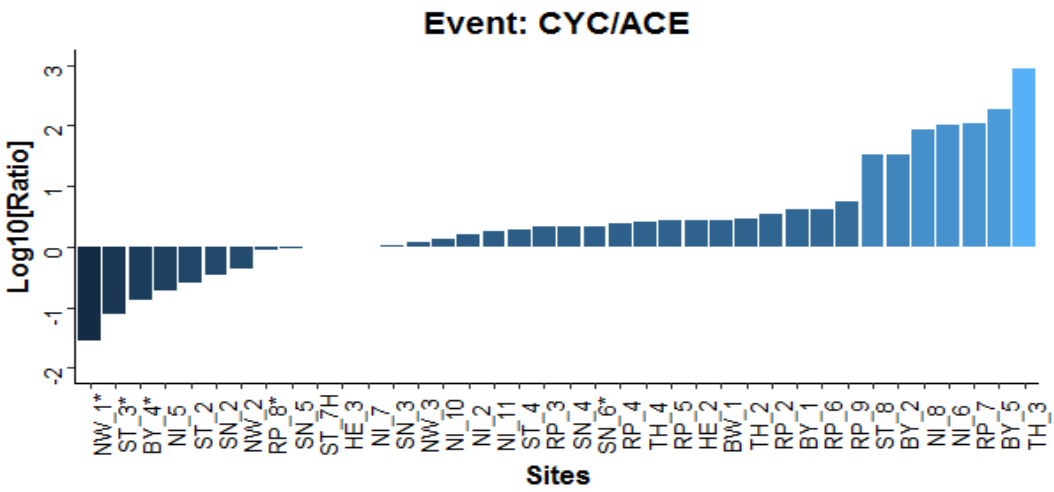


PICTURE 17. Results of the ratio Caffeine/Acesulfame

For the ratio of Caffeine versus Acesulfame 5 of 6 WWTP-sites result in a ratio below 0, indicating treated wastewater influence, as well as 8 others. 29 sites apparently contained traces of untreated wastewater.

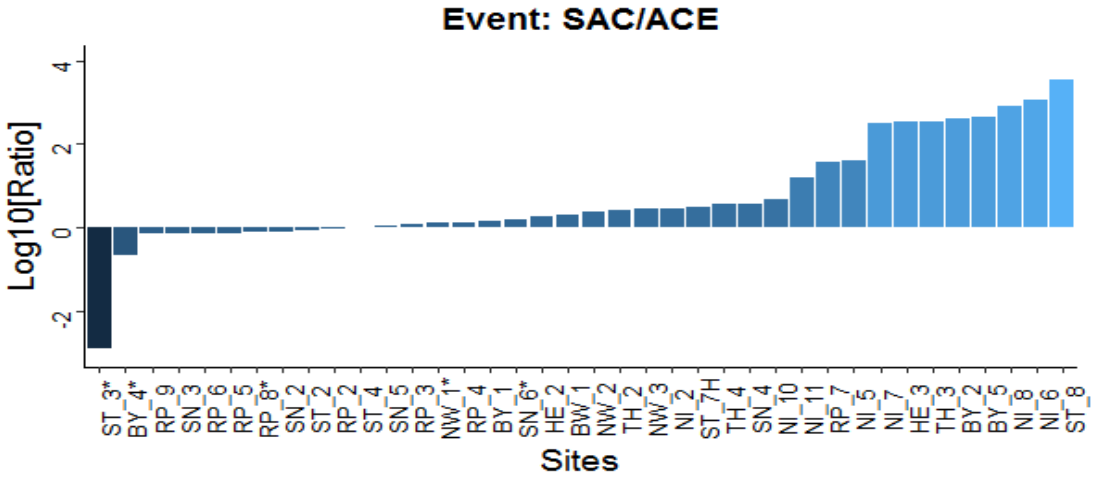
All three ratios result in a different ranking of the sites. In both, the CYC/ACE and the SAC/ACE ratios, the influence of treated wastewater at the WWTP-sites was identified, but so were plenty of others. In case of the CYC/ACE, less sites resulted in a ratio below 0. The CF/ACE ratio classified the least number of sites as affected by treated wastewater, but thereby only included 5 of 6 actual WWTP-sites.

4.3.2 Event samples



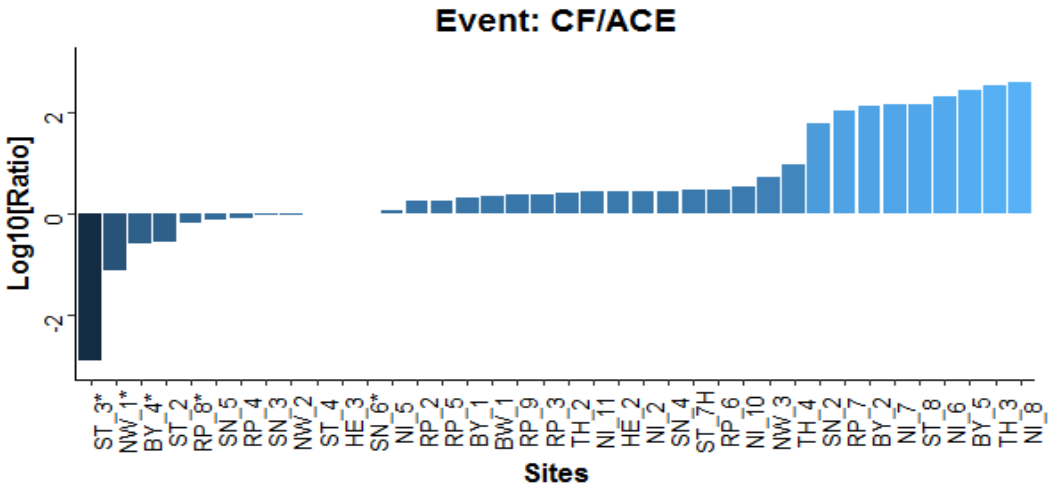
PICTURE 18. Results of the ratio Cyclamate/Acesulfame

Of the 38 sites, ratio Cyclamate/Acesulfame of event samples indicates 26 sites as under influence of untreated wastewater, including one WWTP. Only 9 sites show a clear ratio below 0 and point at the influence of treated wastewater, including 4 of 5 WWTP-sites.



PICTURE 19. Results of the ratio Saccharin/Acesulfame

Picture 19 depicts the outcome of the ratio SAC/ACE. Only three of five WWTP-sites were classified as affected by treated wastewater, making this ratio unreliable. The tendency towards untreated wastewater of this ratio is clearly to see in the diagram.

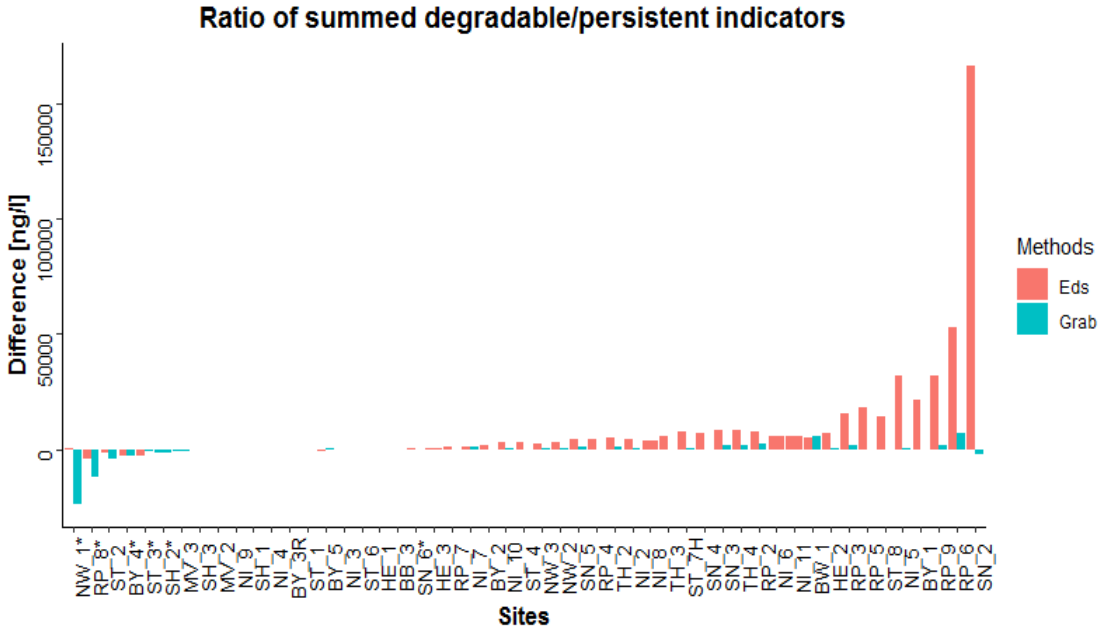


PICTURE 20. Results of the ratio Caffeine/Acesulfame

In terms of the WWTP sites, ratio CF/ACE (picture 20) considers all five measurement locations near-by a WWTP influenced by treated wastewater, four of them even amongst the five lowest ratio rates. 27 of the analysed 38 sites clearly show untreated wastewater influence. This ratio depicts the expected outcome the best.

The event sample ratios resemble the outcome of the grab sample ratios. The WWTP-sites are mostly assessed as under treated wastewater influence, but SN_6* and NW_1* did not quite fit the models. The results of CF/ACE are the closest to the expected outcome.

4.4 Summarized Comparison



PICTURE 21. Ratio of summed degradable markers – sum of persistent markers

Picture 21 shows the overall ratio, dividing the summed concentration of all degradable marker substances by the sum of persistent marker concentrations per site. The sampling methods are depicted with the help of two coloured columns per each site. Successfully, 5 of 6 WWTP-sites are ranked among the 6 sites with highest treated wastewater concentration, resembling the CF/ACE ratio of both methods. Site SN_6* shows signs of a spike.

Altogether, the ratio CF/ACE, as well as the summed ratio of degradable versus persistent indicators showed the most authentic outcome. Considering SN_6* as a spike, the result becomes even more obvious. The ratios of the grab samples indicated a higher number of sites as affected by untreated wastewater, while the event ratios showed a tendency towards treated water assessment.

5 DISCUSSION

Water bodies are influenced by a great variety of parameters. This turns the research of aquatic systems into a complex challenge with many sources of errors. In case of this study, first mistakes could already occur during sampling.

Samples might have been contaminated easily during the collection, especially by CF or AS, although blank samples suggest otherwise. Furthermore, the constant cool storage of samples cannot be assured entirely during sampling trips like those of the KgM-project. Above all, grab bottle sampling is not recommended for the evaluation of wastewater contaminants due to their fluctuating occurrence (Lee 2010). This recommendation is strengthened by the observation of James et al. (2016) and Madoux-Humery et al. (2013), confirming greatly fluctuating marker concentrations. Hence, a frequent automized composite sampling method is more reliable when observing wastewater marker.

Although the eds samples aimed on the determination of peak values, the average marker concentrations are much below reference values (table 4) of other studies on surface water contamination. Considering the strong agricultural influence at the sites while most studies take place in urban areas, the measured mean concentrations seem reasonable.

TABLE 4. Comparison of mean concentrations of wastewater indicator substances and corresponding reference values

Compound	concentration (in ng/l)	Maximum concentration in surface water (in ng/l)
Acesulfame	422	53.700[20]
Sucralose	400	10.000[21]
Saccharin	803	810[22]
Cyclamate	463	1406 [22]
Caffeine	668	14418[22]
Theophylline	308	3430[23]
Acetaminophen	139	1163[22]
Diclofenac	129	180[24]
Sulfamethoxazole	15	130[25]
Carbamazepine	24	190[21]
1H-Benzotriazole	208	1400[26]
5-Methyl-1H-benzotriazole	80	200[26]

[20]Ordonez (2012); [21]Oppenheimer et al. (2011); [22]Tran et al. (2013b); [23]Nakada et al. (2016); [24]Rio et al. (2013); [25]Sgroi et al. (2017); [26]LAWA (2016)

An increased marker concentration during wet weather was observed in many studies and complies with the results of this work. (Zirlewagen et al. 2016; Madoux-Humery et al. 2013; Rio et al. 2013; Buerge et al. 2003; Priegnitz, 2007; Poopipattana et al. 2018; Ekklesia et al. 2015; Tran et al. 2013a; Benotti & Brownawell 2007) The KgM-project's sampling system was aiming to measure maximal contamination of pesticides, not to monitor wastewater pollutants. Thus, the eds samples depict only maximum values, while grab samples resemble a brief extract of contamination in a water depth between 10 and 30cm. Considering that an assessment of wastewater marker was not planned, the shown difference between grab and eds measurements emphasizes that the sampling methods were chosen well by the project's researchers.

The indicators occurred in widely varying magnitude and frequency, complicating the statistical analysis. In wastewater marker research, the gathered data usually subjects sever restrictions, especially concerning the measurement frequency and concentration magnitude. Since the detection limit provides a restriction concerning the magnitude, researchers set frequency limitation between 20% (James et al. 2016) and 80% (Dickenson et al. 2010) in terms of indicator occurrences. With the exclusion of one-time measurements per method and location, the available samples were reduced from 513 to 414 or 19,3% respectively. This also means, 17 sites were not included in the analyses, resembling 24,6% of the available sites, complying with statistical methods of other marker studies.

CF, AS and a comparison of persistent versus degradable marker were often found to be useful indicators for untreated/treated wastewater. (Tran et al. 2013a, 2014; Schramm et al. 2006; Scheurer et al. 2011; Sun et al. 2016; Zirlewagen et al. 2016) Since manufacturers in use of coffee and AS are not known at the sites, a broad influence of untreated domestic wastewater can be concluded. On the other hand, Schramm et al. (2006) found cesspools to be an agricultural source of CF. But the magnitude and frequency of AS emphasize domestic wastewater origin. Comparing the ratios method-dependently indicates an increased influence of treated wastewater in event samples. The most common reasons for this result could be an enhanced input by WWTP overflows or sewage leakages. (Zirlewagen et al. 2016; Madoux-Humery et al. 2013; Rio et al.; Poopipattana et al. 2018; Ekklesia et al. 2015; Benotti & Brownawell 2007)

A common critic of the ratios is their exclusion of effects like indicator consumption patterns, soil absorption, weather conditions, population, land use, sampling method or the original indicator utilisation. (Ekklesia et al. 2015, Madoux- Humery et al. 2013; Kreitler 1969, Bremner et al. 1966, 1973)

The weather conditions were considered thanks to the two compared sampling methods. By limiting the measurement sites to agricultural water streams, land use effects were taken into account or rather certain pollutants statistically excluded from the beginning. In doing so, the ratio of summarized WWTP-extractable versus persistent indicators is an exceptionally good example of including differing land use pattern. This overall comparison considers the agricultural activity on site while delivering statistically stable results by including numerous indicators. Thereby, the ratio appears to be a widely applicable wastewater source identification method.

Furthermore, the compared substances occurred in similar frequencies and magnitudes as recommended by Yang et al. (2017). Nonetheless, more thorough knowledge about the present WWTPs, population, near-by manufacturers or food producing industry would lead to more precise results and finer data selection.

When assessing the toxicity, the lack of comparable data hinders the evaluation. Using the found PNEC-values, the marker substances do not occur in environmentally hazardous amounts. Only DCF exceeded its PNEC. On the other hand, a DCF-frequency of 7 measurements in 513 samples overall becomes neglectable with slightly severer statistical restrictions.

A comparison with resembling studies is rather difficult since wastewater marker studies preferably take place in and around municipalities and are conducted in cooperation with local WWTPs. Additionally, the type of examined water differ as well. The study results of drinking water springs in the Alpes can hardly be compared with those of the sea water at Jamaica bay. For this reason, the reference studies are limited to researches on surface water without saltwater influence but similar statistical approach.

6 CONCLUSIONS

The KgM-project at the UFZ examines agricultural small water bodies in order to assess the toxic influence of pesticides. The aim is to establish a water monitoring system and thereby improve the national water quality according to the EU Framework Directive 2009/128/EC on the sustainable use of pesticides. This work is analyzing the data of two different sampling methods, collected within the project, to evaluate the influence of urban wastewater at the tested sites.

Altogether, 412 of 513 samples contained a marker for industrial or domestic wastewater. A frequent presence of indicator substances was determined at 51 of 69 measurement sites. All 13 substances were detected, 12 of them frequently, but in widely differing concentrations and frequencies. CF occurred in the highest concentration and frequency, implying a general influence of domestic, untreated wastewater. The result is fortified by the high concentrations of SUC and SAC as well as the high frequency of CMZP. 1H-BTZ was detected in a similar high frequency as CF, suggesting a common industrial wastewater influence in the agricultural streams.

Furthermore, a weather-dependent, significant difference of indicator concentrations was determined. Overall, the collected event samples contained a greater variety and higher concentration of the selected marker compounds than the dry weather samples. This outcome does not necessarily depict the marker pattern of every individual measurement site. Nonetheless, the effects due to precipitation should be taken into account for similar measurements and researches.

Although the concentrations differ significantly between methods, the ratio CF/ACE assessed the WWTP contribution on sites in the most reliable manner for both sampling types. The ratio of summarised degradable versus persistent substances shows a similar outcome and confirms the results. These ratios help to quickly assess the contribution of untreated or treated wastewater at the measurement sites.

This work shows, even small agricultural streams with minimal urban influence are contaminated with municipal and industrial wastewater. The concentration of the studied marker substances generally increases during precipitation. Furthermore, the selected indicators allow an identification of the contributing sources in form of untreated or treated wastewater origin, helping to narrow further analyses.

However, wastewater marker and ratios are no stable tool for the assessment of wastewater contribution and sources yet. It is recommended to use marker substances in perspective of land use, season, precipitation, soil and water flow properties. For a more detailed identification of the polluting sources, assessment with a variety of ratios and correlations (population, pathogens, chloride) is necessary. Furthermore, an automatized sampling method at several locations along the water body and further research considering the present WWTPs or population distribution could help to identify polluting sources more thoroughly.

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APPENDIX

Appendix 1. Measurement sites with according mean concentrations of wastewater indicator substances in ng/l 1 (10)

Site	ACE	SUC	SAC	CYC	CF	THEO	AC	DCF	ASMX	SMX	CMZP	1H-BTZ	5M-BTZ	Additional
BB_3														
Grab	93.0		26.0	30.0	185.0	27.0					99.7	30.0		
Event														
BW_1														
Grab	169.0		232.3	1043.3	486.3							69.5		
Event	187.0		359.0	513.7	415.7	2233.0					6.0	451.3		
BY_1														
Grab						56.5					1.2			
Event	594.5	292.0	866.0	2428.5	1221.7	1176.0		64.5			18.5	411.0		
BY_2														
Grab					69.6	27.5								
Event			404.6	34.0	135.0	115.3						125.6		
BY_3R														Reference site
Grab					61.6									
Event														
BY_4*														WWTP presence
Grab	955.0		112.6	299.3	253.5	255.0					12.7	228.7	25.5	
Event	854.2	167.3	175.0	111.2	222.2	233.2						337.0		

Appendix 1. Measurement sites with according mean concentrations of wastewater indicator substances in ng/l 2 (10)

Site	ACE	SUC	SAC	CYC	CF	THEO	AC	DCF	ASMX	SMX	CMZP	1H-BTZ	5M-BTZ	Additional
BY_5														
Grab			18.0	18.3	169.5	55.3					4.6	28.5		
Event			448.2	183.0	293.5	1624.7					3.5	159.6	42.5	
HE_1														
Grab	67.0				141.0	157.0					5.2			
Event														
HE_2														
Grab						114.3					1.6			
Event	237.6		438.3	643.0	651.3	164.5		21.5			1.6	259.6		
HE_3														
Grab			344.6			31.0						126.5		
Event														
MV_1														
Grab						19.5								
Event														
MV_2														
Grab					31.0	29.6								
Event														

Appendix 1. Measurement sites with according mean concentrations of wastewater indicator substances in ng/l 3 (10)

Site	ACE	SUC	SAC	CYC	CF	THEO	AC	DCF	ASMX	SMX	CMZP	1H-BTZ	5M-BTZ	Additional
MV_3														
Grab	97.0		17.6		88.0	45.5					4.0	54.3		
Event														
NI_3														
Grab					109.5	66.0					1.2			
Event														
NI_4														
Grab											1.3			
Event														
NI_5														
Grab	102.0	204.6	28.3	52.5		71.3					2.3	19.0		
Event	156.6	175.5	6375.0	30.0		130.5					2.0	100.3		
NI_6														
Grab														
Event			1094.6	100.6	210.5	131.0	57.5				1.0	135.0		
NI_7														
Grab					237.3	71.6								
Event			313.0		149.0	227.0						139.0		

Appendix 1. Measurement sites with according mean concentrations of wastewater indicator substances in ng/l 4 (10)

Site	ACE	SUC	SAC	CYC	CF	THEO	AC	DCF	ASMX	SMX	CMZP	1H-BTZ	5M-BTZ	Additional
NI_8														
Grab						14.5								
Event			833.0	84.0	404.8	749.2						166.4		
NI_9														
Grab			22.6	53.5	47.3									
Event														
NI_10														
Grab	104.5		51.6	103.6	163.3	88.3					3.0	24.5		
Event	75.0		362.0	102.0	259.0	95.5					6.0	186.5		
NI_11														
Grab						34.5								
Event	75.0		1158.3	133.2	200.0	50.6						74.5		
NW_1*														
Grab	4057.7	1769.0	1428.0	308.5	198.2	86.0					84.5			WWTP presence
Event	3829.0	1417.0	5029.5	107.5	293.5	122.5					72.5	922.0		
NW_2														
Grab	231.6			146.5	231.0	122.5						125.0		
Event	409.0	446.5	984.0	174.0	394.3	160.3						259.0		

Appendix 1. Measurement sites with according mean concentrations of wastewater indicator substances in ng/l 5 (10)

Site	ACE	SUC	SAC	CYC	CF	THEO	AC	DCF	ASMX	SMX	CMZP	1H-BTZ	5M-BTZ	Additional
NW_3														
Grab					271.5	109.7					1.0	21.7		
Event	67.6		190.0	82.6	350.2	150.6					1.5	141.6		
RP_2														
Grab			281.5	278.5	316.2	37.5					60.4	42.6		
Event	287.6	140.0	265.4	977.6	509.6	537.6					26.2	191.4		
RP_3														
Grab	140.8		84.0	347.6	75.4	91.5					6.4	48.5	20.0	
Event	640.8	278.0	731.8	1365.1	1540.7	1519.1					64.4	323.2	437.0	
RP_4														
Grab					149.2						2.8	20.3	12.0	
Event	297.7		400.3	701.8	239.8	201.0					12.2	106.0		
RP_5														
Grab	190.5		25.3	118.7	80.5	49.0					14.7	18.5		
Event	868.8	589.3	638.6	2333.7	1604.1	880.0					92.7	295.6		
RP_6														
Grab	97.3		58.3	134.7	1363.5						2.4	42.0		
Event	1410.4	914.2	1001.6	5929.0	4326.2	1528.8					78.0	566.6		

Appendix 1. Measurement sites with according mean concentrations of wastewater indicator substances in ng/l 6 (10)

Site	ACE	SUC	SAC	CYC	CF	THEO	AC	DCF	ASMX	SMX	CMZP	1H-BTZ	5M-BTZ	Additional
RP_7														
Grab					89.0							39.5		
Event			37.0	112.0	112.3	110.0					2.6			
RP_8*														
Grab	629.3	2084.6	342.3	212.0	239.3						18.1	1047.0	74.5	WWTP presence
Event	991.0	1936.4	763.7	862.2	642.2	225.0					43.1	783.8	105.0	
RP_9														
Grab				546.5	308.5	222.5								
Event	749.7	456.4	519.2	4270.0	1794.0	1010.7					1.1	217.8		
SH_1														
Grab														
Event					91.7	30.0								
SH_2*														
Grab	527.7		58.6	252.0	64.0	31.0						114.7		WWTP presence
Event														
SH_3														
Grab	79.0			23.0		30.5								
Event														

Appendix 1. Measurement sites with according mean concentrations of wastewater indicator substances in ng/l 7 (10)

Site	ACE	SUC	SAC	CYC	CF	THEO	AC	DCF	ASMX	SMX	CMZP	1H-BTZ	5M-BTZ	Additional
SN_2														
Grab	568.7	477.0	81.5	198.5	430.7	101.3					5.7	166.7		
Event	444.0	374.0	345.3	153.6	28029.3	661.6					5.3	184.6		
SN_3														
Grab	946.5	149.0	250.0	795.0	385.0	132.6					4.5	1170.2		
Event	1025.4	269.3	722.9	1071.0	953.1	1697.4					15.4	1437.7	302.0	
SN_4														
Grab			22.6		79.5	68.5								
Event	204.0	88.0	731.3	442.0	574.6	500.2						154.2		
SN_5														
Grab	558.5	361.3	194.2	547.5	361.2	308.6					4.0	186.7		
Event	570.4	296.1	601.8	542.0	431.1	347.7		90.0		19.0	4.0	418.0	25.5	
SN_6*														
Grab	78.6	171.0	18.0		103.3							66.5		WWTP presence
Event	128.3		192.0	280.5	133.3	85.3						137.6		
ST_1														
Grab					66.0									
Event														

Appendix 1. Measurement sites with according mean concentrations of wastewater indicator substances in ng/l 8 (10)

Site	ACE	SUC	SAC	CYC	CF	THEO	AC	DCF	ASMX	SMX	CMZP	1H-BTZ	5M-BTZ	Additional
ST_2														
Grab	708.2	199.0	57.2	174.7	93.5	42.0					66.7	292.5		
Event	577.4	410.4	489.8	147.2	156.5	109.0					74.6	336.1	148.5	
ST_3*														
Grab	617.5	114.5		489.0	51.6	31.3					48.3	132.0		WWTP presence
Event	830.5			65.0							73.0	157.0		
ST_4														
Grab			59.0	28.0	52.5						5.0			
Event	262.0		253.3	491.5	260.0	190.2					34.0	128.1		
ST_6														
Grab											1.5			
Event														
ST_7H														
Grab	69.0		31.5	110.5	98.5	22.5					2.7	44.6		
Event	250.8		786.0	250.5	729.4	377.2					4.2	240.4		
ST_8														
Grab					197.6							21.0		
Event			3392.0	33.3	153.3	69.0					1.0	105.0	7.0	

Appendix 1. Measurement sites with according mean concentrations of wastewater indicator substances in ng/l 9 (10)

Site	ACE	SUC	SAC	CYC	CF	THEO	AC	DCF	ASMX	SMX	CMZP	1H-BTZ	5M-BTZ	Additional
TH_1R														
Grab						23.0								References site
Event														
TH_2														
	68.0		30.5	121.0	187.6	108.0					45.2	118.5		
Event	149.0	100.0	369.5	426.0	392.0	220.0					59.0	258.0		
TH_3														
Grab					60.6						3.5			
Event			350.0	895.0	347.5						3.0	109.0		
TH_4														
Grab			68.0	93.6	365.0						5.5	82.0		
Event	112.5		393.4	285.0	1087.5	215.2					6.5	205.8		

Appendix 1. Measurement sites with according mean concentrations of wastewater indicator substances in ng/l 10 (10)

Excluded sites due to statistical requirements														
BB_1														
BB_2														
BY_3R														Reference site
NI_1														
NI_1R														Reference site
NI_2														
NI_12														
RP_1														
RP_2R														Reference site
RP_3R														Reference site
RP_5R														Reference site
RP_8R														Reference site
RP_9R														
RP_10														
SN_1R														References site
ST_5R														Reference site
ST_7B														

Appendix 2. R-Script 1(14)

```
# Importing data
meinpfad <- "C:\\Users\\eglinski\\Desktop\\CHRISSI_Thesis"
master_c <- read.table(file.choose("Rohdata"),
                       header=TRUE, sep="\t", dec=".",quote = "",stringsAsFactors =FALSE);
head(dataframe)

referencesites<- c("NI_1R", "TH_1R", "RP_5R","RP_6R", "RP_8R", "RP_9R");
print(referencesites)
wwtpsites<- c("BY_4*", "NW_1*", "RP_8*","SH_2*", "ST_3*", "SN_6*")

# Subset from the master data frame of sewage indicators
master_c <- subset(master_c, subKgM=="Acesulfame" | subKgM=="Sucralose" |
subKgM=="Saccharin" | subKgM=="Cyclamate" | subKgM=="Caffeine" |
subKgM=="Theophyllin" | subKgM=="Acetaminophen" | subKgM=="Diclofenac" |
subKgM=="Acetyl-sulfamethoxazole" | subKgM=="Sulfamethoxazole" |
subKgM=="Carbamazepine" | subKgM=="1H-Benzotriazole" | subKgM=="5-Methyl-
1H-benzotriazole"); unique(master_c$subKgM)

# Delete all rows that contain NA in concentration column
master_c <- master_c[complete.cases(master_c$concentration),]; nrow(master_c)

#amount of samples
Samples <- master_c%>%
dplyr::group_by(siteID, date)%>%
dplyr::select(siteID, date, method)%>%
dplyr::count(siteID, date, method)
```

CALCULATIONS

```
# Mean concentration for all substances
master_c <- group_by(master_c, siteID, subKgM, method) %>%
  mutate(., mean(concentration))

# Excluding one-time concentrations:
attach(master_c)
master_c %>%
  group_by(siteID) %>%
  duplicated(.,siteID, subKgM, incomparables = FALSE, fromLast = TRUE)

#Turn siteID into factor for grouping
master_c$siteID<-as.factor(master_c$siteID)

#Check for one time measurements
smartie <- master_c %>%
  dplyr::count(siteID, subKgM) %>%
  filter(n>1); sum(master_c$n)
```

Appendix 2. R-Script 2(14)

```
# Grouping site, mean and substance and calculate mean concentration
Question2<-
ddply(master_c,.(subKgM,siteID,method),function(x){data.frame(siteID=x$siteID[1],
 #subKgM=x$subKgM[1], meanconcentration=mean(x$concentration) #sub =
x$subKgM[mean(x$concentration)])})

#Connecting one time measurements and mean concentration
barplotdata<-paste(smartie$siteID,smartie$subKgM)
barplotdata <- merge(Question2, smartie, by=c("siteID", "method", "subKgM")); head(barplotdata)

#Logarithm of concentration into new column
barplotdata$Logmeanconcentration <- log10(barplotdata$meanconcentration)

#Calculate total sum of sewage indicators for each site, so plot can be ordered
barplot1 <- barplotdata
barplot1 <- barplot1 %>% dplyr::group_by(siteID,method) %>% dplyr::select(siteID, method, Logmeanconcentration) %>% dplyr::summarise(., sum = sum(Logmeanconcentration, na.rm = TRUE)) %>% spread(key = method, value = sum)

#Spread mean concentration according to methods
barplotdata1 <- barplotdata %>% group_by(siteID, subKgM, method, Logmeanconcentration) %>% spread(key = method, value = Logmeanconcentration)

#Merge total sum and individual mean concentration to order plot
barplotdata2 <- merge(barplotdata1, barplot1, by="siteID"); head(barplotdata2)
```

PLOTTING

```
#Mean concentration of single subKgM
Submean <- barplotdata %>% group_by(subKgM) %>% dplyr::summarise(., mean_conc = mean(meanconcentration))

ggplot(Submean[which(Submean$mean_conc>0),], aes(x = reorder(subKgM,mean_conc), y = mean_conc))+ geom_bar(stat= "identity", aes(fill = subKgM))+ labs(x="Indicator", y="Concentration [ng/l]", title="Mean concentration of marker substances")+ theme_classic()+ theme(axis.text.x = element_text(family= "Times", color="black", size=8, face="bold",angle=90,margin = margin(t = 10, r = 0, b = 0, l = 0)), axis.title.x = element_blank(), axis.text.y = element_text(family= "Times", color="black", size=10, face="plain",angle=90), axis.title.y = element_text(family="Times", color = "black", size = 10, face = "bold"), plot.title = element_text(hjust=0.5, family="Times", color="black", size=12, face="bold"))+ coord_cartesian(xlim = c(1:13))+ scale_fill_manual( "Indicator substances", values=c("1H-Benzotriazole"="red2","5-Methyl-1H-benzotriazole"="grey45", "Acesulfame"="gold2", "Acetaminophen"="darkgreen","Acetyl-sulfamethoxazole"="olivedrab2", "Caffeine"="limegreen", "Carbamazepine"="turquoise2", "Cyclamate" = "royalblue", Diclofenac"="blue4", "Saccharin"="darkorchid", "Sucralose"="lightpink1", "Sulfamethoxazole"="deeppink4", "Theophyllin"="orangered1")) + scale_x_discrete(labels=c("SMX", "CMZP", "AC", "DCF", "5M-1H-BTZ","1H-BTZ","THEO","ACE", "CYC", "SUC", "SAC", "CF"))
```

Appendix 2. R-Script 3(14)

```
#Frequency
frequ <- barplotdata %>%
  dplyr::group_by(subKgM) %>%
  dplyr::summarise(., Frequency = sum(n, na.rm = TRUE))

ggplot(frequ[which(frequ$Frequency>0),], aes(x = reorder(subKgM,Frequency), y = Frequency))+ geom_bar(stat= "identity", aes(fill = subKgM))+ labs(x="Indicator", y="Frequency", title="Frequency of marker substances")+ theme_classic()+ theme(axis.text.x = element_text(family= "Times", color="black", size=10, face="bold",angle=90,margin = margin(t = 10, r = 0, b = 0, l = 0)), axis.title.x = element_blank(), axis.text.y = element_text(family= "Times", color="black", size=10, face="plain",angle=90), axis.title.y = element_text(family="Times", color = "black", size = 10, face = "bold"), plot.title = element_text(hjust=0.5, family="Times", color="black", size=12, face="bold"))+ coord_cartesian(xlim = c(1:13))+ scale_fill_manual( "Indicator substances", values=c("1H-Benzotriazole"="red2","5-Methyl-1H-benzotriazole"="grey45", "Acesulfame"="gold2", "Acetaminophen"="darkgreen","Acetyl-sulfamethoxazole"="olivedrab2", "Caffeine"="limegreen", "Carbamazepine"="turquoise2", "Cyclamate" = "royalblue", Diclofenac"="blue4", "Saccharin"="darkorchid", "Sucralose"="lightpink1", "Sulfamethoxazole"="deeppink4", "Theophyllin"="orangered1"))+ scale_x_discrete(labels=c("AC","SMX","DCF", "5M-1H-BTZ","SUC", "ACE", "CYC", "SAC", "THEO", "CMZP", "1H-BTZ","CF"))

#Difference plot between event and grab samples per site
diff <- barplotdata2

#Calculate difference between schoepf and eds in new column
diff$diff <- diff$eds.y / diff$schoepf.y

#Unique the columns siteID and difference
diff1 <- unique(diff[c("siteID","diff")])
```

Appendix 2. R-Script 7(26)

```
# Plot
ggplot(diff1[which(diff1$diff>0),], aes(x=reorder(siteID, diff), y=diff, fill=diff))+ geom_bar(stat = "identity")+ theme_classic()+ labs(x="Sites", y="Log10[ng/l]", title="Method-dependent difference of marker concentrations")+ theme(axis.text.x = element_text(angle=90))+ theme(axis.text.x = element_text(family= "Times", color="black", size=12, face="plain",angle=90), axis.title.x = element_text(family="Times", color = "black", size = 14, face = "bold"), axis.text.y = element_text(family= "Times", color="black", size=10, face="plain",angle=90), axis.title.y = element_text(family="Times", color = "black", size = 14, face = "bold"), plot.title = element_text(family="Times", color="black", size=14, face="bold"))
```

Appendix 2. R-Script 4(14)

T-TEST

```
#Setting NA as 0
is.na(diff$diff)
diff[is.na(diff)] <- 0

#Is the data distribution normal?
shapiro.test(diff$diff)
data: diff$diff W = 0.63908, p-value < 2.2e-16 #p<0,05
 data normally distributed, paired t-test possible
ts = replicate(1000,t.test(rnorm(490),rnorm(490))$statistic) range(ts) pts = seq(-4,4,length=100) plot(pts,dt(pts, df=30), col='red',type='h', lwd=2,xlab="", ylab="", main="Distribution of measured values, df=30") lines(density(ts), lwd=3, type='l') labs(x="T-distribution", y=) legend("topright", c("measurements","t-test distribution"), fill=c("black","red"))

#Paired t-test
t.test(diff$eds.y,diff$schoepf.y,paired=TRUE, alternative="two.sided")
data:  diff$eds.y and diff$schoepf.y t = 20.086, df = 489, p-value < 2.2e-16 alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:  5.046483 6.140828
sample estimates:  mean of the differences 5.593655

ggplot(diff1[which(diff1$diff>0),], aes(x=reorder(siteID, diff), y=diff, fill=diff))+
 geom_bar(stat = "identity")+theme_classic()+ labs(x="Sites", y="Log10[ng/l]", title="Method-dependent difference of marker concentrations", subtitle = "t = 20.086, df = 489, p-value < 2.2e-16, mean of the differences= 5.59")+ theme(axis.text.x = element_text(angle=90))+ theme(axis.text.x = element_text(family= "Times", color="black", size=12, face="plain",angle=90), axis.title.x = element_text(family="Times", color = "black", size = 12, face = "bold"), axis.text.y = element_text(family= "Times", color="black", size=10, face="plain",angle=90), axis.title.y = element_text(family="Times", color = "black", size = 12, face = "bold"), plot.title = element_text(family="Times", color="black", size=14, face="bold"),  legend.position = "none")
```

GRAB SAMPLES

```
#Order acording to sum of all grab samples via siteID and subKgM
barplotdata3 <- barplotdata2[order(barplotdata2$schoepf.y, barplotdata2$siteID, barplotdata2$subKgM),]; head(barplotdata3)
```

Appendix 2. R-Script 5(14)

```
#Stacked Bar Plot with ggplot2
ggplot(barplotdata3[which(barplotdata3$schoepf.y >0),], aes(x = reorder(siteID, schoepf.y), y = schoepf.x, fill="Indicators"))+ geom_bar(stat= "identity", aes(fill = subKgM, width =0.8))+ labs(x="Sites", y="Log10[ng/l]", title="Marker concentrations in grab samples per site")+ theme_classic()+ theme(axis.text.x = element_text(family= "Times", color="black", size=10, face="plain",angle=90), axis.title.x = element_text(family="Times", color = "black", size = 12, face = "bold"), axis.text.y = element_text(family= "Times", color="black", size=10, face="plain",angle=90), axis.title.y = element_text(family="Times", color = "black", size = 12, face = "bold"), plot.title = element_text(hjust=0.5, family="Times", color="black", size=14, face="bold"))+ coord_cartesian(xlim = c(1:51))+ scale_fill_manual( "Indicator substances", values=c("1H-Benzotriazole"="red2","5-Methyl-1H-benzotriazole"="grey45", "Acesulfame"="gold2", "Acetaminophen"="darkgreen","Acetyl-sulfamethoxazole"="olivedrab2", "Caffeine"="limegreen", "Carbamazepine"="turquoise2", "Cyclamate" = "royalblue", Diclofenac"="blue4", "Saccharin"="darkorchid", "Sucralose"="lightpink1", "Sulfamethoxazole"="deeppink4", "Theophyllin"="orangered1"))
```

EVENT SAMPLES

```
# Order according to sum of all events
barplotdata4<-
barplotdata2[order(barplotdata2$eds.y,barplotdata2$siteID,barplotdata2$subKgM),];
head(barplotdata4)

ggplot(barplotdata4[which(barplotdata4$eds.y>0),], aes(x = reorder(siteID, eds.y), y = eds.x))+ geom_bar(stat= "identity", aes(fill = subKgM, width =0.8))+ labs(x="Sites", y="Log10[ng/l]", title="Marker concentrations in event samples per site")+ theme_classic()+ theme(axis.text.x = element_text(family= "Times", color="black", size=10, face="plain",angle=90), axis.title.x = element_text(family="Times", color = "black", size = 12, face = "bold"), axis.text.y = element_text(family= "Times", color="black", size=10, face="plain",angle=90), axis.title.y = element_text(family="Times", color = "black", size = 12, face = "bold"), plot.title = element_text(hjust=0.5, family="Times", color="black", size=14, face="bold"))+ coord_cartesian(xlim = c(1:38))+ scale_fill_manual( "Indicator substances", values=c("1H-Benzotriazole"="red2","5-Methyl-1H-benzotriazole"="grey45", "Acesulfame"="gold2", "Acetaminophen"="darkgreen","Acetyl-sulfamethoxazole"="olivedrab2", "Caffeine"="limegreen", "Carbamazepine"="turquoise2", "Cyclamate" = "royalblue", Diclofenac"="blue4", "Saccharin"="darkorchid", "Sucralose"="lightpink1", "Sulfamethoxazole"="deeppink4", "Theophyllin"="orangered1"))
```

Appendix 2. R-Script 6(14)

BOXPLOTS

```
#New column with information about method
Boxplotdata<-barplotdata4
Boxplotdata$method <- ifelse(Boxplotdata$eds.x > 0, "eds", "schoepf") Box-
plotdata$method[is.na(Boxplotdata$method)] <- "schoepf"
Boxplotdata$conc <- NA Boxplotdata$conc[is.na(Boxplotdata$conc)] <- Box-
plotdata$eds.x Boxplotdata$conc <- ifelse(is.na(Boxplotdata$conc), Box-
plotdata$schoepf.x, Boxplotdata$eds.x)
Boxplotdata$reference <- ifelse(Boxplotdata$siteID %in% referencesites, "Reference",
"Non-Reference"); print(df$reference); head(df)
Boxplotdata$wwtp <- ifelse(Boxplotdata$siteID %in% wwtpsites, "WWTP", "Non-
WWTP"); print(df$wwtp); head(df)

#Plotting Referencesites from Grab samples
ggplot(Boxplotdata, aes(x =reference, y = schoepf.y))+ geom_boxplot()+
theme_classic()+ fill=schoepf.y+ labs(x="Non-Reference/Referencesites",
y="log10[ng/l]", title="Comparison of marker concentrations in grab samples ")+
scale_fill_hue(c=100,l=70)+ theme(axis.text.x = element_text(angle=90))

#with subKgM
ggplot(Boxplotdata, aes(x = reference, y = schoepf.y, fill=subKgM))+ geom_boxplot()+
labs(x = element_blank(),y="Log10[ng/l]", title="Marker concentrations in Refer-
ence/Non-Reference sites (Grab)")+  theme_classic()+ theme(axis.text.x = ele-
ment_text(family= "Times", color="black", size=10, face="bold"), axis.title.x = ele-
ment_text(family="Times", color = "black", size = 12, face = "bold"), axis.text.y = ele-
ment_text(family= "Times", color="black", size=10, face="plain",angle=90), axis.title.y
= element_text(family="Times", color = "black", size = 12, face = "bold"), plot.title =
element_text(hjust=0.5, family="Times", color="black", size=12, face="bold"))+
scale_fill_manual( "Indicator substances", values=c("1H-Benzotriazole"="red2","5-
Methyl-1H-benzotriazole"="grey45", "Acesulfame"="gold2", "Acetamino-
phen"="darkgreen","Acetyl-sulfamethoxazole"="olivedrab2", "Caffeine"="limegreen",
"Carbamazepine"="turquoise2", "Cyclamate" = "royalblue", Diclofenac"="blue4",
"Saccharin"="darkorchid", "Sucralose"="lightpink1", "Sulfamethoxa-
zole"="deeppink4", "Theophyllin"="orangered1"))
```

Appendix 2. R-Script 7(14)

```
#Plotting WWTP from Grab samples
ggplot(Boxplotdata, aes(x = wwtp, y = schoepf.y, fill=subKgM))+ geom_boxplot()+ labs(x = element_blank(),y="Log10[ng/l]", title="Marker concentrations in WWTP/Non-WWTP sites (Grab)")+ theme_classic()+ theme(axis.text.x = element_text(family= "Times", color="black", size=10, face="bold"), axis.title.x = element_text(family="Times", color = "black", size = 12, face = "bold"), axis.text.y = element_text(family= "Times", color="black", size=10, face="plain",angle=90), axis.title.y = element_text(family="Times", color = "black", size = 12, face = "bold"), plot.title = element_text(hjust=0.5, family="Times", color="black", size=12, face="bold"))+ scale_fill_manual( "Indicator substances", values=c("1H-Benzotriazole"="red2","5-Methyl-1H-benzotriazole"="grey45", "Acesulfame"="gold2", "Acetaminophen"="darkgreen","Acetyl-sulfamethoxazole"="olivedrab2", "Caffeine"="limegreen", "Carbamazepine"="turquoise2", "Cyclamate" = "royalblue", Diclofenac"="blue4", "Saccharin"="darkorchid", "Sucralose"="lightpink1", "Sulfamethoxazole"="deeppink4", "Theophyllin"="orangered1"))

#Plotting Referencesites from event samples
ggplot(Boxplotdata, aes(x = reference, y = eds.y, fill=subKgM))+ geom_boxplot()+ labs(x = element_blank(),y="Log10[ng/l]", title="Marker concentrations Reference/Non-Reference sites (Event)")+ theme_classic()+ theme(axis.text.x = element_text(family= "Times", color="black", size=10, face="bold"), axis.title.x = element_text(family="Times", color = "black", size = 12, face = "bold"), axis.text.y = element_text(family= "Times", color="black", size=10, face="plain",angle=90), axis.title.y = element_text(family="Times", color = "black", size = 12, face = "bold"), plot.title = element_text(hjust=0.5, family="Times", color="black", size=12, face="bold"))+ scale_fill_manual("Indicator substances", values=c("1H-Benzotriazole"="red2","5-Methyl-1H-benzotriazole"="grey45", "Acesulfame"="gold2", "Acetaminophen"="darkgreen","Acetyl-sulfamethoxazole"="olivedrab2", "Caffeine"="limegreen", "Carbamazepine"="turquoise2", "Cyclamate" = "royalblue", Diclofenac"="blue4", "Saccharin"="darkorchid", "Sucralose"="lightpink1", "Sulfamethoxazole"="deeppink4", "Theophyllin"="orangered1"))
```

Appendix 2. R-Script 8(14)

```
#Plotting WWTP-sites of Events
ggplot(Boxplotdata, aes(x = wwtp, y = eds.y, fill=subKgM))+  geom_boxplot()+
  labs(x = element_blank(),y="Log10[ng/l]", title="Marker concentrations WWTP/Non-WWTP sites (Event)")+ theme_classic()+ theme(axis.text.x = element_text(family= "Times", color="black", size=10, face="bold"), axis.title.x = element_text(family="Times", color = "black", size = 12, face = "bold"), axis.text.y = element_text(family= "Times", color="black", size=10, face="plain",angle=90), axis.title.y = element_text(family="Times", color = "black", size = 12, face = "bold"), plot.title = element_text(hjust=0.5, family="Times", color="black", size=12, face="bold"))+ scale_fill_manual("Indicator substances", values=c("1H-Benzotriazole"="red2","5-Methyl-1H-benzotriazole"="grey45", "Acesulfame"="gold2", "Acetaminophen"="darkgreen","Acetyl-sulfamethoxazole"="olivedrab2", "Caffeine"="limegreen", "Carbamazepine"="turquoise2", "Cyclamate" = "royalblue", Diclofenac"="blue4", "Saccharin"="darkorchid", "Sucralose"="lightpink1", "Sulfamethoxazole"="deeppink4", "Theophyllin"="orangered1"))
```

RATIOS

```
ratio <- subset(master_c, subKgM=="Acesulfame" |subKgM=="Sucralose" |subKgM=="Saccharin" |subKgM=="Cyclamate" |subKgM=="Caffeine" |subKgM=="Carbamazepine"); unique(ratio$subKgM)
ratio <- ratio[.,c("siteID","date","method","subKgM", "concentration")]; head(ratio)

# Excluding one-time measurements
onetime <- ratio %>%  dplyr::count(siteID, subKgM) %>%  filter(n>1)
# Merge with subset of ratios
ratio1<-paste(onetime$siteID, onetime$subKgM, onetime$method)
ratio1 <- merge(ratio, onetime, by=c("siteID","subKgM", "method"), all.x = FALSE, all.y = FALSE)

# Mean concentration into columns for ratio calculation
ratagg <- ratio1 %>%  group_by(siteID,subKgM, method) %>% dplyr::summarise(., mean_conc = mean(concentration)) %>% spread(key = subKgM, value = mean_conc)
```

Appendix 2. R-Script 9(14)

```
# Turn NAs in 0
ratagg[is.na(ratagg)] <- 1

# Calculate conventional Ratios
ratagg$Cyc_Ace <- ratagg$Cyclamate / ratagg$Acesulfame
ratagg$Caf_Cmzp <- ratagg$Caffeine / ratagg$Carbamazepine
ratagg$Sac_Suc <- ratagg$Saccharin / ratagg$Sucralose
ratagg$Sac_Ace <- ratagg$Saccharin / ratagg$Acesulfame
ratagg$Caf_Ace <- ratagg$Caffeine / ratagg$Acesulfame
ratagg$Cyc_Suc <- ratagg$Cyclamate / ratagg$Sucralose

#Subesetting for plots
rataggEds <-subset( ratagg, ratagg$method=="eds")
rataggEds$LogCYC_ACE <- log10(rataggEds$Cyc_Ace)
rataggEds$LogCAF_CMZP <- log10(rataggEds$Caf_Cmzp)
rataggEds$LogSAC_SUC <- log10(rataggEds$Sac_Suc)
rataggEds$LogSAC_ACE <- log10(rataggEds$Sac_Ace)
rataggEds$LogCAF_ACE <- log10(rataggEds$Caf_Ace)
rataggEds$LogCYC_SUC <- log10(rataggEds$Cyc_Suc)

rataggGrab <-subset( ratagg, ratagg$method=="schoepf")
rataggGrab$LogCYC_ACE <- log10(rataggGrab$Cyc_Ace)
rataggGrab$LogCAF_CMZP <- log10(rataggGrab$Caf_Cmzp)
rataggGrab$LogSAC_SUC <- log10(rataggGrab$Sac_Suc)
rataggGrab$LogSAC_ACE <- log10(rataggGrab$Sac_Ace)
rataggGrab$LogCAF_ACE <- log10(rataggGrab$Caf_Ace)
rataggGrab$LogCYC_SUC <- log10(rataggGrab$Cyc_Suc)
```

Appendix 2. R-Script 10(14)

```
#Plotting of Events, using log10
#CYC/ACE
ggplot(rataggEds, aes(x=reorder(siteID, LogCYC_ACE), y=LogCYC_ACE, fill=LogCYC_ACE))+ geom_bar(stat = "identity")+ theme_classic()+ theme(axis.text.x = element_text(family= "Times", color="black", size=10, face="plain",angle=90), axis.title.x = element_text(family="Times", color = "black", size = 12, face = "bold"), axis.text.y = element_text(family= "Times", color="black", size=10, face="plain",angle=90), axis.title.y = element_text(family="Times", color = "black", size = 12, face = "bold"), plot.title = element_text(hjust=0.5, family="Times", color="black", size=14, face="bold"), legend.position = "none")+ labs(x="Sites", y="Log10[Ratio]", title="Event: CYC/ACE")+
  coord_cartesian(xlim = c(1:38),ylim = (-2:3))

[annotate("text", x=c("BY_4*", "NW_1*", "RP_8*","SH_2*", "ST_3*", "SN_6*"),y=-2, label="*", colour="red", size=5)]

#CAF/CMZP
ggplot(rataggEds,aes(x= reorder (siteID, LogCAF_CMZP), y=LogCAF_CMZP, fill=LogCAF_CMZP))+ geom_bar(stat = "identity")+ theme_classic()+ labs(x="Sites", y="Log10[Ratio]", title="Event: CAF/CMZP")+ theme(axis.text.x = element_text(family= "Times", color="black", size=10, face="plain",angle=90), axis.title.x = element_text(family="Times", color = "black", size = 14, face = "plain"), axis.text.y = element_text(family= "Times", color="black", size=10, face="plain",angle=90), axis.title.y = element_text(family="Times", color = "black", size = 14, face = "plain"), plot.title = element_text(hjust=0.5, family="Times", color="black", size=14, face="bold"), legend.position = "none")+ coord_cartesian(xlim = c(1:38), ylim = (-2:4))
```

Appendix 2. R-Script 11(14)

```
#SAC/SUC
ggplot(rataggEds, aes(x= reorder (siteID, LogSAC_SUC), y=LogSAC_SUC, fill=LogSAC_SUC))+ geom_bar(stat = "identity")+ theme_classic()+
 labs(x="Sites", y="Log10[Ratio]", title="Event: SAC/SUC")+ theme(axis.text.x = element_text(family= "Times", color="black", size=10, face="plain",angle=90), axis.title.x = element_text(family="Times", color = "black", size = 14, face = "plain"),
      axis.text.y = element_text(family= "Times", color="black", size=10, face="plain",angle=90), axis.title.y = element_text(family="Times", color = "black", size = 14, face = "plain"), plot.title = element_text(hjust=0.5, family="Times", color="black", size=14, face="bold"), legend.position = "none")+ coord_cartesian(xlim = c(1:38), ylim = (-2:4))

#SAC/ACE
ggplot(rataggEds, aes(x= reorder (siteID, LogSAC_ACE), y=LogSAC_ACE, fill=LogSAC_ACE))+ geom_bar(stat = "identity")+ theme_classic()+ labs(x="Sites", y="Log10[Ratio]", title="Event: SAC/ACE")+ theme(axis.text.x = element_text(family= "Times", color="black", size=10, face="plain",angle=90), axis.title.x = element_text(family="Times", color = "black", size = 14, face = "plain"), axis.text.y = element_text(family= "Times", color="black", size=10, face="plain",angle=90), axis.title.y = element_text(family="Times", color = "black", size = 14, face = "plain"), plot.title = element_text(hjust=0.5, family="Times", color="black", size=14, face="bold"), legend.position = "none")+coord_cartesian(xlim = c(1:38), ylim = (-3:4))

#CF/ACE
ggplot(rataggEds, aes(x= reorder (siteID, LogCAF_ACE), y=LogCAF_ACE, fill=LogCAF_ACE))+geom_bar(stat = "identity")+ theme_classic()+labs(x="Sites", y="Log10[Ratio]", title="Event: CF/ACE")+theme(axis.text.x = element_text(family= "Times", color="black", size=10, face="plain",angle=90), axis.title.x = element_text(family="Times", color = "black", size = 12, face = "bold"), axis.text.y = element_text(family= "Times", color="black", size=10, face="plain",angle=90), axis.title.y = element_text(family="Times", color = "black", size = 12, face = "bold"), plot.title = element_text(hjust=0.5, family="Times", color="black", size=14, face="bold"), legend.position = "none")+ coord_cartesian(xlim = c(1:38), ylim = (-3:3))
```

Appendix 2. R-Script 12(14)

```
#CYC/SUC
ggplot(rataggEds, aes(x= reorder (siteID, LogCYC_SUC), y=LogCYC_SUC, fill=LogCYC_SUC))+ geom_bar(stat = "identity")+ theme_classic()+ labs(x="Sites", y="Log10[Ratio]", title="Event: CYC/SUC")+ theme(axis.text.x = element_text(family= "Times", color="black", size=10, face="plain",angle=90), axis.title.x = element_text(family="Times", color = "black", size = 14, face = "plain"), axis.text.y = element_text(family= "Times", color="black", size=10, face="plain",angle=90), axis.title.y = element_text(family="Times", color = "black", size = 14, face = "plain"), plot.title = element_text(hjust=0.5, family="Times", color="black", size=14, face="bold"), legend.position = "none")+ coord_cartesian(xlim = c(1:38), ylim = (-3:4))

#Grab samples using log10
#CYC/ACE
ggplot(rataggGrab, aes(x= reorder (siteID, LogCYC_ACE), y=LogCYC_ACE, fill=LogCYC_ACE))+ geom_bar(stat = "identity")+ theme_classic()+ labs(x="Sites", y="Log10[Ratio]", title="Grab sample: CYC/ACE")+ theme(axis.text.x = element_text(family= "Times", color="black", size=10, face="plain",angle=90), axis.title.x = element_text(family="Times", color = "black", size = 12, face = "bold"), axis.text.y = element_text(family= "Times", color="black", size=10, face="plain",angle=90), axis.title.y = element_text(family="Times", color = "black", size = 12, face = "bold"), plot.title = element_text(hjust=0.5, family="Times", color="black", size=14, face="bold"), legend.position = "none")+ coord_cartesian(xlim = c(1:47), ylim = (-2:3))

#CAF/CMZP
ggplot(rataggGrab, aes(x= reorder (siteID, LogCAF_CMZP), y=LogCAF_CMZP, fill=LogCAF_CMZP))+ geom_bar(stat = "identity")+ theme_classic()+ labs(x="Sites", y="Log10[Ratio]", title="Grab sample: CAF/CMPZ")+ theme(axis.text.x = element_text(family= "Times", color="black", size=10, face="plain",angle=90), axis.title.x = element_text(family="Times", color = "black", size = 14, face = "plain"), axis.text.y = element_text(family= "Times", color="black", size=10, face="plain",angle=90), axis.title.y = element_text(family="Times", color = "black", size = 14, face = "plain"), plot.title = element_text(hjust=0.5, family="Times", color="black", size=14, face="bold"), legend.position = "none")+ coord_cartesian(xlim = c(1:47), ylim = (-1:3))
```

Appendix 2. R-Script 13(14)

```
#SAC/SUC
ggplot(rataggGrab, aes(x= reorder (siteID, LogSAC_SUC), y=LogSAC_SUC, fill=LogSAC_SUC))+ geom_bar(stat = "identity")+ theme_classic()+ labs(x="Sites", y="Log10[Ratio]", title="Grab sample: SAC/SUC")+ theme(axis.text.x = element_text(family= "Times", color="black", size=10, face="plain",angle=90), axis.title.x = element_text(family="Times", color = "black", size = 14, face = "plain"), axis.text.y = element_text(family= "Times", color="black", size=10, face="plain",angle=90), axis.title.y = element_text(family="Times", color = "black", size = 14, face = "plain"), plot.title = element_text(hjust=0.5, family="Times", color="black", size=14, face="bold"), legend.position = "none")+ coord_cartesian(xlim = c(1:47), ylim = (-2:3))

#SAC/ACE :-)
ggplot(rataggGrab, aes(x= reorder (siteID, LogSAC_ACE), y=LogSAC_ACE, fill=LogSAC_ACE))+ geom_bar(stat = "identity")+ theme_classic()+ labs(x="Sites", y="Log10[Ratio]", title="Grab sample: SAC/ACE")+ theme(axis.text.x = element_text(family= "Times", color="black", size=10, face="plain",angle=90), axis.title.x = element_text(family="Times", color = "black", size = 12, face = "bold"), axis.text.y = element_text(family= "Times", color="black", size=10, face="plain",angle=90), axis.title.y = element_text(family="Times", color = "black", size = 12, face = "bold"). plot.title = element_text(hjust=0.5, family="Times", color="black", size=14, face="bold"), legend.position = "none")+ coord_cartesian(xlim = c(1:47), ylim = (-3:3))

#CAF/ACE
ggplot(rataggGrab, aes(x= reorder (siteID, LogCAF_ACE), y=LogCAF_ACE, fill=LogCAF_ACE))+ geom_bar(stat = "identity")+ theme_classic()+ labs(x="Sites", y="Log10[Ratio]", title="Grab sample: CF/ACE")+ theme(axis.text.x = element_text(family= "Times", color="black", size=10, face="plain",angle=90), axis.title.x = element_text(family="Times", color = "black", size = 12, face = "bold"), axis.text.y = element_text(family= "Times", color="black", size=10, face="plain",angle=90), axis.title.y = element_text(family="Times", color = "black", size = 12, face = "bold"), plot.title = element_text(hjust=0.5, family="Times", color="black", size=14, face="bold"), legend.position = "none")+coord_cartesian(xlim = c(1:47), ylim = (-2:3))
```

Appendix 2. R-Script 14(14)

```
#CYC/SUC
ggplot(rataggGrab, aes(x= reorder (siteID, LogCYC_SUC), y=LogCYC_SUC, fill=LogCYC_SUC))+ geom_bar(stat = "identity")+ theme_classic()+ labs(x="Sites", y="Log10[Ratio]", title="Grab sample: CYC/SUC")+ theme(axis.text.x = element_text(family= "Times", color="black", size=10, face="plain",angle=90), axis.title.x = element_text(family="Times", color = "black", size = 14, face = "plain"), axis.text.y = element_text(family= "Times", color="black", size=10, face="plain",angle=90), axis.title.y = element_text(family="Times", color = "black", size = 14, face = "plain"), plot.title = element_text(hjust=0.5, family="Times", color="black", size=14, face="bold"), legend.position = "none")+ coord_cartesian(xlim = c(1:47), ylim = (-3:3))

#Test with combined ratios for persistence and degradable
rcomb <- ratio %>% group_by(siteID, subKgM, method) %>% dplyr::summarise(., mean = mean(concentration)) %>% spread(key = subKgM, value = mean)
ratio1 <- ratio %>% dplyr::count(siteID, subKgM) %>% filter(n>1); sum(ratio$n)
ratio1plot<- paste(ratio1$siteID, ratio1$subKgM)
ratio1plot <- merge(rcomb, ratio1, by=c("siteID", "method")); head(barplotdata)
ratio1plot <- ratio1plot %>% group_by(siteID,method) %>% dplyr::mutate(., sumpers = sum(Acesulfame, Carbamazepine, Sucralose, na.rm = T)) %>% dplyr::mutate(., sumdeg = sum(Cyclamate, Caffeine, Saccharin, na.rm = T)) %>% mutate(., ratio1plot = sumdeg - sumpers)

ggplot(ratio1plot, aes(x=reorder(siteID, ratio1plot), y=ratio1plot, fill=method))+ geom_bar(stat = "identity", position = "dodge")+ theme_classic()+ scale_fill_discrete(name = "Methods", labels = c("Eds", "Grab"))+ labs(x="Sites", y="Difference [ng/l]", title="Ratio of summed degradable/persistent indicators")+ theme(axis.text.x = element_text(family= "Times", color="black", size=10, face="plain",angle=90), axis.title.x = element_text(family="Times", color = "black", size = 12, face = "bold"), axis.text.y = element_text(family= "Times", color="black", size=10, face="plain",angle=90), axis.title.y = element_text(family="Times", color = "black", size = 12, face = "bold"), plot.title = element_text(hjust=0.5, family="Times", color="black", size=14, face="bold")
```