Fate of organic micropollutants in a karst aquifer system – Implications for sustainable raw water management

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Abstract

The fundamental understanding of karst aquifers is vital for the sustainable management of raw water quality and eventually the access to clean drinking water for up to one quarter of the world's population. In order to improve this understanding the storage and attenuation potential of a karst aquifer was investigated in the presented work, employing organic micropollutants as indicators for transport paths, attenuation and attenuation processes.

As a prerequisite for reliable data acquisition, suitable stabilisation and storage strategies for organic micropollutants in water samples have been evaluated: addition of the biocides (i) copper sulphate and (ii) sodium azide to water samples directly after sampling with subsequent sample storage as liquid phase and (iii) direct solid phase extraction (SPE), stabilising the samples by reducing the water content. River water and treated effluent were chosen as commonly investigated matrices with a high potential of biodegradation activity. Analyses were carried out for sample storage temperatures of 4 and 28 °C for water samples stored as liquid phase and for sample storage temperatures of 4, 20 and 40 °C for SPE cartridges. Cooling of water samples alone was not sufficient for longer storage times (> 24 h). While copper sulphate caused detrimental interferences with azole- and imidazole-like compounds, sodium azide proved to be a suitable stabilising agent. The best results could be obtained for SPE cartridges stored coolly. Recommendations for sample preservation are provided.

In the following chapter the long-term storage potential of a karst aquifer was investigated. To achieve a sustainable raw water quality for drinking water production, the understanding of this potential is highly essential. The transport dynamics of the two herbicides metazachlor and atrazine as well as a degradation product of the latter (desethylatrazine) were investigated at a karst spring over one year. Even 20 years after its ban in Germany, atrazine and its degradation product were almost always detectable in the spring water in the low ng L^{-1}. Metazachlor could only be detected after precipitation events and the observed concentrations were significantly higher than atrazine or desethylatrazine. Comparing the dynamics of the herbicides with the inorganic ions Ca^{2+}, Mg^{2+} and the electrical conductivity, a positive correlation of atrazine with these parameters could be observed. From this observation, atrazine is concluded to be located within the aquifer matrix, deteriorating the raw water quality for decades.

In order to identify the attenuation potential within the conduits of karst aquifers *in-situ* and to estimate the risk posed by micropollutants, a dualtracer experiment was conducted to investigate differential transport in the subsurface: the reactive compound caffeine was used as a tracer to indicate the attenuation potential within the aquifer *in-situ*. Due to the low limit of quantification, only small amounts of caffeine needed to be injected. To calibrate a model and to visualise the attenuation of caffeine a conservative reference tracer (uranine) was injected simultaneously. The methodology was tested in a well characterised

karst system in southwest Germany. The results indicate a significantly higher attenuation rate than was expected for karst aquifers. The attenuation was described as a first-order process. The corresponding half-life was 104 h. This low half-life suggests that a generally assumed low natural attenuation potential of karst aquifers is unjustified. The observed mass loss of caffeine illustrates the potential of caffeine to be used as reactive tracer for indicating *in-situ* attenuation potential within hydraulically highly conductive systems, such as karst aquifers. Due to the high attenuation rate of caffeine it does not pose a threat as a long-time contaminant. In combination with a conservative reference tracer an economical and environmentally benign method is presented in this chapter for the *in-situ* determination of the attenuation potential of highly conductive aquifer systems.

Based on the results of the dualtracer experiment, a multitracer experiment was performed for verifying the results, examining the transferability of the attenuation potential of caffeine to other substances and to specify the attenuation processes responsible for the observed mass loss. Uranine, acesulfame and carbamazepine were injected into a sinkhole as reference tracers together with the reactive compounds atenolol, caffeine, cyclamate, ibuprofen and paracetamol. The breakthrough curves of the reactive compounds were interpreted relative to the reference substances. No significant retardation was observed for any of the investigated micropollutants. The determined half-lives of the reactive compounds range from 38 to 1400 h (i. e. persistent within the investigation period) in the following order (from high to no observed attenuation): paracetamol > atenolol ≈ ibuprofen > caffeine >> cyclamate. The attenuation rates are generally in agreement with studies from other environmental compartments and with the results from the dualtracer experiment. The occurrence of the biotransformation product atenolol acid served as evidence for the occurrence of *in-situ* biodegradation within the aquifer system.

Zusammenfassung

Das grundsätzliche Verständnis von Karstgrundwasserleitern ist essentiell für das nachhaltige Management der Rohwasserqualität und letztendlich für sauberes Trinkwasser für bis zu 25 Prozent der Weltbevölkerung. Um dieses Verständnis zu verbessern, wird in der vorliegenden Arbeit das Speicher- und Attenuationspotential eines Karstgrundwasserleiters untersucht. Hierbei werden organische Spurenstoffe als Indikatoren für Transportpfade, Attenuation und Attenuationsprozessen eingesetzt.

Als Voraussetzung für die Erfassung belastbarer Daten, wurden geeignete Stabilisierungsstrategien für organische Spurenstoffe in Wasserproben bewertet: Zugabe der Biozide (i) Kupfersulphat und (ii) Natriumazid zu Wasserproben nach der Probenahme und anschließende Lagerung der Proben in flüssiger Form sowie (iii) sofortige Festphasenextraktion (SPE), was zu einer Stabilisierung der Proben durch eine Reduktion des Wassergehaltes führt. Es wurden Fluss- und behandeltes Abwasser untersucht. Diese zeichnen sich üblicherweise durch ein hohes Potential für biologische Aktivität und demnach hohe Biotransformationsraten aus. Analysiert wurde der Einfluss der Lagerungstemperatur von 4 und 28° C für die Proben, die in flüssiger Form gelagert wurden und von 4, 20 und 40° C für die Lagerung der SPE-Kartuschen. Kühlen der Wasserproben allein reichte nicht aus, um die Proben für längere Zeit (> 24 h) zu stabilisieren. Die Zugabe von Kupfersulphat führte zu Problemen mit Azol- und Imidazol-ähnlichen Verbindungen. Natriumazid erwies sich als geeigneter Stabilisierungszusatz. Die besten Ergebnisse konnten für kühl gelagerte SPE-Kartuschen beobachtet werden.

Im darauffolgenden Kapitel wird das Langzeitspeicherpotential von Karstgrundwasserleitern untersucht. Um eine nachhaltige Rohwasserqualität zu gewährleisten ist das Verständnis dieses Potentials essentiell. Die Transportdynamik der zwei Herbizide Metazachlor und Atrazin sowie dessen Abbauprodukt (Desethylatrazin) wurde an einer Karstquelle untersucht. Sogar 20 Jahre nach dessen Anwendungsverbot konnten Atrazin und dessen Abbauprodukt nahezu immer im Quellwasser in geringen Konzentrationen (wenige ng L^{-1}) nachgewiesen werden. Metazachlor dagegen tritt nur nach Niederschlagsereignissen auf und die beobachteten Konzentrationen sind deutlich höher. Ein Vergleich der Dynamik der zwei Herbizide mit der der anorganischen Kationen Ca^{2+}, Mg^{2+} und der elektrischen Leitfähigkeit zeigte, dass Atrazin mit diesen Parametern korreliert. Aus dieser Beobachtung konnte abgeleitet werden, dass Atrazin innerhalb der Gesteinsmatrix vorliegt und die Rohwasserqualität für Jahrzehnte beeinflusst.

Um das *in-situ* Attenuationspotential innerhalb des Röhrensystems eines Karstgrundwasserleiters zu identifizieren und das Risiko, das von organischen Spurenstoffen ausgeht, abzuschätzen, wurde ein Doppeltracer-Experiment durchgeführt: Der reaktive Stoff Coffein wurde als Markierungsstoff genutzt um das *in-situ* Attenuationspotential des untersuchten Grundwasserleiters zu bewerten. Aufgrund der

niedrigen Bestimmungsgrenze konnten sehr geringe Mengen eingesetzt werden. Um ein Modell zu kalibrieren und die Attenuation des Coffeins zu visualisieren wurde der konservative Markierungsstoff Uranin simultan eingegeben. Diese Methodik wurde in einem gut charakterisierten Karstgrundwasserleiter in Baden-Württemberg getestet. Die Ergebnisse zeigten eine deutlich höhere Attenuationsrate als für einen Karstgrundwasserleiter erwartet wurde. Die Attenuation wurde als Prozess erster Ordnung beschrieben; die bestimmte Halbwertszeit betrug 104 h. Diese geringe Halbwertszeit deutet darauf hin, dass das generell angenommene geringe Attenuationspotential nicht gerechtfertigt ist. Der beobachtete Massenverlust des Coffeins zeigt auf, dass Coffein als reaktiver Markierungsstoff in hydraulisch hochdurchlässigen Systemen, wie Karstgrundwasserleitern, zur Untersuchung des *in-situ* Attenuationspotentials geeignet ist. Aufgrund der hohen Attenuationsrate des Coffeins, ist nicht mit einer Langzeitkontamination zu rechnen. In der Kombination mit einem konservativen Referenzmarkierungsstoff wird in diesem Kapitel eine ökonomische und ökologisch ungefährliche Methode zur Bestimmung des *in-situ* Attenuationspotentials vorgestellt.

Aufgrund der Ergebnisse des Doppeltracer-Experiments wurde ein Multitracer-Experiment durchgeführt um das ermittelte Attenuationspotential zu verifizieren, dessen Übertragbarkeit auf andere Stoffe zu überprüfen und die Attenuationsprozesse zu spezifizieren. Als Referenzsubstanzen wurden Uranin, Acesulfam und Carbamazepin gemeinsam mit den reaktiven Markierungsstoffen Atenolol, Coffein, Cyclamat, Ibuprofen und Paracetamol in eine Doline eingegeben. Die Durchbruchskurven der reaktiven Markierungsstoffe wurden relativ zu den Referenzsubstanzen ausgewertet. Für keinen der Stoffe konnte eine signifikante Retardation beobachtet werden. Die ermittelten Halbwertszeiten betrugen 38 bis 1400 h (d. h. stabil innerhalb des Beobachtungszeitraums) in der folgenden Reihenfolge (von hoher zu keiner Attenuation absteigend sortiert): Paracetamol > Atenolol ≈ Ibuprofen > Coffein >> Cyclamat. Die Attenuationsraten stimmen generell mit denen aus anderen Studien, die andere Umweltkompartimente untersuchten, und den Ergebnissen des Doppeltracer-Experiments überein. Das Auftreten des Biotransformationsproduktes Atenololsäure diente dem Nachweis von *in-situ* Biotransformation innerhalb des Karstgrundwasserleitersystems.

Danksagung

Den Betreuern meiner Arbeit, Tobias Licha und Martin Sauter, die mir die Möglichkeit gegeben haben dieses interessante Forschungsthema zu bearbeiten, gilt mein Dank in erster Linie. Besondere Erwähnung verdient hierbei Tobias Licha, der bei meiner Betreuung einen sehr angenehmen Mittelweg zwischen richtungsweisenden Ratschlägen, erleuchtenden Diskussionen und dem Einräumen wissenschaftlicher Freiräume fand. Vielen Dank! Ebenso möchte ich mich natürlich auch bei den übrigen Mitgliedern meiner Prüfungskommission Gudrun Massmann, Hans Ruppert, Chicgoua Noubactep und Roland Suchenwirth bedanken.

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Nur indirekt mit der Promotion verbunden aber nicht weniger wichtig, gilt mein Dank all meinen Freunden und meinen jetzigen und zukünftigen Familienmitgliedern von deren Seite ich bewusste und unbewusste Unterstützung und Rückhalt erfahren durfte. Vielen herzlichen Dank dafür!

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Chapter 1

1 Introduction

1.1 Motivation of the work

For mankind the access to clean water in sufficient quantity has been declared a human right, as it *is essential for the full enjoyment of life and all human rights* (UN General Assembly, 2010). Sighting the global reserves, it becomes clear that groundwater, amounting to around 96% of the global liquid fresh water (Hölting and Coldewey, 2005), is likely to play a key role to ensure this access. Currently around 2.5 billion people worldwide rely solely on groundwater for their daily needs (Schneegans, 2013) and 40% of the worlds' food is produced by irrigated agriculture with water largely obtained from aquifers (Morris et al., 2003).

As a consequence of human activities, e. g. accidental or intentional release of contaminants (Bucheli et al., 1998; Moran et al., 2007; Schwarzbauer et al., 2002; Shih et al., 2004), treated or untreated wastewater (Asano and Cotruvo, 2004; Buerge et al., 2006; Drewes et al., 2003; Gasser et al., 2010; Foppen, 2002; Hillebrand et al., 2012; Paul et al., 1997) into natural environments or applying manure, pesticides and fertilisers (Baran et al., 2008; Lapworth and Gooddy, 2006; Masaka et al., 2013; Mullaney et al., 2009) on arable areas, various compounds of anthropogenic origin can be detected in groundwater systems. A common group of compounds, that recently aroused great interest in the scientific community, are the so-called *organic micropollutants*. This term is used referring to numerous trace organic compounds of anthropogenic origin such as human and veterinary pharmaceuticals, herbicides or personal care products and will be used accordingly throughout this work. They have been found ubiquitously in all compartments of the aquatic environment (Hughes et al., 2013; Loos et al., 2009; Loos et al., 2010; Schwarzenbach et al., 2006). While the presence of organic micropollutants in the environment is certainly undesirable, the observed concentrations are typically below the health-oriented guidance value of 0.1 µg L^{-1} (Dieter, 2011). However, they have great potential to be used as indicators, allowing for the identification of processes and the characterisation of eco- and geosystems (Licha, 2013). Especially in karst aquifers, that are difficult to characterise by conventional hydrogeological approaches (Bakalowicz, 2005), the application of organic micropollutants as indicators for e. g. flow components and attenuation processes may contribute significantly to the fundamental understanding of flow

and transport in these highly heterogeneous and vulnerable (Baran et al., 2008; Schwarz et al., 2011; Vesper et al., 2001) systems. This fundamental understanding is vital for sustainable raw water management as stated by the World Health Organisation (WHO, 2010).

1.2 Karst aquifer

Karst systems are common geological systems all over the world (Figure 1.1) and karst aquifers serve largely or entirely as water source for 20–25% of the global population (Ford and Williams, 2007). These systems are set apart from other aquifers by the tendency of the host rock to be dissolved by water. All typical karst features arise from the dissolution of the rock material by flowing water. Karst may evolve in carbonate rocks (e. g. limestone and dolomite) as well as in evaporites, anhydrite, gypsum and partly even in quartzite (Bakalowicz, 2005). In the following only karst in carbonate rocks will be considered.

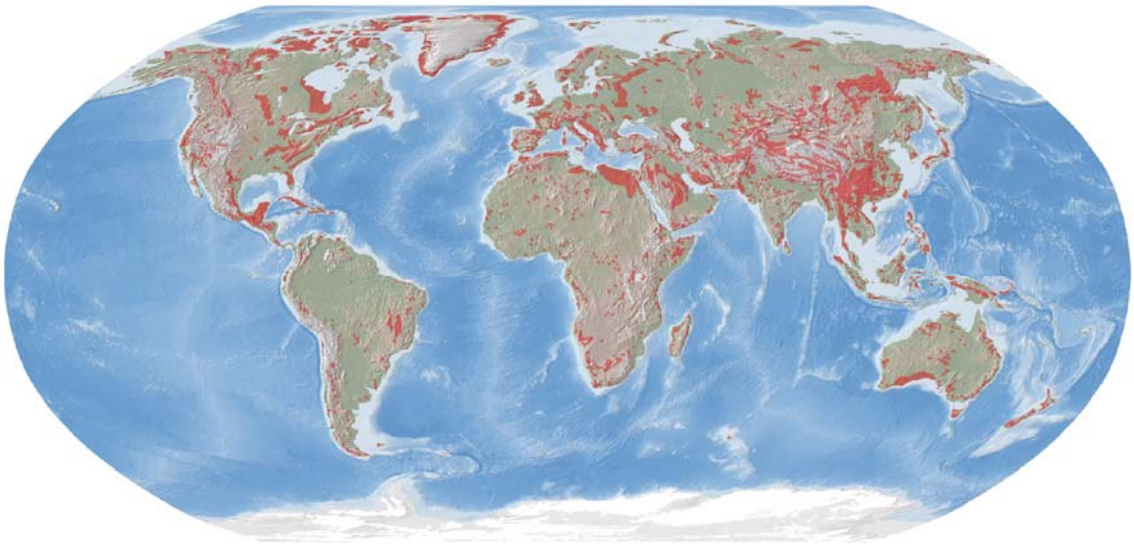

Figure 1.1. Global distribution of carbonate rock outcrops (red areas; Hollingsworth et al., 2008, modified after Ford and Williams, 2007).

1.2.1 Conceptual Model of karst aquifers

The solubility of the host rock leads to a widening of existing fissures or other pathways provided by the geological structure (Ford and Williams, 2007) and consequently to an enhanced hydraulic conductivity. Typical features are karst conduits and dolines (cf. Figure 1.2). Contrary, areas that have not been fissured before do not experience dissolution in the same extend, resulting in a spatial heterogeneity of the hydraulic parameters.

The conceptual structure of a karst aquifer is illustrated in Figure 1.2, picturing its features and general vertical build-up. The top layer is the soil layer, typically thin or missing in karst regions. Consequently contaminants can easily enter the aquifer system. It is underlain by

the epikarst (subcutaneous zone). This zone is characterised by its high secondary permeability (Williams, 1983); rain water percolating through the soil layer is enriched in CO_2, leading to an effective dissolution of the soluble host rock. This dissolution becomes less effective with depth. As a result, perched aquifers may evolve in this zone, allowing for lateral flow towards preferential flow paths (Williams, 1983). From the epikarst water flows through the lower part of the vadose (unsaturated) zone, where water flow is generally oriented vertically (Kaufmann, 2003). As soon as water reaches the phreatic (saturated) zone, the main flow is directed towards the local outlet. As a result of the high hydraulic conductivity of karst conduits, water flow is directed towards them in their vicinity.

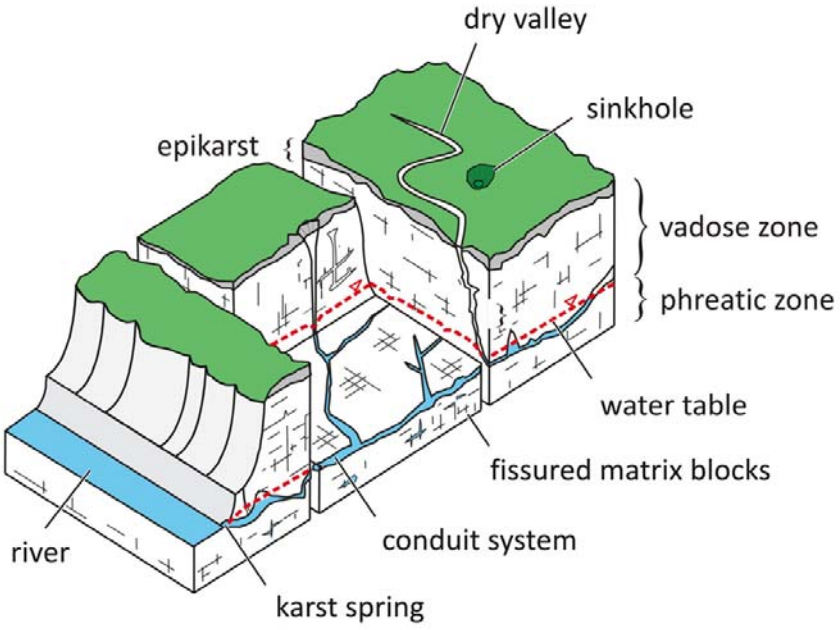

Figure 1.2. Conceptual structure of a karst aquifer, illustrating typical components and features (modified after Geyer, 2008).

As a consequence of their heterogeneous nature, a characterisation of karst systems is difficult. The unknown distribution and structure of the karst conduit system, multiple flow components interacting with each other and the size make it difficult to characterise karst systems by conventional hydrogeological methodologies (Bakalowicz, 2005). An elegant and promising approach to deal with the complexity of karst is the investigation of spring water dynamics (White, 2002), comprising physical and chemical signals to allow for the characterisation of karst systems at the catchment scale (e. g. Geyer, 2008).

1.2.2 Duality of karst aquifers

In the context of karst aquifers the term *duality* is often used, referring to several peculiarities of and a model concept for these systems. As an approximation, the highly complex karst aquifers may be described by a double permeability approach: a high

permeability mesh immersed in a low permeability fractured limestone volume (Kiraly, 1998). In the following the term duality will be used to refer to this double permeability approach, which can be transferred to the infiltration process and the flow and storage of karst aquifers:

(i) The infiltration of precipitation may either occur diffusely into the fissured matrix blocks or as point recharge into conduits, widened by solution. The epikarst can have a large influence on the infiltration process, allowing for lateral flow towards vertical shafts, joints, dolines or other preferential flow paths (Williams, 1983). If the recharge occurs as point recharge, its effect on the spring discharge can be recognised rapidly. It is therefore termed rapid recharge or concentrated recharge.

(ii) The fissured matrix accounts for more than 90% of the aquifer storage, but less than 10% of the flow (derived from the hydraulic conductivities of the conduits and the fissured matrix). The opposite applies to the conduit system: flow mainly occurs in the highly conductive conduits, while the proportion of storage is less than 5% (Worthington et al., 2000). As a consequence of this duality the residence time for the two flow components varies drastically. For karst conduits flow velocities of several km d^{-1} have been observed (Seiler et al., 1989), resulting in residence times of a few days (Pronk et al., 2009), while the residence time in the fissured karst matrix is typically in the order of years (Einsiedl, 2005).

It becomes evident that both components of this conceptual model need consideration for the fundamental understanding of karst aquifers.

1.3 Attenuation of organic micropollutants in karst aquifers

The term attenuation comprises all processes lowering the concentration of a compound in groundwater. Some of these processes affect all compounds, including e. g. dilution, dispersion and dual porosity effects (COST Action 620, 2004) and are sufficient to describe the fate of a hypothetical conservative contaminant.

All non-conservative compounds may undergo additional attenuation processes, such as sorption, degradation, precipitation or volatilisation. Apart from the properties of the compounds, the properties of the respective system or layer may have a great impact and need to be taken into account. In the following the term attenuation will refer only to this latter class of attenuation processes. For organic contaminants, three processes are stated to be key processes in karst aquifers (COST Action 620, 2004), which will be elucidated in the following:

(1) sorption

(2) degradation

(3) volatilisation

Sorption describes the tendency of a compound, to interact with the aquifer material or solids in general leading to a net enrichment of the compound on the surface of the solid phase (Schaffer, 2013) and retardation of the sorbed compounds. Depending on the compound's and solid's surface charge in groundwater, the process is mainly related to non-polar interaction of compounds with mineral surfaces and organic carbon in particular, cation exchange or anion exchange. For cationic and anionic compounds, the cation exchange capacity (CEC) and anion exchange capacity (AEC) of the subsurface system need to be considered respectively (e. g. Schaffer et al., 2012a; Tülp et al., 2009). Since, the AEC is typically reversely correlated with the ambient pH (Pansu and Gautheyrou, 2006) insignificant anion sorption is expected in carbonate buffered systems, such as karst aquifers.

The term degradation comprises chemically or biologically induced transformation processes, such as oxidation/reduction and hydrolysis. Although knowledge of all sub-processes may be of interest, a breakdown of the degradation process is difficult, since many transformation reactions are mediated by microorganisms (e. g. Radjenovic et al., 2008). It may be understood as a cumulative parameter including all named sub-processes.

Volatilisation describes the process of evaporation of a compound. For various polar and thus highly water-soluble organic compounds, this process is insignificant; Henry's Law constants are often low. For all micropollutants investigated in the course of this work, volatilisation is negligible.

If an unambiguous distinction of the above named attenuation processes is not possible, the term attenuation is used.

For the determination of the tendency of organic compounds to exhibit sorption and to determine degradation rates (often expressed as half-life) for organic compounds various approaches exist. Sorption of non-polar compounds may be estimated from empirical log K_{OW}–log K_{OC} correlations (e. g. Karickhoff, 1981) and the fraction of organic carbon of the investigated system. Similarly, adapted correlations were developed for ionic compounds accounting for their polarity (Franco and Trapp, 2008; Schaffer et al., 2012a). Experimentally, sorption and degradation rates may be determined by laboratory experiments, such as batch (Barbieri et al., 2012; Yamamoto et al, 2009) and column experiments (Schaffer et al., 2012b; Scheytt et al., 2006), evaluation of field data (Panno and Kelly, 2004; Swartz et al., 2006) or from tracer tests (Kunkel and Radke, 2011; Thierrin et al., 1995). The latter represents the most reliable approach for a specific system as attenuation rates (sorption and degradation rates) are determined *in-situ.* However, no such studies have been conducted with organic micropollutants in karst aquifers.

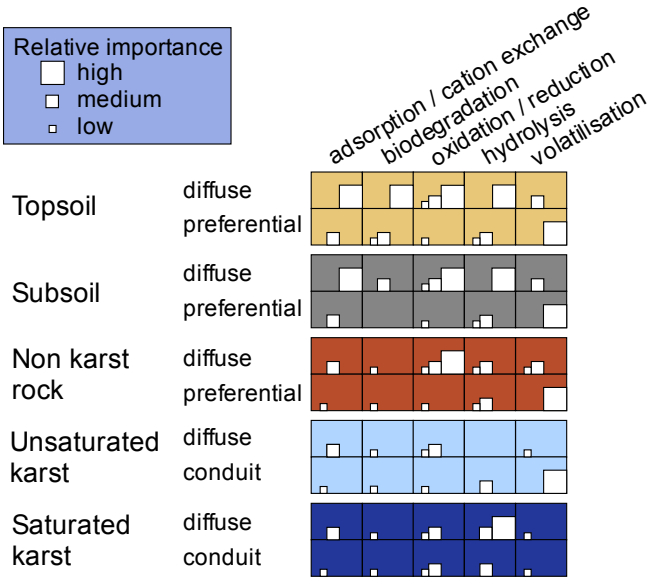

Figure 1.3. Key processes affecting transport of organic contaminants and their estimated relative importance in different layers of karst systems (modified after COST Action 620, 2004). The terms oxidation/reduction and hydrolysis refer to these processes, independent of the microbiological activity in each respective layer.

In Figure 1.3 the estimated relative importance of each process in several layers of karst systems is presented. It becomes obvious, that the largest attenuation is expected in the soil layer, where a high content of organic carbon (e. g. humus) and microorganisms allow for effective sorption of contaminants and their degradation. However, soil layers are typically thin or absent in karst regions and some contaminants may be introduced into a system below the soil zone (e. g. wastewater leakage from sewer system). In the deeper layers sorption and degradation processes become more and more ineffective, especially in the conduit system. Consequently the vulnerability of karst systems is often assessed by residence time distributions (e. g. Einsiedl et al., 2009). A low residence time indicates high vulnerability and vice versa. This approach aims for the determination of the intrinsic vulnerability of karst systems as defined in COST Action 620 (2004), not considering the actual attenuation potential of karst aquifers for individual contaminants or contaminant classes. Few studies exist, investigating attenuation processes, such as sorption or degradation, in karst aquifers.

Panno and Kelly (2004) investigated the mass flux of nitrate and two herbicides out of a catchment and observed significantly lower relative mass fluxes for the two herbicides, than for nitrate. An effective retention of the herbicides was concluded. Taking into account the results from Johnson et al. (1998), who observed only little and varying potential for sorption in the unsaturated and saturated zone, it is likely that the observed retention of the herbicides is related to sorption in the soil layer.

Isotopic data (Einsiedl and Mayer, 2005; Einsiedl et al., 2005; Panno et al., 2001) and the occurrence of the atrazine degradation product desethylatrazine (e. g. Börger and Poll,

1998) suggest that degradation processes are active within karst hydrologic systems. This has been further specified by Byl et al. (2002), who observed biodegradation in batch experiments with raw karst water from contaminated sites and concluded from biological, chemical and hydrological data, that biodegradation processes were active in the investigated karst aquifer. When distinguishing the different layers and their potential for degradation, inconsistent data are reported. In some publications degradation of contaminants is reported only to occur in the soil layer (Johnson et al., 2000), others report that in the unsaturated zone degradation of contaminants may occur as well (Börger and Poll, 1998; Gooddy et al., 2001), but significantly less effective (Chilton et al., 2005; Johnson et al., 1998). For the saturated zone no or ineffective degradation processes were observed (Börger and Poll, 1998; Johnson et al., 1998).

1.4 Scope, objective and further outline of the thesis

For the sustainable raw water management of karst aquifers a fundamental understanding of these systems is vital (WHO, 2011). A holistic understanding can only be achieved, if the duality of karst aquifers is considered. In order to characterise the long-term storage potential for contaminants within the fissured rock matrix of karst aquifers on the one hand and the attenuation potential within the conduit system on the other hand, pesticides, pharmaceuticals and life-style products can be used as indicators or employed as tracers to characterise different hydraulic compartments and attenuation processes. The interpretation of the dynamics of the integrating spring signals allows for the characterisation of the whole catchment or, in case of tracer tests, for a characterisation of the connection between the injection and the sampling location. A more detailed outline of each chapter is provided in the following.

Chapter 2 deals with the stabilisation and storage of micropollutants in water samples. As a prerequisite for the reliable determination of organic micropollutants' concentrations in water samples without the possibility of immediate sample analysis, an effective way for sample stabilisation and storage was determined. Different stabilisation techniques and storage temperatures were investigated and recommendations for sample preservation are provided.

In **Chapter 3** the long-term storage potential of karst aquifers is investigated. On the example of the herbicides metazachlor and atrazine and the atrazine degradation product desethylatrazine the different time-series of recently and formerly applied herbicides are highlighted. This chapter addresses the characterisation of the slow flow component and the respective transport in the matrix component of the aquifer and its long-term effect on spring water quality.

In **Chapter 4** a method for the identification of the attenuation potential within karst conduits and the related rapid flow and transport is presented, employing caffeine as a reactive tracer in the course of a dualtracer experiment.

The insights gained in Chapter 4 are extended in **Chapter 5**. The results of a multitracer experiment are presented and discussed. A total of 8 (reactive and conservative) tracers were injected into a karst aquifer to investigate differentiated transport and attenuation, verify the reproducibility and transferability of the determined attenuation potential of the aquifer system and specify the responsible attenuation processes.

In **Chapter 6** a summarising conclusion of the thesis is provided together with suggestions for further research.

In **Appendix A** additional information regarding this dissertation are provided.

In **Appendix B** a list of journal articles, conference abstracts and miscellaneous publications was compiled authored or co-authored by me and directly related to the presented work.

Please note that, as a result of the cumulative nature of this thesis, references are provided at the end of each chapter.

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Chapter 2

2 The challenge of sample-stabilisation in the era of multi-residue analytical methods: a practical guideline for the stabilisation of 46 organic micropollutants in aqueous samples

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Abstract

Water sample storage and stabilisation may affect data quality, if samples are managed improperly. In this study three stabilising strategies are evaluated for 46 relevant organic micro-pollutants: addition of the biocides (i) copper sulphate and (ii) sodium azide to water samples directly after sampling with subsequent sample storage as liquid phase and (iii) direct solid phase extraction (SPE), stabilising the samples by reducing the activity of water. River water and treated effluent were chosen as commonly investigated matrices with a high potential of biotransformation activity. Analyses were carried out for sample storage temperatures of 4 and 28 °C for water samples stored as liquid phase and for sample storage temperatures of 4, 20 and 40 °C for SPE cartridges. Cooling of water samples alone was not sufficient for longer storage times (>24 h). While copper sulphate caused detrimental interferences with nitrogen containing heterocyclic compounds, sodium azide proved to be a suitable stabilising agent. The best results could be obtained for SPE cartridges stored cool. Recommendations for samples preservation are provided.

2.1 Introduction

Within the last 20 years, researchers increasingly investigated the occurrence and fate of organic compounds in trace concentrations (µg L^{-1} to ng L^{-1}). These so-called micro-contaminants or micro-pollutants, such as pharmaceuticals and personal care products, endocrine disrupting compounds, pesticides and/or industrial chemicals at low concentrations were detected in virtually all parts of the water cycle (Focazio et al., 2008; Heberer, 2002; Schwarzenbach et al., 2006; Ternes, 2007; Weigel et al., 2001). Due to the diversity of these compounds, analytical methods focussing on only one class of compounds do not meet the requirements of current research undertaken in environmental sciences (Estévez et al., 2012; Nödler et al., 2011; Reh et al., 2013). However, thanks to significant progress in the field of analytical science several multi-residue analytical methods were developed (e. g. Huntscha et al., 2012; Nödler et al., 2010; Nurmi and Pellinen, 2011; Wode et al., 2012).

Although the diversity of compounds can nowadays be handled analytically by multi-residue analysis, the wide spectrum of compounds with various stabilities and reactivities (e. g. Nödler et al., 2010; Wode et al., 2012) results in a challenge for sample preservation. In cases when the immediate sample analysis is difficult or impossible (e. g. remote areas) or the sampling is intended to be realised over longer periods (e. g. weekly-integrated sampling; Kylin, 2013), the storage conditions become highly relevant (Barceló and Alpendurada, 1996; U.S. EPA, 2010; Vanderford et al., 2011). Especially for easily degradable compounds, their reliable determination largely depends on proper sample storage conditions. Various processes such as microbial degradation, chemical reactions, volatilisation or adsorption may occur even during relatively short sample storage times resulting in low analyte recoveries. For example, caffeine, ibuprofen and paracetamol (acetaminophen) are commonly investigated micro-contaminants and known to be easily degradable in wastewater treatment plants (WWTPs) and in the environment (e. g. Halling-Sørensen et al., 1998; Joss et al., 2006) while carbamazepine is known to be a very stable compound (Clara et al., 2004; Gasser et al., 2010). Acknowledging the large range of stability encountered for compounds in multi-residue analysis, it is obvious that a proper sample pre-treatment and storage is essential to obtain reliable results. Thus, sample stabilisation methods should be applied to minimise concentration changes between sampling and analysis. These methods are most common in inorganic analysis and include addition of chemicals, cooling, pH-modifications and choice of storage container.

For micro-contaminants the influence of storage temperatures, the material of the storage container and different quenching agents have been investigated for water samples, stored as liquid phase (U.S. EPA, 2010; Vanderford et al., 2011). As stabilising agents sodium azide and sulphuric acid have been tested (Vanderford et al., 2011). However, these recent

investigations focussed on the sample treatment of water samples stored as liquid phase, although the advantages of using SPE-cartridges for sample stabilisation has been recognized several years ago (Barceló and Alpendurada, 1996).

To inhibit biological degradation in water samples, two biocidal additives which can be used are sodium azide and copper sulphate. Sodium azide is frequently used in laboratory studies (e. g. Vanderford and Snyder, 2006), especially to produce abiotic reference samples in degradation experiments (e. g. Margesin et al., 2000; Ying et al., 2008) and has been described by Vanderford et al. (2011) as the most benign of the investigated preservatives for sample stabilisation. Copper sulphate is particularly applied for the stabilisation of phenols and phenolics (DIN 38409-16; Hossain and Salehuddin, 2009). A common non-chemical stabilisation technique is solid phase extraction (SPE). By reducing the water activity, the microbial growth can be controlled (Madigan et al., 2003).

The aim of this study was to evaluate the influence of the water sample matrix, the storage temperature, the addition of two selected chemical preservatives and the direct application of SPE on the recovery of 46 analytes. The here investigated micro-contaminants comprise of a large variety of different compound-classes including readily degradable and highly persistent compounds.

Table 2.1. Investigated analytes and their application/origin.

Application or origin	Compound	Application or origin	Compound
Analgesics / Anti-inflammatories	Diclofenac	Lipid regulators	Bezafibrate
	Ibuprofen		Clofibric acid
	Naproxen		Gemfibrozil
	Paracetamol	Antihistamines	Cetirizine
	Phenazone		Loratadine
Stimulants / Caffeine metabolites	Caffeine	Anticonvulsants / Sedatives	Carbamazepine
	Paraxanthine		Diazepam
	Theobromine		Primidone
	Theophylline		Tetrazepam
	1-Methylxanthine	Selective serotonin reuptake inhibitors	Citalopram
	3-Methylxanthine		Fluoxetine
Antihypertensive agents	Atenolol		Sertraline
	Metoprolol	Herbicides / Herbicide metabolites	Atrazine
	Sotalol		Desethylatrazine
Iodinated contrast media	Iohexol		Desisopropylatrazi
	Iomeprol		Diuron
	Iopamidol		Isoproturon
	Iopromide		Mecoprop
Antibiotics	Clarithromycin		Metazachlor
	Erythromycin	Corrosion inhibitors	1H-benzotriazole
	Roxithromycin		Tolyltriazole
	Sulfamethoxazole	Cocaine metabolite	Benzoylecgonine
	Trimethoprim	Gastric acid regulator	Pantoprazole

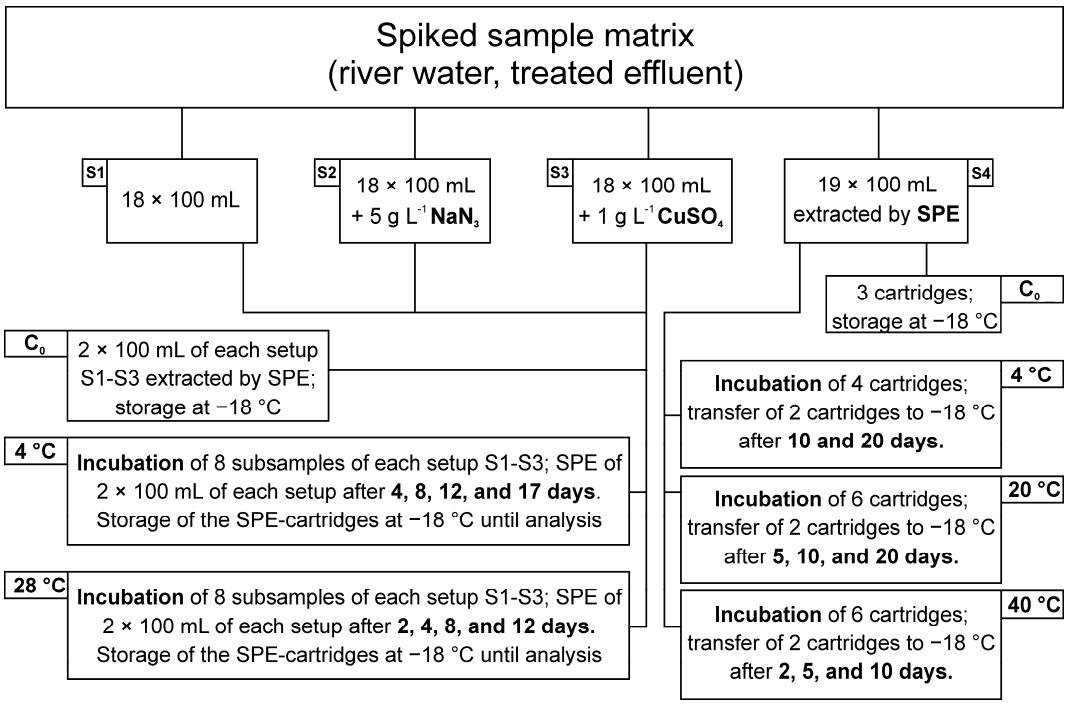

Figure 2.1. Schematic overview of the experiments to investigate the influence of different stabilisation techniques (c_0= initial concentration).

2.2 Methods and materials

2.2.1 Chemicals

Sodium azide (NaN_3) and copper sulphate pentahydrate ($CuSO_4 \cdot 5\ H_2O$) were purchased from Fisher Scientific (Schwerte, Germany). The suppliers of all target analytes, the internal standards (IS), the SPE cartridges, and all other reagents were published previously (Nödler et al., 2010). The investigated trace organic compounds are presented in Table 1.

2.2.2 Sample preparation

A schematic overview of the experiments is presented in Figure 2.1. Water samples were collected by using 1 L and 2 L clear-glass bottles, pre-rinsed with the respective water sample. The samples were taken from the effluent of the wastewater treatment plant (WWTP) Göttingen (Germany, ~120,000 inhabitants) and the Leine River (Göttingen, Germany). Under dry weather discharge conditions, the mean hydraulic residence time within the WWTP was 20–24 h. The treatment processes consisted of a mechanical treatment for the separation of solid material followed by activated sludge treatment, including nitrification and denitrification. Additionally, chemical P-removal was performed. During a previously published study, the treated effluent was analysed on a daily basis for 27 days and easily degradable compounds such as ibuprofen, caffeine and its degradation products were not detected (Nödler et al., 2011). Therefore, the presence of highly adapted

micro-organisms can be assumed, which underlines the big challenge of stabilising these compounds in this sample matrix. The presence of anthropogenic micro-pollutants in the Leine River was also demonstrated in previous studies (Nödler et al., 2010; 2011). Therefore, adapted micro-organisms were expected in both matrices.

Sample subsets S1–S3: Composite samples of 6.5 L river water and treated effluent, respectively, were prepared and spiked with 650 µL stock solution containing all analytes. The stock solution was prepared in 50/50 water/methanol (v/v); the final methanol concentration in the water samples was therefore 0.005% (v/v). Spike levels of 2 µg L^{-1} of each individual iodinated contrast media and the individual concentration of 1 µg L^{-1} of all other compounds were applied. The spiked composite sample was stirred for 30 min by a magnetic stirrer. Aliquots of 100 mL sample were taken by a 100 mL glass pipette and transferred into 100 mL clear-glass and screw cap bottles. As the samples were not filtered, stirring was applied to enable the transfer of representative aliquots including dispersed particles. 54 100 mL sub-samples were prepared. Of each sample matrix 18 sub-samples were spiked with 1 mL of an aqueous NaN_3 stock solution resulting in a final concentration of 5 g L^{-1} NaN_3 (Wender et al., 2000; Ying et al., 2008). Another 18 aliquots were spiked with 1 mL of an aqueous $CuSO_4 \cdot 5\ H_2O$ solution resulting in a concentration of 1 g L^{-1} $CuSO_4$ (DIN 38409-16). However, acidification of the Cu-stabilised samples as recommended by the DIN standard (DIN 38409-16) was not applied, as some of the analytes are sensitive to low pH-values. To the remaining 18 aliquots 1 mL ultrapure water was added to keep the sample volumes comparable to the stabilised samples. Because some of the analytes were already present in the native samples (Nödler et al., 2010; 2011), duplicates of each spiked sample matrix were immediately extracted by SPE to determine the here applied initial concentration of the analytes (c_0).

Atenolol acid was identified by Radjenovic et al. (2008) and Barbieri et al. (2012) as a microbial transformation product (TP) of atenolol, generated by hydrolysis of its amide bond. Therefore, the compound was monitored to evaluate the fate of atenolol in the prepared subsets. The analysis was performed according to Reh et al. (2013).

To simulate the impact of the preservatives depending on the storage temperature, samples were stored in a refrigerator (4 °C) and in an incubator (28 °C), respectively. All samples were covered to prevent photodegradation. The incubation of the samples was terminated according to the schedule presented in Figure 2.1 and samples were immediately extracted. For the extraction, the sample (100 mL) was spiked with 10 µL of an IS-mix (for details on the used internal standards please refer to Nödler et al., 2010; Reh et al., 2013 or Table A.1) and 1 mL of a phosphate buffer concentrate (pH 7) and extracted by SPE (OASIS® HLB) according to Nödler et al. (2010). After extraction the cartridges were sealed with parafilm, covered in alumina foil, and stored in a freezer at −18 °C until elution and analysis. It is assumed that storing the SPE cartridges at −18 °C stabilises all analytes. Alterations of the samples during this storage phase are not part of this manuscript.

Sample subset S4: River and treated effluent matrix were spiked and 19 100 mL subsamples of each matrix were extracted by SPE similar to the other subsets. However, the samples were not spiked with the above mentioned IS-mix prior to the SPE. The loaded cartridges were incubated according to Figure 2.1 in a GC-oven (40 °C; Chrompack CP 9001), in a temperature-controlled laboratory (20 °C; protected from light) and in a refrigerator (4 °C). The minimum and maximum temperatures were monitored and the deviation did not exceed 1 °C. In comparison to the native water sample the SPE process reduces the water activity. As this is a well-known strategy in microbial growth control (Madigan et al., 2003), the effect of biotransformation on the analytes was suspected to be significantly lower than in the subsets S1–S3. Therefore, in comparison with S1–S3 a higher maximum incubation temperature (40 °C) was chosen.

2.2.3 Elution from the SPE-cartridge and analysis of the analytes

The analytes (subsets S1–S3) were eluted with methanol and ethyl acetate under vacuum (flow rate ~1 mL min^{-1}). The solvents were evaporated to dryness at 40 °C with a gentle stream of nitrogen and re-dissolved in 1 mL of aqueous 5 mM ammonium acetate solution, containing 4% methanol. The extract was transferred into an autosampler vial and centrifuged for 10 min (4000 rpm). The compounds were analysed with a multi-residue analytical method based on high performance liquid chromatographic separation coupled to an electrospray ionisation with tandem mass spectrometric detection (HPLC/MS–MS; Nödler et al., 2010). The extracts of subset 4 were spiked with 10 µL of the above mentioned IS-mix before the evaporation step of the solvents. The further procedure and analysis was according to the subsets S1–S3.

2.3 Results and discussion

A significance level of 80% is assumed in all experiments i. e. if the recovery of the analyte is reduced by less than 20% over the period of observation, it is declared to be insignificant and acceptable.

2.3.1 Water samples stored as liquid phase

2.3.1.1 Stability of compounds in non-stabilised water samples (subset 1)

Pantoprazole exhibited incomparable duplicates for river water (RW) and was therefore discarded from further analysis. For the treated effluent matrix (WW) a significantly low recovery could be observed at 28 °C for the stabilised as well as the non-stabilised samples. Out of the remaining 45 micro-pollutants, 18 proved to be stable (recovery ≥ 80%) in both water matrices (river and WWTP effluent) at 4 and 28 °C although non-stabilised.

The stable substances are, among others, all but one investigated contrast media, both antihistamines and all anticonvulsants and sedatives (cf.

Table 2.1). This was expected since their stability is well known. The persistence of carbamazepine, for example, was demonstrated in previous studies (Castiglioni et al., 2006; Clara et al., 2004). For tables with all spiked compounds and their respective recoveries, see Table A.2 to Table A.5.

The analytes, for which unacceptable recoveries have been observed at the end of the investigation period, were generally the same for both water matrices.

It can be assumed that, in sewage, more micro-organisms are present and they readily cause a more efficient transformation, whereas in natural water (e. g. river water) the bacteria require a longer lag phase to adapt to changed conditions (Madigan et al., 2003). Thus, it can be expected that recoveries from WW are generally lower than from RW. This is partially confirmed by the presented study. However, for the compounds atenolol, metoprolol, iomeprol, sulfamethoxazole, bezafibrate, fluoxetine, sertraline, desisopropylatrazine, 1H-benzotriazole and benzoylecgonine a lower recovery could be observed in the RW samples.

The concentration of atenolol acid in the RW stored at 28 °C increased from 25 ng L^{-1} (present in the native sample) to 250 ng L^{-1} at the end of the incubation period. Assuming the TP being stable within the investigated period, ~30% of the atenolol loss can be attributed to the formation of atenolol acid.

Typically, higher temperatures (within the physical range of micro-organisms) promote the microbial growth and activity, whereas lower temperatures are inhibitory (Castiglioni et al., 2006; Kang and Kondo, 2002; Vieno et al., 2005). Accordingly, except for clofibric acid, sertraline, diuron and isoproturon in WW as well as tolyltriazole in RW, all compounds demonstrated higher recoveries in the cooled samples. The substances with the lowest recovery were methylxanthines (caffeine, paraxanthine, theobromine, theophylline, 1-methylxanthine, 3-methylxanthine), ibuprofen and paracetamol; rapidly decreasing recoveries were observed in the WW samples for both temperatures (Figure 2.2). In the RW samples ibuprofen and paracetamol exhibited unacceptably low recoveries at both temperature levels at the end of the observation period. For the methylxanthines, this holds only true for the higher temperature level of 28 °C (Figure 2.3).

2.3.1.2 Stabilisation with sodium azide (subset 2)

Sodium azide inhibits microbial activity and growth (e. g. Margesin et al., 2000; Ying et al., 2008). After two days of incubation at 28 °C, the non-stabilised samples exhibited a clearly visible turbidity. In contrast, the stabilised samples hardly manifested any turbidity. This may be interpreted as an indication for the reduced microbial activity of the stabilised samples.

The addition of sodium azide generally led to higher recoveries of the analytes in the samples, relative to non-stabilised samples. This was observed for all analytes in the WW samples. However, anomalies were observed in the RW samples for naproxen, iomeprol, iopamidol, bezafibrate, clofibric acid, gemfibrozil, sertraline and diuron at 4 °C and for tolyltriazole at 28 °C. Several authors describe interferences of sodium azide with some analytes resulting in a transformation (Chefetz et al., 2006; Lichtenstein et al., 1968; Sharom et al., 1980), which may explain the observations. Chefetz et al. (2006) observed a nucleophilic aromatic substitution reaction: the chlorine atom of the atrazine was replaced by the azide group. This may explain the low recoveries of bezafibrate, clofibric acid, sertraline and diuron in the stabilised samples. However, it does not explain the low recoveries of the other analytes and why it was observed for 4 °C but not for 28 °C. Grenni et al. (2013) found gemfibrozil and naproxen to be biodegradable in river water, while these compounds were observed to be stable in sterilized river water samples. One may read this as an indication that micro-organisms, responsible for the degradation of gemfibrozil and naproxen, are either affected to a lower extend or not affected at all by sodium azide.

It is noteworthy, that Vanderford et al. (2011) found recoveries to be unacceptable for atenolol and fluoxetine in water samples, stabilised with sodium azide and stored at 4 °C. This cannot be confirmed from the observations of this study. However Vanderford et al. (2011) used higher storage times and a slightly more strict level of significance.

While the easily degradable compounds from the methylxanthines group, ibuprofen and paracetamol demonstrated rapidly decreasing recoveries in the non-stabilised samples, they could be successfully stabilised with sodium azide in both water matrices between 2 and 17 days, depending on the analyte (Figure 2.2 and Figure 2.3). In the stabilised and cooled samples all these compounds exhibit acceptably high recoveries over the entire investigated period. For paracetamol and 1-methylxanthine a higher, but still unacceptably low recovery, was observed over the observation period in the WW samples at 28 °C. Thus, it can be assumed that biodegradation is not the only effect, which influences the stability of these compounds in the aqueous phase.

For tables with all spiked compounds and their respective recoveries, see Table A.6 to Table A.9.

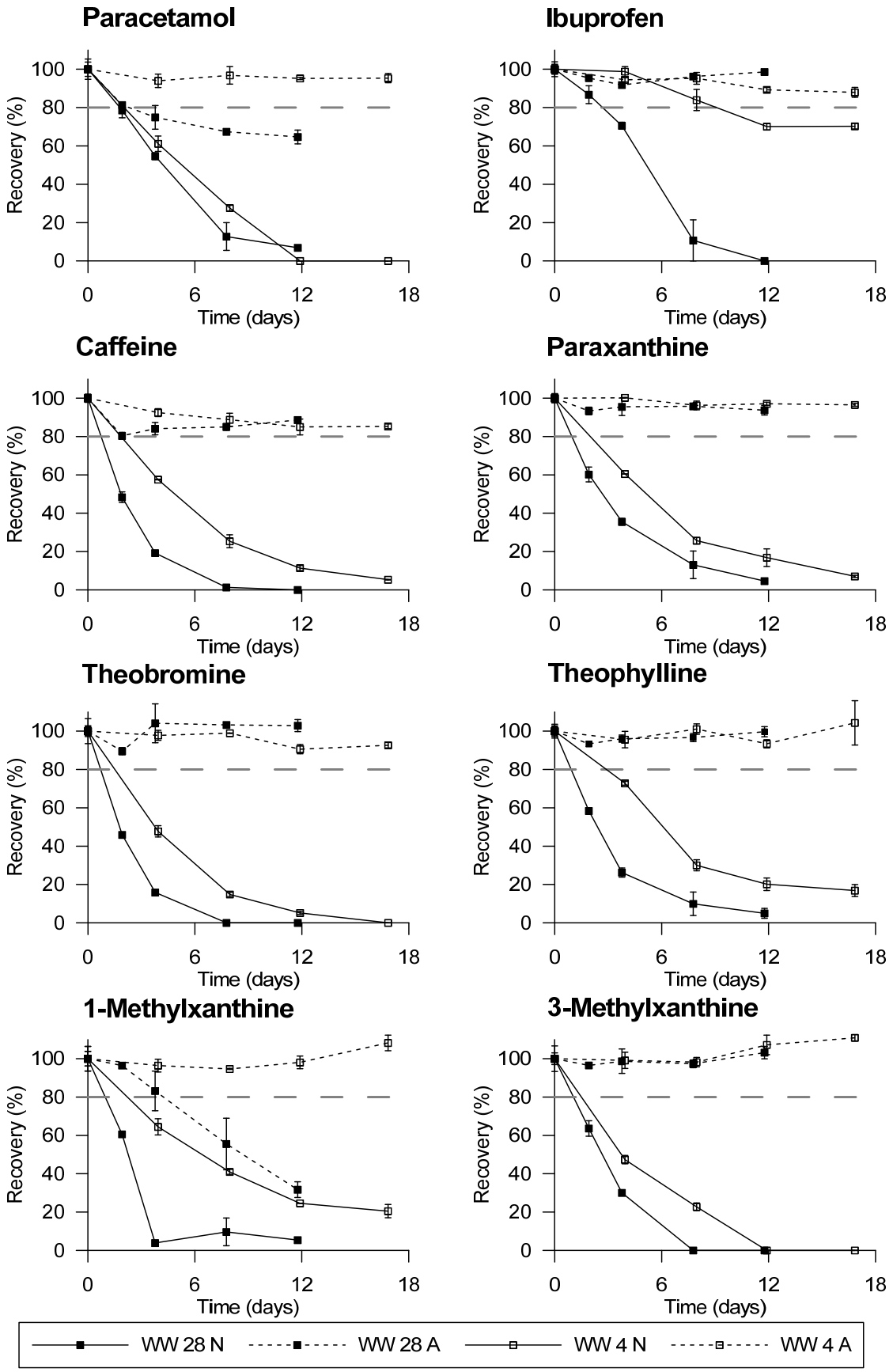

Figure 2.2. Recoveries of selected analytes in WWTP treated effluent with respect to storage time; stored as liquid (WW 28 N= non-stabilised wastewater sample stored at 28 °C; WW 28 A= wastewater sample stored at 28 °C, stabilised with NaN_3; WW 4 N= non-stabilised wastewater sample stored at 4 °C; WW 4 A= wastewater sample stored at 4 °C, stabilised with NaN_3; the dashed grey line at 80% indicates the significance threshold).

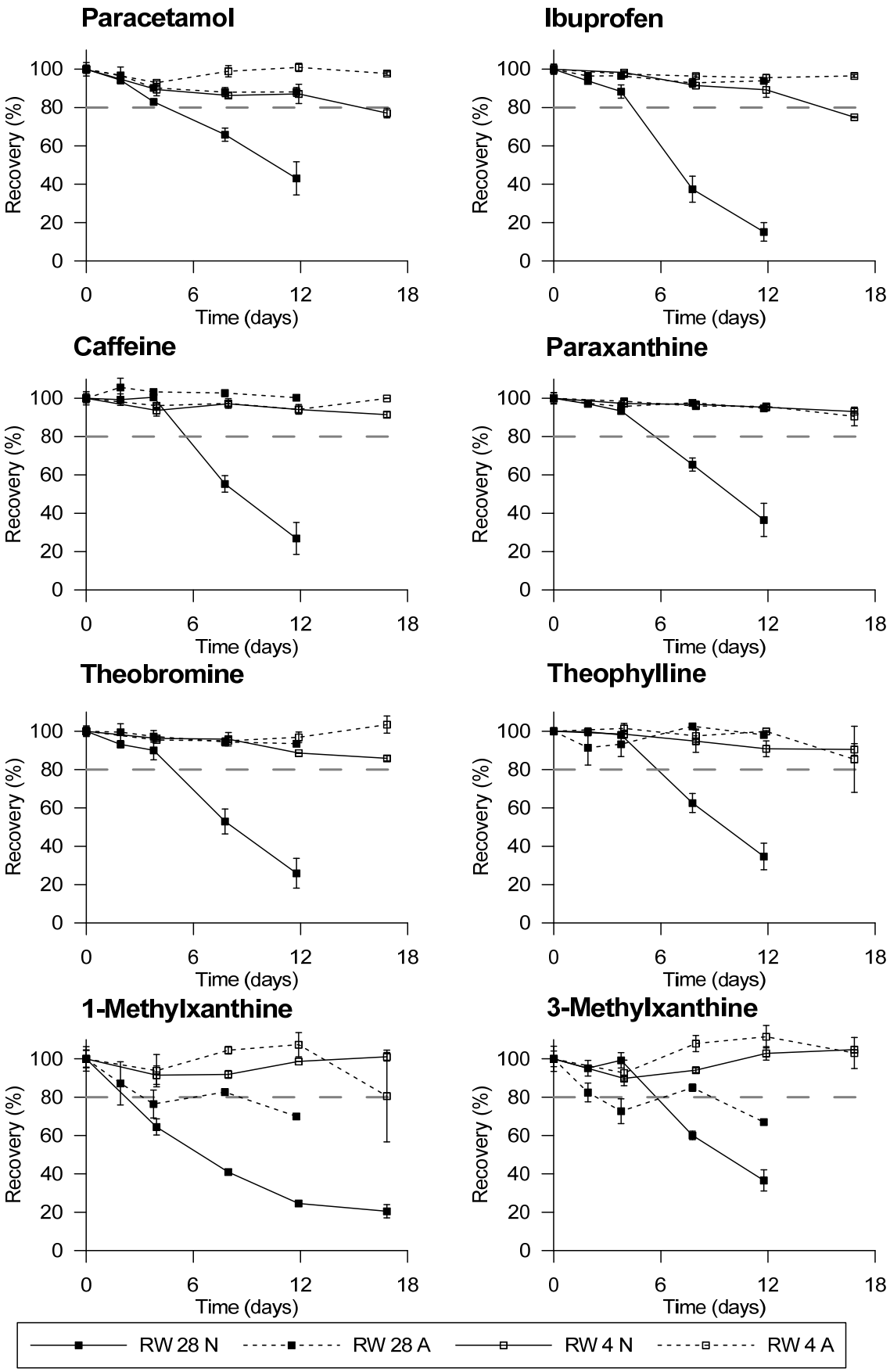

Figure 2.3. Recoveries of selected analytes in river water with respect to storage time; stored as liquid (RW 28 N= non-stabilised river water sample stored at 28 °C; RW 28 A= river water sample stored at 28 °C, stabilised with NaN_3; RW 4 N= non-stabilised river water sample stored at 4 °C; RW 4 A= river water sample stored at 4 °C, stabilised with NaN_3; the dashed grey line at 80% indicates the significance threshold).

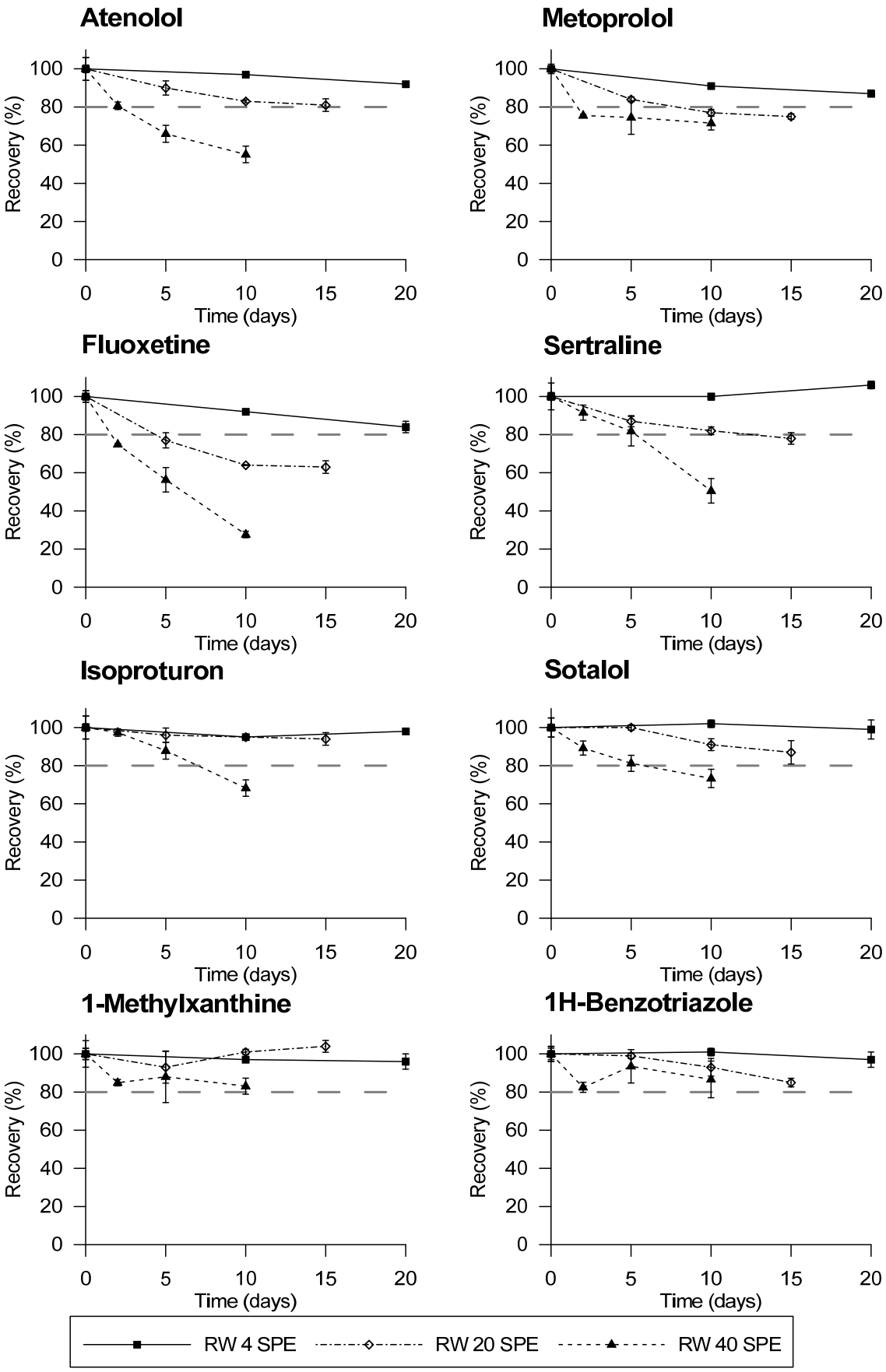

Figure 2.4. Observed recoveries of selected analytes after solid phase extraction of spiked wastewater samples (WW 4 SPE= cartridges stored at 4 °C; WW 20 SPE= cartridges stored at 20 °C; WW 40 SPE= cartridges stored at 40 °C; the dashed grey line at 80% indicates the significance threshold).

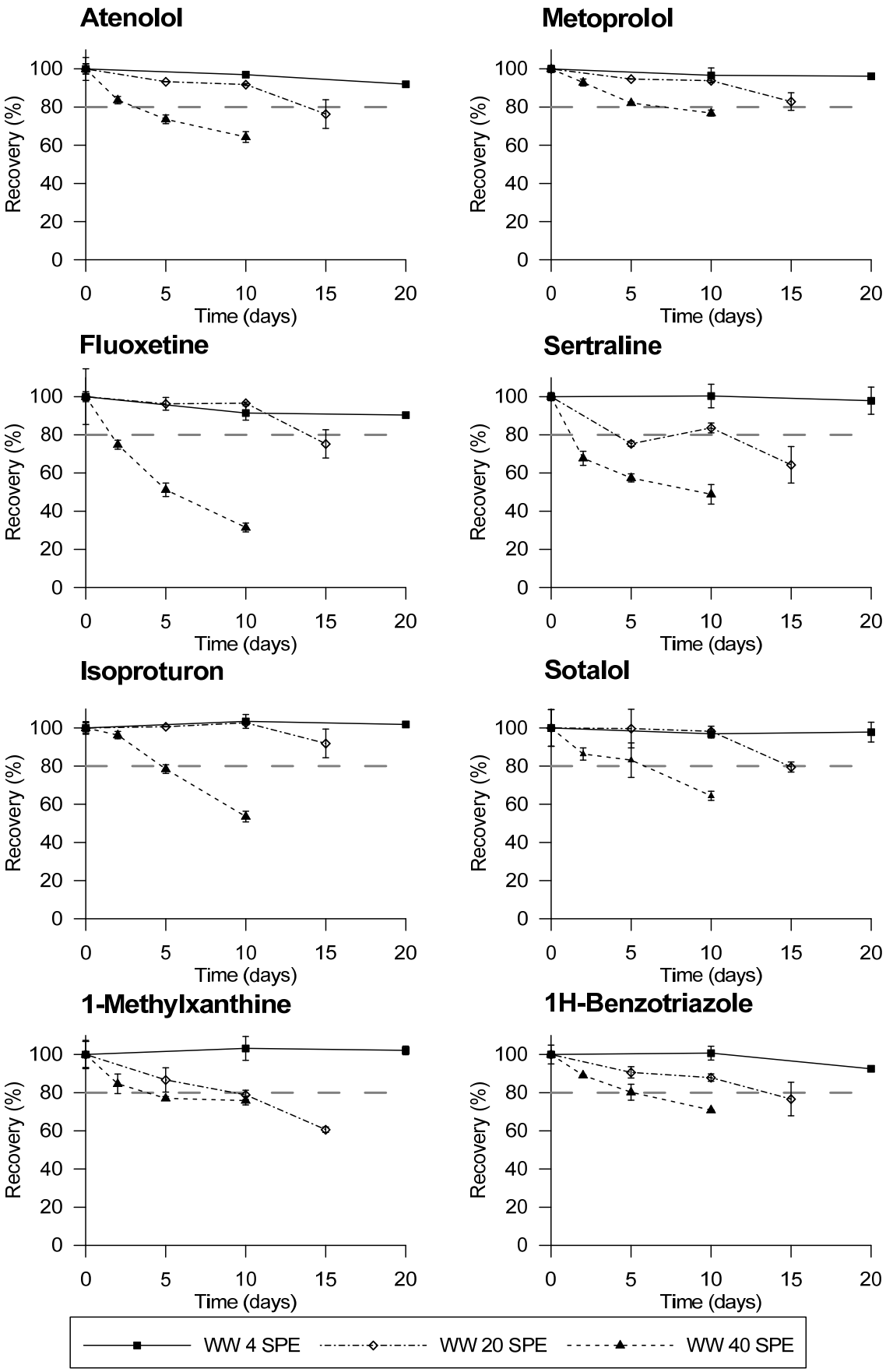

Figure 2.5. Observed recoveries of selected analytes after solid phase extraction of spiked river water samples (RW 4 SPE= cartridges stored at 4 °C; RW 20 SPE= cartridges stored at 20 °C; RW 40 SPE= cartridges stored at 40 °C; the dashed grey line at 80% indicates the significance threshold).

2.3.1.3 Stabilisation with copper sulphate (subset 3)

The stabilisation of the samples with copper sulphate led to significant analytical problems. On the one hand, the addition of the stabilising additive led to a milky-blue precipitate which is assumed to be copper-(II)-hydroxide ($Cu(OH)_2$) given its low solubility ($K_{SP} = 2.20 \cdot 10^{-20}$; Patnaik, 2003). Due to this precipitate SPE was difficult without prior filtration. On the other hand, the methylxanthines exhibited poor recoveries in all samples. Tolyltriazole could hardly and pantoprazole and 1H-benzotriazole could not be detected at all. Probably this is caused by complexation with copper: all the above mentioned compounds comprise an azole structure. 1H-benzotriazole and tolyltriazole are used as corrosion inhibitors for metals including copper. After adsorption of the inhibitor on the copper surface a copper-azole-complex is formed (Subramanian and Lakshminarayanan, 2002). In a third step, polymerisation can occur (Antonijevic and Petrovic, 2008). Imidazole and its derivatives are also efficient copper corrosion inhibitors in various media (Stupnisek-Lisac et al., 2002; Subramanian and Lakshminarayanan, 2002).

In conclusion, it can be assumed that the methylxanthines and pantoprazole containing imidazole-like structures can also form complexes with copper resulting in the observed low to very low recoveries. Due to these problems a further discussion of the results from these samples is excluded.

2.3.2 Stabilisation by SPE (subset 4)

By SPE of the water samples, the water activity and the concentration of inorganic nutrients were reduced. Although for the high storage temperature (at 40 °C) low recoveries were expected, 33 of the 46 analytes could be stabilised over the complete observation period of 10 days. Compounds that showed recoveries lower than the significance level of 80% include all antihypertensive agents, all SSRIs, both corrosion inhibitors, 1-methylxanthine, 3-methylxanthine, loratadine, diuron and isoproturon. The most labile compounds in the non-stabilised samples stored as liquid phase (methylxanthines, paracetamol and ibuprofen) exhibit much higher (1-methylxanthine and 3-methylxanthine) or acceptable recoveries after SPE, whereas fluoxetine, sertraline and atenolol show the lowest recovery of all analytes at the end of the investigation period (28, 49 and 55% respectively; Figure 2.4 and Figure 2.5). Despite the relatively low recovery of atenolol, increasing concentrations of atenolol acid were not observed.

At 4 °C and over the entire investigation period of 20 days no significant decrease in recovery could be observed for any of the investigated compounds. Comparing the water matrices with one another, in general no differences can be observed. In fact, samples from WW and RW showed rather similar recoveries for some of the compounds (e. g. fluoxetine and atenolol). For tables with all spiked compounds and their respective recoveries after SPE, see Table A.10 to Table A.15.

2.4 Conclusion

In the water samples stored as liquid phase the methylxanthines, ibuprofen and paracetamol were ascertained to be among the lowest recovered micro-contaminants in non-stabilised samples of both investigated water matrices, RW and WW. These compounds are valuable indicators for untreated sewage (Bound and Voulvoulis, 2006; Buerge et al., 2006; Hillebrand et al., 2012) and immediate sample preparation and analysis would be the best option to prevent low recoveries due to storage. However, depending on the infrastructure this option may not be feasible and the transport of the samples to the laboratory may take a considerable time.

Stabilising the samples with sodium azide led to significantly higher recoveries in both water matrices. Nevertheless, for some analytes unacceptable recoveries were observed.

The stabilisation of the water samples with copper sulphate caused detrimental interferences with all the methylxanthines, the corrosion inhibitors and pantoprazole, most likely due to the formation of copper-azole-complexes. It can be concluded that copper sulphate is an unsuitable stabilising additive for micro-pollutants in water samples when stored as liquid phase especially, if azole- or imidazole-like compounds are to be included in the list of analytes.

Processing the water samples by SPE showed the best results of all stabilising strategies. While for some analytes recoveries ≤ 80% could be observed at 20 and 40 °C, storing the SPE cartridges at 4 °C led to acceptable recoveries over the whole observation period of 20 days for all investigated analytes.

Concluding from our presented results, the following recommendations for sample preparation and storage can be derived (from best, to worst alternative):

(1) Immediate analysis of the samples

(2) SPE directly after sampling with SPE cartridge, store as cool as possible

(3) Stabilisation of the samples with sodium azide and store as cool as possible

(4) Storage of non-stabilised samples as cool as possible

If no immediate analysis is possible, the storage time should be minimised. Depending on the water matrix sampled a ranking can be set up, reflecting its need for sample stabilisation. Firstly, WW samples need to be stabilised. Due to their high number of adapted micro-organisms, the stabilisation of these samples is most urgent. Secondly, RW samples need to be analysed or otherwise stabilised.

Although groundwater and drinking water have not been investigated in the course of this manuscript, the following can be assumed: for groundwater, which is known to be less loaded with micro-organisms (Schijven et al., 2003; Toze, 2004) as well as drinking water, much less alteration of the analytes is expected. Hence, the analysis or stabilisation of respective samples need to be performed the least urgent.

Acknowledgements

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Chapter 3

3 Investigating the dynamics of two herbicides at a karst spring in Germany: consequences for sustainable raw water management

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Abstract

While karst aquifers are considered as rapid flow and transport systems, their high potential for long-term storage is often ignored. However, to achieve a sustainable raw water quality for drinking water production, the understanding of this potential is highly essential. In this study, the transport dynamics of the two herbicides metazachlor and atrazine as well as a degradation product of the latter (desethylatrazine) were investigated at a karst spring over one year. Even 20 years after its ban in Germany, atrazine and its degradation product were almost always detectable in the spring water in the low ng L^{-1} range (up to 5.2 ng L^{-1}). Metazachlor could only be detected after precipitation events and the observed concentrations (up to 82.9 ng L^{-1}) are significantly higher than atrazine or desethylatrazine. Comparing the dynamics of the herbicides with the inorganic ions Ca^{2+}, Mg^{2+} and the electrical conductivity, a positive correlation of atrazine with these parameters could be observed. From this observation, atrazine is concluded to be located within the aquifer matrix. To achieve a sustainable raw water management at karst springs, the rapidness of these systems needs to be highlighted as well as their long-term storage potential. Persistent substances or transformation products are prone to deteriorate the raw water quality for decades.

3.1 Introduction

In the *Guidelines for drinking-water quality,* the World Health Organisation emphasises the advantages and necessities of effective catchment management, i. e. understanding an aquifer and identifying possible water pollution scenarios affecting the raw water quality (WHO, 2011). The understanding of karst aquifers is particularly challenging, due to their specific characteristics (e. g. dolines, conduits flow). Still, these highly dynamic and heterogeneous aquifer systems are important drinking water sources all over the world. The complex interaction between developed karst conduits including the related rapid flow and transport processes in them (residence time of a few days, e. g. Pronk et al., 2009; Hillebrand et al., 2012a) and the high-volume porous rock matrix (characterised by slow matrix flow and long residence times of several years, e. g. Einsiedl, 2005) is not yet fully understood and thus still subject to research. Investigating the recharge mechanisms at a shallow karst system rapid preferential flow and diffuse matrix flow (which is characterised by much slower flow rates) were observed (Atkison, 1977; Haria et al., 2003). However, for some deep aquifers only slow matrix flow could be identified (Haria et al., 2003; Chilton et al., 2005).

It is a long established fact that recharge events in karst systems lead to strong variations in spring water quality (Jakucs, 1959). Monitoring these spring signals in terms of physical or chemical parameters allows for the integral characterisation of the total catchment area. This feature has been used to e. g. determine the mean residence time of water within aquifer systems based on tritium data (Maloszewski et al., 2002) or to estimate the total amount of wastewater infiltrating such systems by employing caffeine as a semi-quantitative indicator (Hillebrand et al., 2012a). Stueber and Criss (2005) derived the primary immediate sources for water quality components depending on their covariance with the electrical conductivity (EC) or the turbidity. A positive covariance of components time-series with the EC implies diffuse (matrix) flow being its primary source, while a positive covariance with turbidity suggests that the components immediate source was from agricultural fields.

In the presented work, two herbicides (atrazine and metazachlor) and the degradation product desethylatrazine are employed, in order to improve the understanding of spring water signals after precipitation events and consequently the understanding of the investigated karst aquifer system which are vital for providing measures for sustainable raw water quality. Atrazine is one of the most widely used soil and weed herbicides, whereas its use has been prohibited in Germany since 1992. However, it is well dispersed and can still be found in the environment even after more than 20 years (Jablonowski et al., 2011; Nödler et al., 2013; Reh et al., 2013). The potential of atrazine to be degraded in karst

aquifers is stated to be very little to non-existent (Johnson et al., 2000; Chilton et al., 2005). One of its degradation products is desethylatrazine (Kolpin et al., 1998). However, desethylatrazine is also formed from other triazine herbicides like propazine (Behki and Kahn, 1994). Atrazine is affected by sorption, exhibiting a low desorption rate, which may take several days or even weeks (Dehghani et al., 2005). In contrast to the banned substance atrazine, the weed control agent metazachlor is approved in Germany. Its tendency to adsorb onto soil material is known to be low (Mamy and Barriuso, 2005), while being readily degradable (Allen and Walker, 1987; Beulke and Malkomes, 2001). In the investigated karst system, transport is known to be rapid and an appearance of metazachlor in spring water can still be expected. For reference purposes and to locate the origin of atrazine, desethylatrazine and metazachlor, the time-series of these three compounds are compared to the time-series of the inorganic ions nitrate (NO_3^-), calcium (Ca^{2+}) and magnesium (Mg^{2+}) as well as the EC of the spring water.

The aims of the study are to i) improve the understanding of contaminant migration in karst aquifers under consideration of recent and former herbicide applications, ii) highlight the long-term storage potential of karst aquifers and to iii) draw attention to the consequences of unsustainable herbicide application for the raw water quality. The authors hypothesise that the characteristic residence time distribution of water in karst aquifer systems (days to several decades) is reflected in the occurrence and dynamics of the investigated herbicides.

3.2 Materials and methods

3.2.1 Field work

3.2.1.1 Study area

The investigated karst spring is the Gallusquelle, which is located in Southwest Germany (Figure 3.1). It is used as a drinking water source for 40,000 people. Its average discharge is 500 L s^{-1} draining a rural catchment (4000 inhabitants) of approximately 45 km². Around 40% of the catchment is used for agriculture. These areas are used as grasslands and for the cultivation of crops (approximately 14% of the total catchment area; Sauter, 1992). Despite the thick unsaturated zone (~100 m, Figure 3.1) within the investigated system, precipitation can quickly reach the groundwater through dolines and dry valleys as concentrated recharge. Through these preferential flow paths, the transport of solutes including contaminants from the ground surface towards the spring is enhanced. The occurrence of contaminants only days after precipitation events has been shown in former investigations (Heinz et al., 2009; Hillebrand et al., 2012b). In contrast, a mean groundwater residence time of more than 20 years was determined by Geyer et al. (2011) employing lumped parameter modelling of tritium in spring water.

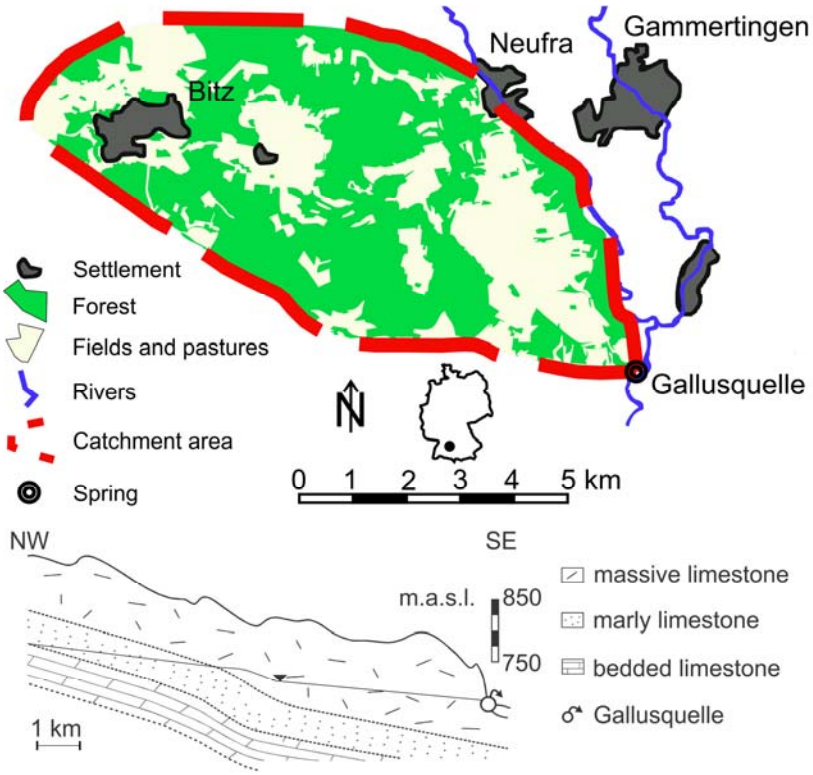

Figure 3.1. The catchment area of the Gallusquelle and its geological cross section (delineation of the catchment and cross-section according to Sauter, 1992).

3.2.2 Sampling

Over the period of nearly one year, a total of 263 spring water samples were collected and analysed for herbicides from March, 6th 2010 until February, 16th 2011. The sampling rate varied between weekly, daily and multiple daily depending on the spring discharge and the occurrence of recharge events. For one recharge event a highly increased sampling rate of up to 8 samples per day was realised. Selected major ions concentrations were determined over a period of 3 months, including the mentioned recharge event (n= 153). Additionally, a rain water sample was collected during that recharge event with a precipitation-totalisator (accumulative precipitation gauge) for the hydrograph separation. To ensure the stability of the analytes, the samples were stored at 4 °C. For herbicides, samples were preconcentrated by solid phase extraction (SPE) within 48 h and the SPE cartridges were stored at –18 °C until analysis (Hillebrand et al., 2013).

3.2.3 Chemicals

Methanol (LC/MS grade) was purchased from Fisher Scientific (Schwerte, Germany), ammonium acetate, ethyl acetate, formic acid, potassium dihydrogen phosphate and disodium hydrogen phosphate dihydrate (all analytical grade) were purchased from VWR (Darmstadt, Germany). Atrazine, atrazine-D_5, desethylatrazine and metazachlor were purchased from Dr. Ehrenstorfer (Augsburg, Germany), carbamazepine-D_{10} from Promochem (Wesel, Germany).

3.2.4 Laboratory and on-site analyses

3.2.4.1 On-site analysis

Hourly data for electrical conductivity (reference temperature: 20 °C) and turbidity of the spring water, as well as the spring water levels were gauged with an installed continuous monitoring system. The water levels were transferred into spring discharge data, applying a rating curve.

3.2.4.2 Inorganic ions

The samples for cation analysis were acidified with methane sulfonic acid (2.5 µL mL^{-1}). The analysis for the inorganic ions was performed by ion chromatography (IC) as described in Nödler et al. (2011).

3.2.4.3 Herbicides

The analytical method for the determination of the herbicides metazachlor and atrazine as well as its degradation product desethylatrazine is based on SPE and high-performance

liquid chromatographic separation coupled with tandem mass spectrometric detection (HPLC/MS-MS). The details of the method have been published earlier (Nödler et al., 2010). Briefly, a sample volume of 500 mL was buffered at neutral pH (phosphate buffer), spiked with 100 ng atrazine-D_5 and carbamazepine-D_{10} and extracted by SPE (500 mg Oasis HLB, Waters, Eschborn, Germany). After extraction, the cartridges were rinsed with ultrapure water, dried, wrapped in aluminium foil and kept frozen (–18 °C) until analysis. Prior to analysis the herbicides were successively eluted from the sorbent with methanol and ethyl acetate. The eluate was evaporated and re-dissolved in a 5 mM ammonium acetate aqueous solution, containing 4% methanol. Unlike Nödler et al. (2010), only 0.8 mL was used to re-dissolve the analytes. Thus, a higher enrichment factor and consequently lower method detection and quantification limits were achieved: the method detection limits (MDL) of atrazine, desethylatrazine and metazachlor were 0.3, 0.4 and 0.5 ng L^{-1}, respectively. The method quantification limits (MQL) were 1.1 ng L^{-1} for atrazine and, 1.4 ng L^{-1} for desethylatrazine and metazachlor. The MDLs and MQLs were determined according to DIN 32645 (2008).

3.2.5 Hydrograph separation

The hydrograph separation technique was employed for estimating the amount of rainwater reaching the spring over rapid recharge. Typically, isotopic data (e. g. Malík and Michalko, 2010), inorganic ions (e. g. Dreiss, 1989) or the EC (e. g. Laudon and Slaymaker, 1997) are used. On the basis of end-member mixing the variations of the rainwater tracers are utilised in estimating the fraction of rapidly transported rainwater to the spring. The calculation procedure is shown in the appendix (Appendix A.1). Please note, that the application of end-member mixing is discussed controversially (e. g. Nakamura, 1971; Pilgrim et al., 1979). The approach assumes the conservative behaviour of both end-members, i. e. the investigated components do not change. This is obviously not true for any of the investigated parameters here. On the example of EC, one can assume that the amount of dissolved solids in the rainwater and consequently the EC increases the moment it comes into contact with the earth's surface and with increasing contact time with soil or aquifer material. Taking these uncertainties into account, the hydrograph separation based on end-member mixing of EC can be understood as a lower boundary estimation of the true fraction of rainwater at the spring. The actual amount of rainwater is likely to be higher. Comparable uncertainties must be considered to some extend when performing hydrograph separation by end-member mixing with Ca^{2+}, Mg^{2+}, atrazine and desethylatrazine.

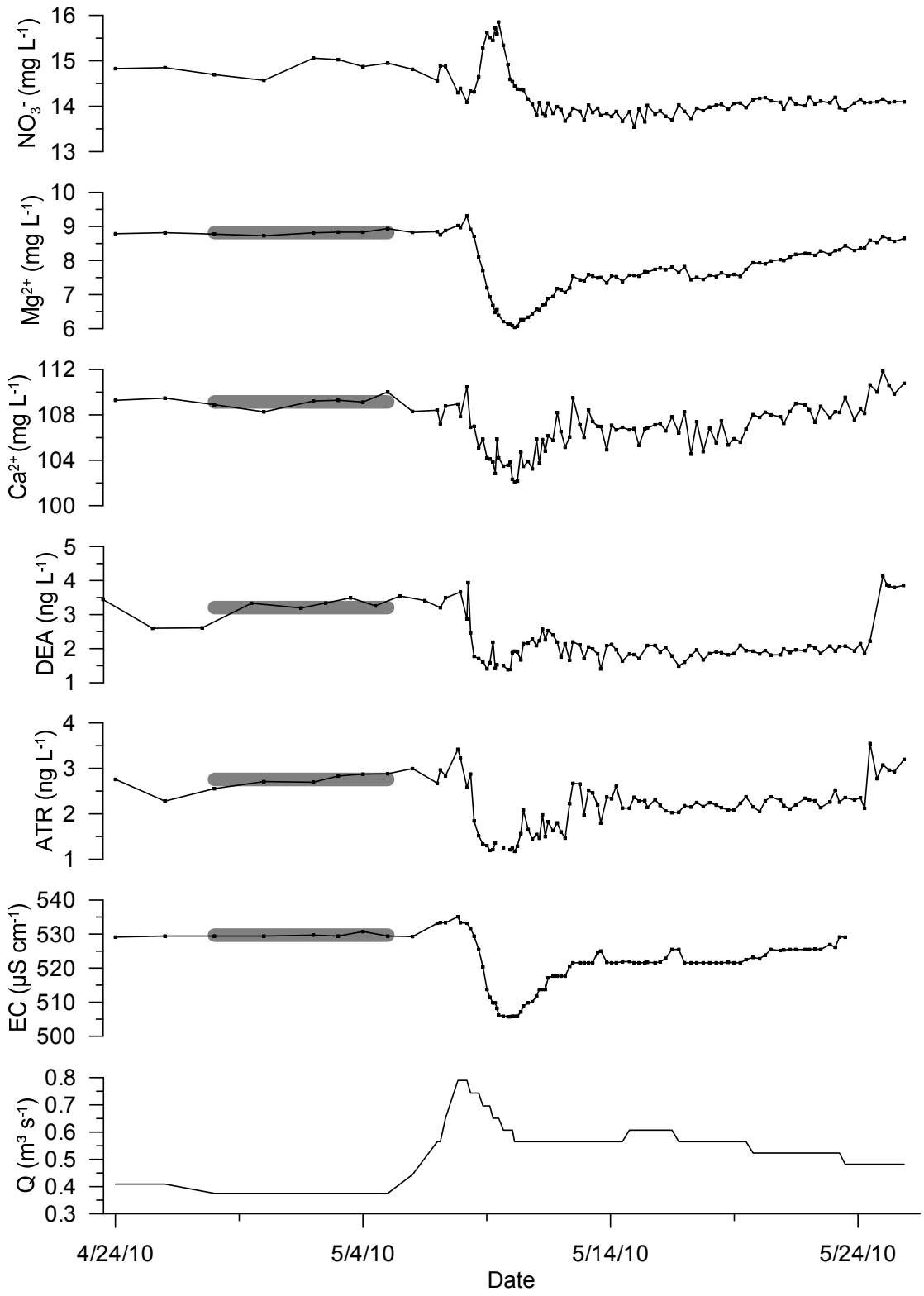

Figure 3.2. Behaviour of the inorganic ions NO_3^-, Mg^{2+} and Ca^{2+}, electrical conductivity (EC), desethylatrazine (DEA) and atrazine (ATR) after a precipitation event (Q= spring discharge). The grey bars highlight the background concentration, used for the discharge separation calculations. The discontinuity at the DEA- and ATR-curves' minimum refer to single samples, where the observed concentrations were below the limit of quantification (LOQ).

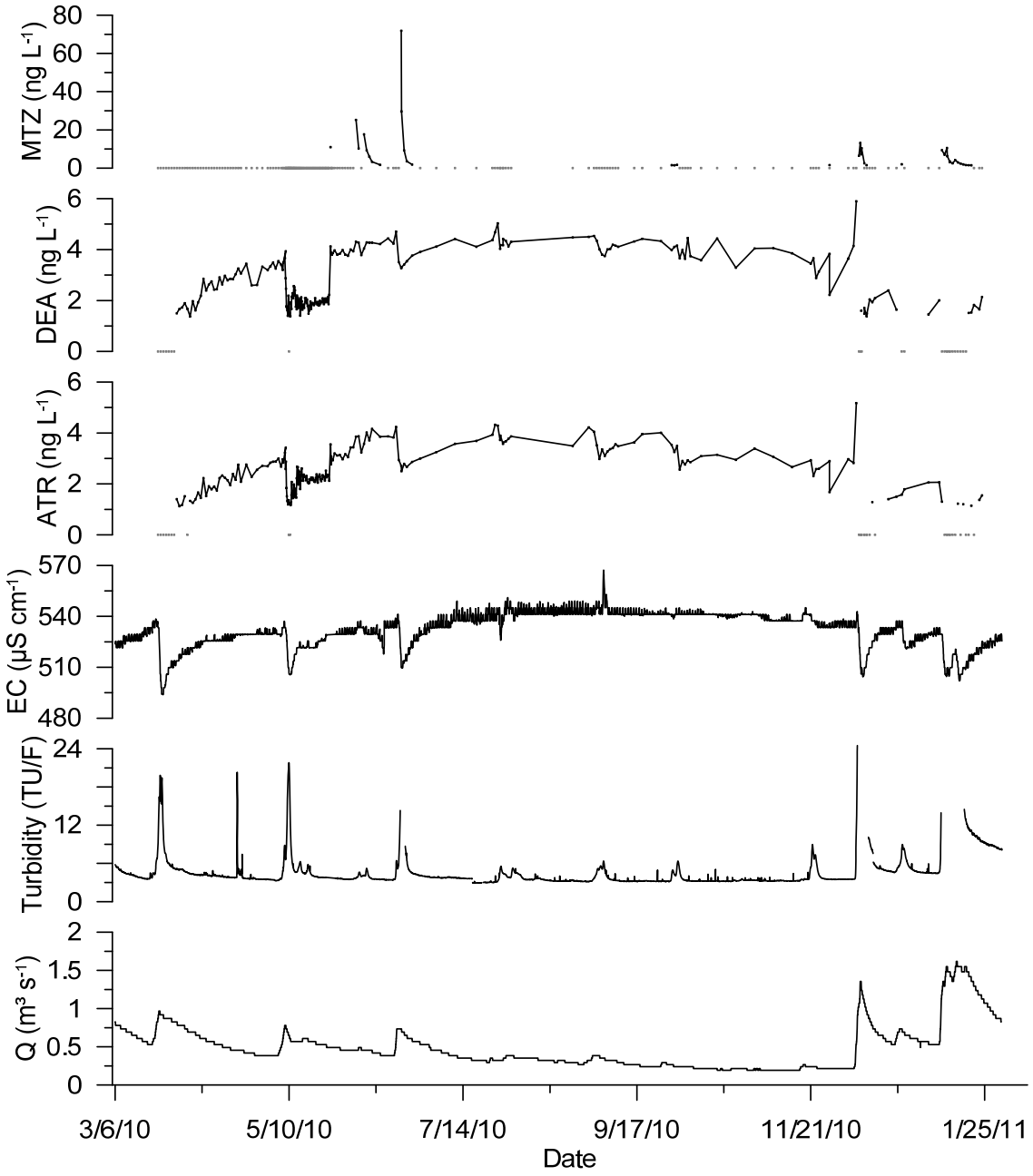

Figure 3.3. Variations of metazachlor (MTZ), desethylatrazine (DEA), atrazine (ATR), electrical conductivity (EC), turbidity and daily average spring discharge (Q) at the Gallusquelle spring over the period of investigation. The grey squares at 0 ng L^{-1} for MTZ, DEA and ATR indicate samples for which the observed concentration was below the LOQ. Interruptions in the turbidity line are caused by data loss.

3.3 Results and discussion

3.3.1 Variations of investigated parameters

The concentration range of Ca^{2+}, Mg^{2+}, NO_3^-, atrazine, desethylatrazine and metazachlor in spring water are presented in Table 3.1.

Although NO_3^- may also originate from urban sources, such as leaky sewers and landfills (Wakida and Lerner, 2005), the agricultural application of fertilisers is its main source,

especially in sparsely populated and rural areas such as the catchment under investigation. After precipitation events, the concentration of NO_3^- increases before returning to a background concentration within a few days, only slightly affected by dilution (Figure 3.2). This is expected for NO_3^- and other substances/ions, which are introduced into the karst system together with the infiltrating rainwater or snow-melt (i. e. recharge events). The same behaviour can be observed for the herbicide metazachlor (Figure 3.3). It does not occur evenly distributed over time, but only after precipitation events at comparatively high concentration (Table 3.1). However, while for NO_3^- a background concentration exists in the spring water, the concentration of metazachlor decreases below the limit of detection (LOD) within a short period of time. The irregular occurrence only after precipitation events indicates the transport of metazachlor with the percolating rainwater through the unsaturated zone to the local karst spring. As metazachlor was not detected in spring water during the winter months, it is unlikely that the occurrence of metazachlor in the spring to autumn months is related to metazachlor sources within the subsurface, but originates from recent application (metazachlor is applied as a post-emergence herbicide few weeks after the sowing in spring or at the end of august for winter oilseed rape). This is supported by the low half-life of metazachlor in the environment (Allen and Walker, 1987; Beulke and Malkomes, 2001).

In contrast, for the parameter 'hardness' the opposite effect (i. e. decreasing concentrations) has been described (Ashton, 1966; Williams, 1983): (i) initial expulsion of phreatic and subcutaneous water, (ii) arrival of flood water, diluting the spring water, (iii) return to pre-event conditions. This pattern occurs for parameters originating from within the aquifer system which are affected by dilution, i. e. hardness, EC, and the inorganic ions dissolved from the rock matrix. At the Gallusquelle spring, this behaviour can be observed for the EC, Ca^{2+} and Mg^{2+} similar to findings of Stueber and Criss (2005). If there were additional sources for Ca^{2+} and Mg^{2+} beside the subsurface/aquifer material, its influence was negligible.

Table 3.1. Concentration range of the investigated inorganic ions, herbicides and the herbicide degradation product in the spring water of the Gallusquelle during the period of investigation. Concentrations of the inorganic ions are expressed in mg L^{-1}.

	Min[c]	Max[d]	Median	DF[e]
Ca^{2+}[a]	102	114	108	100
Mg^{2+}[a]	6.0	9.3	7.0	100
NO_3^-[a]	11.6	17.5	14.4	100
Atrazine[b]	< LOD[f]	5.2	2.3	99.6
Desethylatrazine[b]	< LOD[f]	5.9	2.3	99.6
Metazachlor[b]	< LOD[f]	82.9	< LOD[f]	30.7

[a]n=153

[b]n=263

[c]minimum concentration

[d]maximum concentration

[e]detection frequency in %

[f]limit of detection

Unlike the irregular occurrence of metazachlor at the spring, atrazine and desethylatrazine were detected in nearly all samples throughout the investigation period (Figure 3.3). Their observed concentrations were generally low (Table 3.1), but comparable to values from Switzerland (Morasch, 2013). Storm pulses (i. e. increasing concentrations with increasing discharge) were reported for atrazine after precipitation events in the U.S. (Vesper et al., 2001), only occurring after application of atrazine (Stueber and Criss, 2005). Similarly, in southwest England a positive correlation was observed between increased water levels and atrazine concentrations, which was explained by the remobilisation of historic pollution incidents (Lapworth and Gooddy, 2006). At the Gallusquelle spring a different behaviour can be observed, comparable to that of the EC, Ca^{2+} and Mg^{2+}. In fact, the correlation of normalised (concentrations attain values between 1 and 0 for their maximum and minimum value respectively) atrazine concentrations with these parameters in normalised form yields values for R^2 of 0.6–0.7 (performing the same correlation calculations with NO_3^- leads to values for R^2 of 0; scatter-diagrams for all parameters are provided in the appendix (Appendix A.2). From the correlation of the time-series of Ca^{2+}, Mg^{2+}, the EC and atrazine a similar origin can be deduced (Stueber and Criss, 2005). As Ca^{2+} and Mg^{2+} originate from the aquifer matrix, atrazine is inferred to be located within the aquifer material, i. e. inside the rock matrix. From here it is released slowly into the groundwater. This is in agreement with findings reported by Morasch (2013) who assigned the observed continuous low atrazine concentrations in a Swiss (where atrazine is still applied) karst aquifer system to its slow but steady release from the aquifer matrix. Please note, that sorption may partly affect the long-term fate of atrazine, but that the appearance of atrazine even after more than 20 years in the investigated aquifer is more likely related to the slow groundwater flow rates inside the karst matrix and the resulting long residence times, while hardly or not affected by degradation (Johnson et al., 2000; Chilton et al., 2005).

For desethylatrazine a time-series similar to atrazine, Ca^{2+}, Mg^{2+} and the EC could be observed (Figure 3.2). But yet, it is different; a correlation of desethylatrazine with these parameters was significantly worse (R^2 between 0.2 and 0.4, details can be found in the Appendix A.2.). The arrival of rapid recharge after a precipitation event leads to decreasing concentrations of desethylatrazine and hence its origin can be concluded to be situated within the fissured rock matrix, too. However, the time-series seems to evolve a plateau before returning to its background concentration rapidly some time after the recharge event, instead of a slow and steady concentration increase. The slightly different behaviour of desethylatrazine to atrazine is difficult to assess and beyond the scope of this study.

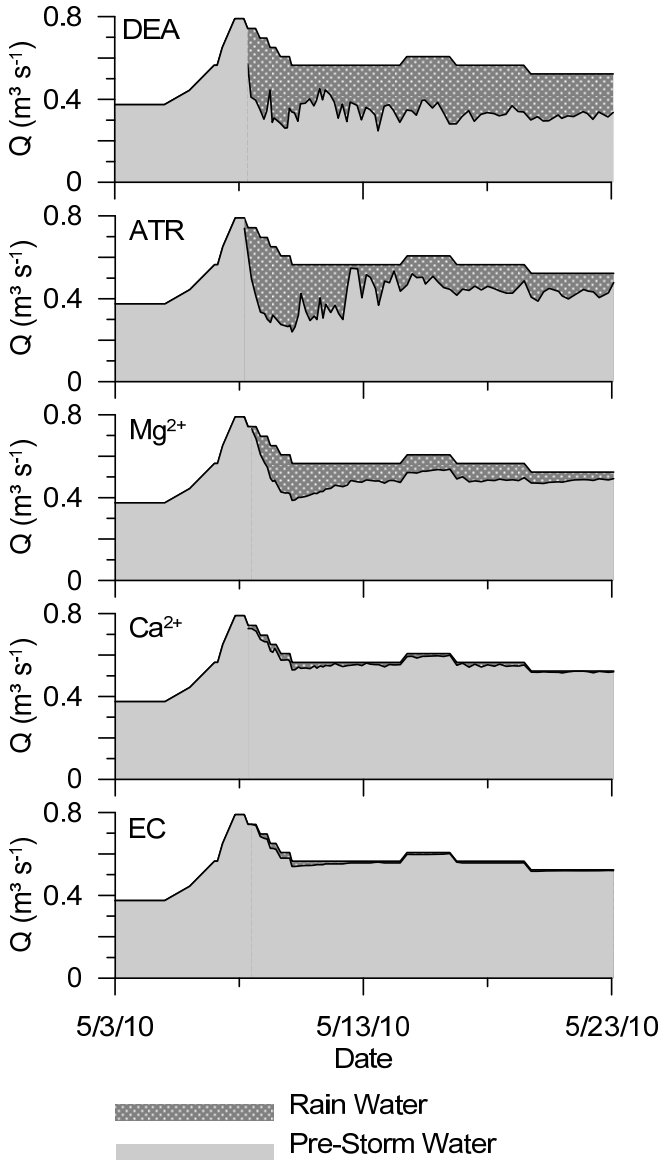

Figure 3.4. Calculated discharge (Q) separation based on end-member mixing for desethylatrazine (DEA), atrazine (ATR), the inorganic ions Mg^{2+} and Ca^{2+} and the electrical conductivity (EC).

3.3.2 Hydrograph separation

For one event a hydrograph separation was performed, based on the dilution of the concentrations of atrazine, desethylatrazine, Ca^{2+} and Mg^{2+} and the EC. As end-member for the pre-storm water the background concentration/value of each parameter was used (illustrated in Figure 3.2). A sample of the precipitation contained 1.7 mg L^{-1} of Ca^{2+} and 0.07 mg L^{-1} of Mg^{2+}. As end-member for the rainwater a concentration/value of 0 is assumed for all parameters. This assumption seems legitimate as the observed concentrations are very low (< 1% of the background concentration) and it does not alter the determined fractions of the rainwater at the spring significantly.

The results are illustrated in Figure 3.4. The calculated amount of rainwater reaching the spring over direct recharge is similar for the EC (maximum: 4.5%) and Ca^{2+} (maximum: 6.5%). These values are in agreement with previously published results from Sauter

(1997), who stated the fraction of rapid recharge to be in the order of 5–10% based on $\delta^{18}O$-data. Using Mg^{2+} the determined amount of freshly introduced recharge is much larger (maximum: 32%). This observation may originate either from (i) unevenly distributed Mg^{2+} minerals (e. g. dolomite) in the subsurface, i. e. disproportional dilution of the Mg^{2+} concentration or (ii) slower dissolution of dolomite relative to calcite (Liu and Dreybrodt, 2001). It is likely, that the extent of the dilution of the Mg^{2+} concentration is affected by both factors. For atrazine and desethylatrazine even higher dilutions can be observed. Consequently, a higher amount of rainfall reaching the spring over rapid recharge was calculated (maximum: 58% and 57% for atrazine and desethylatrazine, respectively). As atrazine was applied as herbicide on agriculturally used areas, the area of application can be estimated to be 14% of the catchment area at maximum (Sauter, 1992). Considering this fraction, it becomes evident that a larger dilution for atrazine is to be expected than for Ca^{2+} and the EC. Furthermore, the rain-component for atrazine is likely to be less influenced by equilibration with the subsurface as a consequence of the restricted areal distribution.

Assuming the maximum dilution of the investigated parameters to be representative for their areal extend, one can estimate the latter (i. e. the magnitude of the maximum dilution is inversely proportional to the fraction of the catchment area over which the parameter is introduced into the system). As Ca^{2+} is believed to occur all over the catchment (i. e. 100%), the area over which atrazine is introduced into the system can be estimated to 11% of the catchment area. This is in good agreement with the reported land use pattern at the time of the application of atrazine (Sauter, 1992). The same applies to desethylatrazine.

3.3.3 Mass-balance for atrazine

Atrazine use has been permitted in Germany from 1958 until its ban in 1992. The total amount of atrazine applied in the investigated area was estimated according to a report from the European Commission (Henriet et al., 1994). The following assumptions were made therein: 11.1% of the agriculturally used area (which is 14% for the investigated catchment (Sauter, 1992)) was used for maize, 90% of the fields were treated with atrazine at a mean dose rate of 1.5 kg ha^{-1}, an additional 10% of atrazine is considered for other uses. Presuming a certain time lag until atrazine was applied as herbicide after its permission, 27 years (1965–1992) were assumed as the duration of application. The resulting total mass of atrazine, being applied in the investigated area was calculated to be approximately 2,800 kg.

To assess the discharge of atrazine the mean concentration of atrazine in the investigation period was used as well as the mean spring discharge of 500 L s^{-1}. Accordingly 37.8 g of atrazine were discharged in the course of the investigated year via the Gallusquelle spring. For an estimation of the total mass of discharged atrazine the following assumptions were made: the concentrations of atrazine were higher in the years 1992–2009 following the

same trend as in the data of Tappe et al. (2002), i. e. declining concentrations following an exponential decline with a decline-rate of 0.26 a^{-1}. For the years 1965–1991 a constant discharge of atrazine is assumed which is equal to the estimated value for 1992 (3.8 kg a^{-1}; a table with the estimated loads for the whole period is provided in the appendix (Table A.16). Over the years 1965–2010 a total discharge of 120 kg (around 4% of the applied mass) of non-metabolised atrazine can be estimated. The remaining 96% of the applied atrazine were either metabolised (e. g. Jablonowski et al., 2009) or still within the aquifer rock matrix. Both possibilities hold true since (i) it could be clearly demonstrated, that even after 20 years without application within the catchment the original compound is still detected in spring water and (ii) the metabolite desethylatrazine has been found in spring water, exhibiting a similar behaviour as atrazine. The estimation of the discharge of atrazine can be refined, taking a quantification of the discharge of desethylatrazine into account. For the calculation all assumptions were made, as stated above for atrazine. However, the concentration of desethylatrazine declines at a different rate, than the concentration of atrazine. The trend has been determined from the results of Tappe et al. (2002) to be 0.22 a^{-1}. The total discharge of desethylatrazine in the years 1965–2010 can be calculated to be 77 kg, corresponding to 88 kg of atrazine. Please note that desethylatrazine is not an unambiguous degradation product, and that the calculation is hence, unambiguous as well. It must be understood as upper boundary estimation. Thus, a total of 120–208 kg of atrazine could be estimated to discharge at the Gallusquelle in the course of 45 years. This corresponds to 4–7.5% of the estimated total atrazine applied. These low values do no surprise, when taking the low to non-existent degradation rates (Johnson et al., 2000; Chilton et al., 2005) and the low leaching rates (Haria et al., 2003; Baran et al., 2008) of atrazine into account. Please note, that further degradation products may occur (Krutz et al., 2003), which have not been considered in the above estimation.

3.4 Conclusion

The concentration of metazachlor in spring water increases after precipitation events and decreases below the LOD within a short period as expected for herbicides. In contrast, the atrazine and desethylatrazine concentrations are diluted after precipitation events and return to their pre-event level. From the correlation with Ca^{2+} and Mg^{2+} it can be concluded, that atrazine is likely to be located within the aquifer matrix. This duality of transport in karst aquifers needs to be considered carefully, in order to achieve a successful and sustainable raw water management of karst springs: on the one hand, drinking water suppliers need to be aware of rapid recharge and the associated strong variations of raw water quality, which may arise from heavy precipitation or snow-melt events. On the other hand, special attention must be drawn to the high potential of karst aquifers for long-term storage. Potentially persistent substances or transformation products are prone to cause long-term contamination. Due to the high residence-time within the rock matrix, persistent

contaminants may influence the raw water quality for decades. Although atrazine was prohibited in Germany more than 20 years ago, its impact on the investigated karst aquifer is still detectable. A similarly long after-effect should be expected in any other region, where atrazine (or any other persistent contaminant) was or is still applied.

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Chapter 4

4 Identification of the attenuation potential of a karst aquifer by an artificial dualtracer experiment with caffeine

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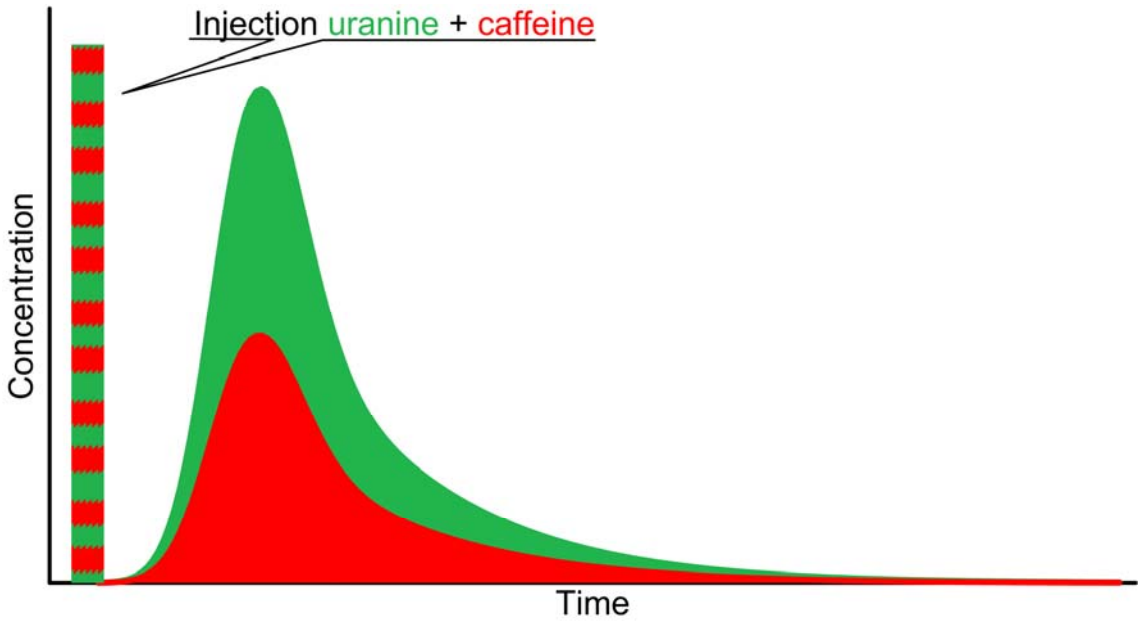

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Abstract

Little is known with respect to the attenuation capacity of karst aquifers. Even less is known about the risk posed by emerging micropollutants in these systems. In order to identify the attenuation potential of karst aquifers *in-situ* and to estimate the risk posed by micropollutants, a dualtracer test was conducted in this study in order to investigate differential transport in the subsurface: the reactive compound caffeine was used as a tracer to indicate the attenuation capacity within the aquifer *in-situ*. Due to the low limit of quantification, only small amounts of caffeine needed to be injected. To calibrate a model and to visualize the attenuation of caffeine a conservative reference tracer (uranine) is injected simultaneously. The methodology is tested in a well characterised karst system in southwest Germany. The results indicate a significantly higher attenuation rate than was expected for karst aquifers. The attenuation is described as a first-order process. The corresponding half-life is 104 h. This low half-life suggests that a generally assumed low natural attenuation capacity of karst aquifers is unjustified. The observed mass loss of caffeine illustrates the potential of caffeine to be used as reactive tracer for indicating *in-situ* attenuation capacity within highly hydraulically conductive systems, such as karst aquifers. Due to the high attenuation rate of caffeine it does not pose a threat as a long-time contaminant. In combination with a conservative reference tracer an economical and environmentally benign method is presented in this manuscript for the *in-situ* determination of the attenuation capacity of highly conductive aquifer systems.

4.1 Introduction

Karst aquifers supply up to one quarter of the world's population with drinking water (Ford and Williams, 2007). Karst springs are referred to as relatively unsafe drinking water sources: along solutionally widened flow paths contaminants can be transported rapidly from the land surface to a karst spring through the subsurface. In these conduits, flow velocities of several km d^{-1} were reported (e. g. Seiler et al., 1989). The resulting low residence times of the rapidly transported water reduces the potential of contaminant attenuation in case of a contamination. Einsiedl et al. (2009) estimated the vulnerability of a karst aquifer based on the residence time distribution.

The biological activity of karst aquifers is believed to be little, as the nutrient offer is low, i. e. karst aquifers are oligotrophic environments (Gibert et al., 1994; Hirsch, 1986). However, very little is known with respect to the natural attenuation capacity of karst aquifers. As important drinking water sources a successful management and an estimation of the risk posed by (potential) contamination of karst aquifers is of public interest.

Within the last decades micropollutants have been ubiquitously registered in all compartments of the environment (Schwarzenbach et al., 2006; Ternes, 2007). Several micropollutants have been used as indicators for contamination (Buerge et al., 2006; Gasser et al., 2010), but so far their fate in karst systems has rarely been addressed (Einsiedl et al., 2010). The lack of knowledge with respect to the fate of micropollutants and the known vulnerability of karst aquifers result in an unknown risk posed by emerging pollutants.

To reliably assess the natural attenuation potential of a karst system, tracer experiments with reactive compounds can be employed. Haggerty et al. (2009) used the organic compound resazurin to quantify the metabolically active transient storage in a stream. Caffeine, as an often discussed micropollutant (Buerge et al., 2003; 2006; Swartz et al., 2006), possesses promising sorption and degradation properties to determine the attenuation potential of a karst aquifer and therefore indicator properties for reactive transport at large. Caffeine is readily degradable in wastewater treatment plants. In lakes and porous aquifers the degradation was observed to be much lower (Buerge et al., 2003; Swartz et al., 2006). The German Federal Environment Agency classified caffeine as lowly water-hazardous (lowest hazard class). Within the context of controlled and specially designed experiments, the mass loss of caffeine has a potential to indicate the attenuation capacity of a karst aquifer along the tracer flow path.

Mass loss of a tracer resulting from degradation and the respective quantification can be uniquely identified from the appearance of metabolic products or by the simultaneous injection of an inert reference tracer (i. e. multitracer test; Geyer et al., 2007). Since primary or secondary metabolites are unlikely to be produced by oligocarbotroph microorganisms

(Wainwright et al., 1993), and laboratory experimental observations indicate that degradation products cannot be expected from the degradation of caffeine (Kurtzman and Schwimmer, 1971; Mazzafera et al., 1996), an inert reference tracer, e. g. uranine, has to be used to determine the mass loss of caffeine in the investigated karst aquifer and therefore demonstrate the natural attenuation capacity of that system.

This study presents results from a dualtracer experiment, employing caffeine as an indicator for the natural attenuation capacity of a karst aquifer. Apart from caffeine, uranine was injected simultaneously as inert reference tracer for model calibration. Transport parameters were estimated with the numerical modelling approach CXTFIT (Toride et al., 1995).

4.2 Materials and methods

4.2.1 Dualtracer test

The selected field site for the dualtracer experiment is located in the catchment area of the Gallusquelle spring in southwest Germany (Fig. 1). The spring drains a catchment area of approximately 45 km². Annual discharge averages to 500 L s^{-1}, ranging from less than 100 to 2500 L s^{-1}. A small fraction of the outflow is expected to occur below the gauging station. Estimations of this discharge component range up to 200 L s^{-1}. The general flow direction in the catchment is NW-SE. Hirsch (1986) stated groundwater to be oligotrophic, based on low concentrations of organic carbon (1–10 mg L^{-1}). These conditions also apply for the investigated aquifer (1–3 mg L^{-1}; Heinz et al., 2009), which is therefore classified to be oligotrophic. However, the accidental, irregular and event-based inflow of wastewater related micro-contaminants was demonstrated in previous studies (Heinz et al., 2009; Hillebrand et al., 2012; Nödler et al., 2012).

A tracer experiment was performed on June, 27th 2011. A sinkhole 3 km northwest of the spring was selected as tracer injection location (Fig. 1). The characteristics of the sinkhole injection site were previously investigated by two artificial tracer tests (Birk et al., 2005; Geyer et al., 2007), which demonstrated the point-to-point connection between injection point and the Gallusquelle spring. The thickness of the unsaturated zone in the area of the sinkhole is approximately 100 m (Geyer et al., 2007). In order to minimize the influence of the unsaturated zone on the tracer injection and facilitate an introduction of the tracers into the conduit system, the sinkhole was flushed with tap water before and after the injection. Before the injection of the tracers 105 m³ of water was used (~4 h with a flow rate of 6.9 L s^{-1}) to temporarily obtain near saturated conditions along the flow path in the vadose zone. Shortly before injection the tracers (30 g of caffeine and 500 g of uranine) were dissolved in 1 m³ of tap water. The tracer injection was followed by 81 m³ of water to flush

the sinkhole over a period of ca. 3.5 h with a flow rate of 6.5 L s^{-1} to force the injected tracer cocktail through the unsaturated into the saturated zone.

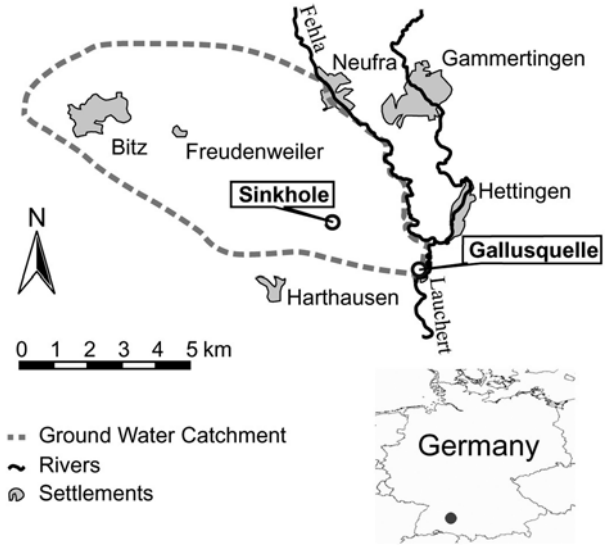

Figure 4.1. Catchment area of the Gallusquelle spring. The sinkhole for the injection of the tracers is located at a distance of 3000 m from the spring (from Birk et al., 2005).

Uranine was simultaneously injected with caffeine since (i) uranine can easily be monitored online by a fluorometer providing an indication for the times when samples for the analysis of the caffeine concentrations need to be taken and (ii) uranine serves as a conservative reference tracer, i. e. it is neither retarded nor degraded (Geyer et al., 2007), to quantify the potential mass loss of caffeine.

4.2.2 Sampling

The uranine concentration was monitored over a period of 16 days with the field spectrofluorometer GGUN-FL30 (excitation: 470 nm, detection: wratten orange filter). The measuring interval was initially set to 10 min, and decreased to 1 min during tracer breakthrough (TBC). The detection limit for uranine in the investigated spring water is stated to be 0.02 µg L^{-1} (Geyer et al., 2007). As quantification limit, a threefold detection limit of 0.06 µg L^{-1} was assumed in this study. Concentrations below this value were set to zero. For the calibration of the device three calibration levels were prepared by subsequently diluting a uranine stock solution (1 mg L^{-1}) with water from the Gallusquelle spring. The calibration levels were 1, 10 and 100 µg L^{-1}. As no natural recharge occurred for the duration of the tracer test (no changes in turbidity) and the calibration was performed with spring water, interferences with fluorescent humic substances can be excluded to affect the quantification of uranine.

Water samples to be analyzed for caffeine and selected metabolites were taken over a period of 7 days. In total 93 spring water samples were taken. The sampling interval for

caffeine varied between several hours and 10 min for the time of the increasing limb of the TBC, achieving a high temporal resolution of the caffeine TBC. The water samples were preconcentrated within a few hours (< 8 h) after sampling by solid phase extraction (SPE) as described in section 2.3.2. The volume of the spring water samples varied between 500, 250 and 200 mL depending on the expected caffeine concentration, estimated from the measured uranine concentrations.

The electrical conductivity and the turbidity of the spring water were monitored every 20 min by a pre-installed multi-parameter probe system. The discharge of the Gallusquelle spring was acquired from a spring gauging station.

4.2.3 Laboratory analysis

4.2.3.1 Chemicals

Methanol (LC/MS grade) and caffeine were purchased from Fisher Scientific (Schwerte, Germany), ethyl acetate and ammonium acetate (all analytical grade) were purchased from VWR (Darmstadt, Germany). Paraxanthine, paraxanthine-D6, theobromine, theophylline, 1-methylxanthine and 3-methylxanthine were obtained from Sigma Aldrich (Steinheim, Germany). Uranine was purchased from Acros Organics (Geel, Belgium).

4.2.3.2 Analysis of caffeine and its metabolites

An analytical method based on SPE and high-performance liquid chromatographic separation with tandem mass spectrometric detection (HPLC/MS-MS) was used for the analysis of caffeine and its metabolites paraxanthine, theobromine, theophylline, 1-methylxanthine and 3-methylxanthine. Details were published previously (Nödler et al., 2010). In brief, 500 mL of sample volume was buffered at neutral pH (phosphate buffer) and extracted by SPE (500 mg OASIS HLB; Waters, Eschborn, Germany). Samples of smaller volume than 500 mL were filled up with ultrapure water. Prior to extraction, 400 ng of paraxanthine-D6 was added as internal standard for the quantification of the analytes.

After extraction the sorbent was rinsed with ultrapure water and dried by drawing air through the cartridges under vacuum. The cartridges were wrapped in aluminium foil and kept frozen (–18 °C) until analysis. The analytes were eluted with methanol and ethyl acetate, successively. The solvents were evaporated and the dry residue was re-dissolved in 1 mL of an aqueous 5 mM ammonium acetate solution, containing 4% methanol. The method quantification limits (MQL) of the analyzed substances were: 4.3 ng L^{-1} (caffeine), 3.2 ng L^{-1} (paraxanthine), 5.1 ng L^{-1} (theobromine), 3.4 ng L^{-1} (theophylline), 21 ng L^{-1} (1-methylxanthine) and 28 ng L^{-1} (3-methylxanthine).

Recovery rates for caffeine were determined by the extraction of 500 mL of the original spring water spiked at levels of 100 and 1000 ng L^{-1}. The results were 109% (± 0.6%) and

100% (± 6.6%), respectively. The influence of uranine on the quantification of caffeine was investigated by analyzing 500 mL of spring water spiked with 1000 ng L^{-1} caffeine and 30,000 ng L^{-1} uranine. No significant influence on the recovery rate of caffeine was observed. All experiments on recovery rates were conducted in duplicates.

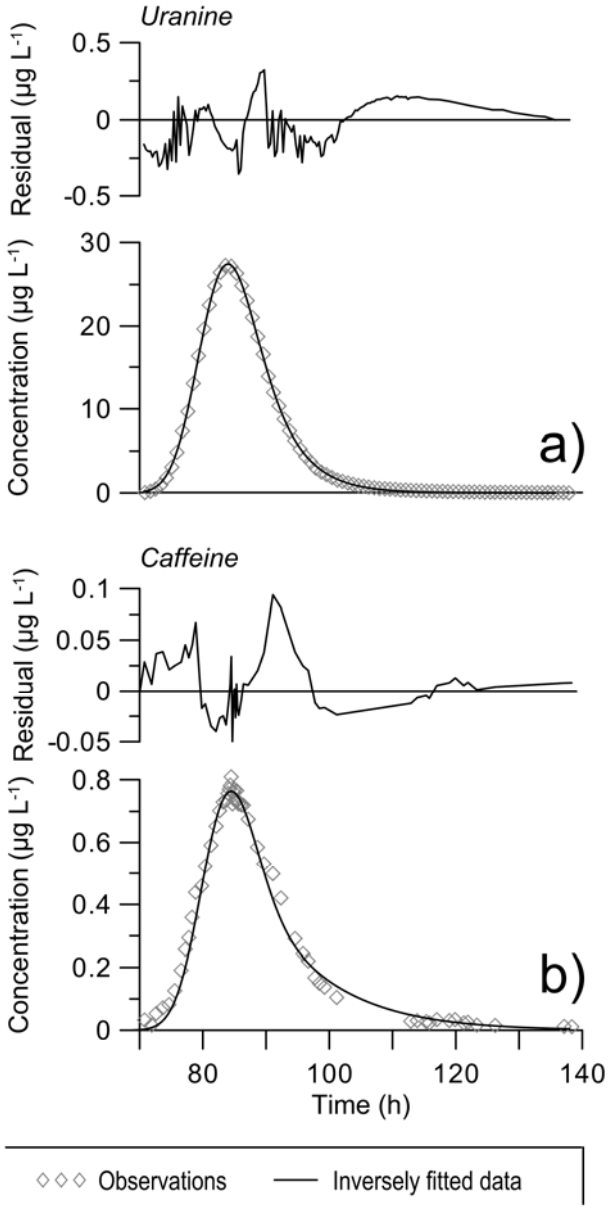

Figure 4.2. Tracer breakthrough curves of uranine (a) and caffeine (b) with their respective fitted models and residuals. For the graphical illustration of the uranine breakthrough only every 50th observation point is displayed.

4.2.4 Modelling

Birk et al. (2005) demonstrated that a simple advection-dispersion model (ADM) fails to reproduce the tailing of TBCs in the investigated karst aquifer. In order to achieve a better model fit and reliably interpret the TBCs of uranine and caffeine, the suggested non-equilibrium ADM was applied for TBC interpretation: CXTFIT 2.0 (Toride et al., 1995) was

used as part of STANMOD (Simunek et al., 1999). The CXTFIT 2.0 code implements a uniaxial, two-region non-equilibrium transport model. Field and Pinsky (2000) introduced the application of two-region non-equilibrium transport models to analyze large scale artificial tracer tests in karst aquifers. The approach considers the fluid in a karst conduit as divided into a mobile and immobile (stagnant relative to the direction of flow) fluid region, described previously (Field and Pinsky, 2000; Hauns et al., 2001; Geyer et al., 2007). Thus immobile fluid regions are characterized by higher residence times, as the water is not displaced by plug flow. Possible immobile fluid regions are vortices and eddies resulting from irregular cross sections of the conduits. As input function for the model a pulse input was used, i. e. the input duration was assumed to be negligible in comparison to the total duration of the tracer test.

Solute transport processes considered in this study include advection, dispersion, mass transfer between the two fluid regions (mobile and immobile), reversible sorption and tracer attenuation. The analytical equations for the one-dimensional, two-region non-equilibrium model are given as follows (modified from van Genuchten and Wagenet, 1989):

$$\beta R \frac{\partial^2 c_m}{\partial t} = D \frac{\partial^2 c_m}{\partial x^2} - v \frac{\partial c_m}{\partial x} - \alpha (c_m - c_{im}) - \beta R \mu_1 c_m \qquad \text{Eq. 4.1}$$

$$(1-\beta) R \frac{\partial c_{im}}{\partial t} = \alpha (c_m - c_{im}) - (1-\beta) R \mu_2 c_{im} \qquad \text{Eq. 4.2}$$

with the retardation coefficient, defined as:

$$R = 1 + \frac{A}{V} K_a \qquad \text{Eq. 4.3}$$

for non-porous matrix blocks. β, the solute partitioning coefficient between mobile and immobile fluid regions is given as:

$$\beta = \frac{\theta_m + f(R-1)}{R} \qquad \text{Eq. 4.4}$$

t is time, x is the space coordinate, D is the dispersion coefficient, v is the average flow velocity, α is a first-order mass transfer coefficient between mobile and immobile fluid regions. c_m and c_{im} are the solute concentrations in, μ_1 and μ_2 are first-order attenuation rates within the mobile and immobile fluid region respectively. In this study a uniform attenuation rate in the mobile and immobile region was considered ($\mu_1=\mu_2=\mu$). θ_m is the volumetric fraction of the mobile fluid region, while $\theta_m+ \theta_{im}=\theta=1$ for a fully saturated conduit, θ_{im} being the volumetric fraction of the immobile fluid region. A/V represents the surface to volume ratio of a karst conduit, K_a is the linear distribution coefficient defined as the ratio of tracer mass per unit surface area of the solid phase to the unit concentration of the tracer

within the conduit. The parameter f refers to the fraction of reversible adsorption sites that equilibrates with the mobile liquid phase. The retardation coefficient R captures the retardation of unpolar sorption as well as from reversible polar interactions as shown by Geyer et al. (2007). Rearranging Eq. 4.4 and inserting physically reasonable values for f (between 0 and 1) allows to constrain β (Geyer et al., 2007):

$$\frac{\theta_m}{R} \leq \beta \leq 1 - \frac{\theta_{im}}{R} \qquad \text{Eq. 4.5}$$

To reliably interpret TBCs of reactive tracers a step-wise calibration strategy can be applied (Geyer et al., 2007). Fitting the TBC of a conservative tracer yields estimates for the parameters v, D, α and θ_m. The application of uranine as conservative tracer in karst hydrology has been shown in several large scale field studies (Birk et al., 2005; Geyer et al., 2007). Conservative transport parameters can be assumed to be equal for conservative and reactive solute tracers (Geyer et al., 2007). Consequently, the calibration of the reactive transport model is reduced to the transport parameters R, β and the decay coefficient μ if a conservative reference tracer is applied simultaneously.

As transport distance, the linear distance of 3000 m between the injection-point and the Gallusquelle spring was used. The initial values for v and D for the calibration of the model were derived from the method of moments, using the software QTRACER (U.S. EPA, 2002). Estimates for α are not generally possible and the initial value for θ_m was obtained from the ratio of the mean tracer velocity and the peak tracer velocity (modified from Goltz and Roberts, 1988; Field and Pinsky, 2000).

4.3 Results and discussion

Precipitation events and associated infiltration can have an impact on the spring discharge and the flow regime in the aquifer, because they impose a temporally variable discharge rate. Therefore, the interpretation of TBCs becomes considerably more complex, since the mass flux of uranine and caffeine are calculated based on the spring discharge. To avoid these complications, the tracer test was performed during a dry spring recession period. During the whole investigation period spring discharge was relatively constant at ca. 175–200 L s^{-1}. Turbidity and electrical conductivity measurements were stable at 0.12 FNU and 650 μS cm^{-1}, demonstrating the absence of disrupting recharge events.

Background effects with respect to caffeine in the spring water originating from wastewater infiltration can be excluded for the tracer test. The caffeine concentrations are comparatively small (Hillebrand et al., 2012) and the wastewater infiltration does not occur evenly distributed over time, but simultaneously to precipitation events (Musolff et al., 2010), which were absent for the duration of the tracer test.

The mass recovery of injected uranine was found to be 49% (246 g). The mass loss of uranine was likely to be caused by groundwater discharge below the gauging station. Geyer et al. (2007) stated for the same catchment area that the proportion of mass loss increases with lower discharge. The observations of this study emphasize this finding.

The recovered mass of injected caffeine was only 27%, indicating an additional mass loss in comparison to uranine, i. e. caffeine shows reactive transport behaviour in the investigated aquifer system. Furthermore, caffeine exhibited a longer tailing (Figure 4.2). While the recovery of the total uranine mass was achieved after 127.5 h, caffeine concentrations took 164 h before dropping below the limit of quantification after the tracer peak. This may be attributed to the lower limit of quantification for caffeine. However, the lower recovered mass and the smaller peak indicate a significant mass loss relative to the conservative tracer uranine. Irreversible sorption is unlikely to occur since caffeine is highly soluble (Gardinali and Zhao, 2002) and has a negative (−0.07; Maeng et al., 2011) log K_{ow} (octanol-water partitioning coefficient). Several authors emphasize the degradability of caffeine and thus being the main process in its attenuation especially in treatment plants, but also in the environment. Buerge et al. (2003; 2006) calculated biodegradation rates of caffeine to be in the order of 0.003 to 0.006 d^{-1} in a lake. Swartz et al. (2006) observed caffeine degradation in a porous aquifer at a rate of 0.07 to 0.014 d^{-1}.

The metabolites of caffeine considered in the analytical method could only be found sporadically and at insignificant levels. The metabolites were either not detectable due to the high dilution, not produced during degradation, or metabolized further at a higher rate than caffeine. The latter is consistent with findings from laboratory experiments (Kurtzman and Schwimmer, 1971; Mazzafera et al. 1996). Moreover, Wainwright et al. (1993) stated the production of primary or secondary metabolites to be unlikely for oligocarbotrophs. Due to the high discharge, a decrease of oxygen concentrations or changes of the redox potential in the spring water cannot be resolved.

In the model a general attenuation rate is determined, which is comprised of all possible mechanisms for the additional mass loss (e. g. degradation, irreversible sorption) of caffeine relative to the conservative reference tracer uranine.

4.3.1 Modelling results

Calibrating the model (using the TBC of uranine) resulted in a very good agreement between observed and fitted concentrations (Figure 4.2). The flow velocity v, dispersion coefficient D, volumetric fraction of the mobile fluid region θ_m as well as the mass transfer coefficient α (Table 4.1) are in good agreement with the results of a previously conducted study by Geyer et al. (2007).

In the model for the caffeine TBC the attenuation rate μ as well as solute-specific values for the retardation coefficient R and the partitioning coefficient β are considered additionally. Figure 4.2 and Table 4.1 show the estimated parameters for uranine and caffeine.

The mass loss of caffeine was modelled by an attenuation rate of 0.0067 h^{-1} (i. e. a half-life of 104 h). This rate is surprisingly high in comparison to degradation values from the literature observed in a lake and a porous aquifer environment (Buerge et al., 2003; 2006; Swartz et al., 2006). The estimations of these authors for the half-life of caffeine range from weeks to months. In general it is assumed that bacteria are associated with sediment and rock surfaces (Holm et al., 1992). For karst aquifers the attenuation rate of caffeine was expected to be lower than the attenuation rate within the porous aquifer, as the contact area of water to the solid matrix, implying a reduced bacteria count for karst aquifers and less reactive interfaces. The relatively high attenuation rate may be related to the influence of wastewater leakage and the redox condition in the subsurface, as proposed by Bradley et al. (2007).

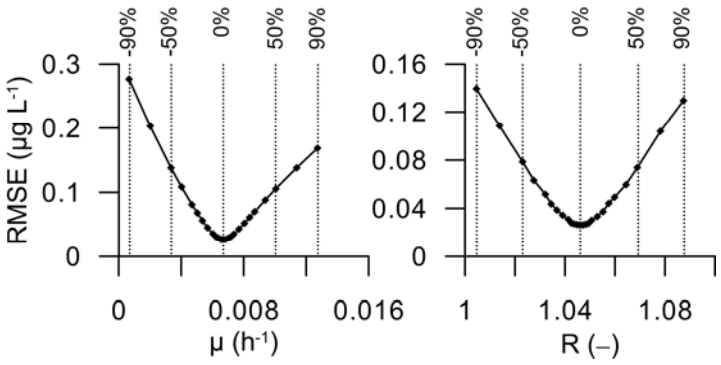

Figure 4.3. Results of the sensitivity analysis for the parameters μ and R. The values were obtained from variations of parameters in the model for caffeine transport. Please note the different scales of the ordinates. μ= attenuation rate, R= retardation coefficient. The percentages above the graphs indicate the magnitude of variation of each parameter.

The literature values on *in-situ* degradation of caffeine mentioned above refer to sub-oxic to anoxic conditions (Swartz et al., 2006) and to conditions with low oxygen (Buerge et al., 2003; 2006). In the investigated aquifer oxic conditions prevail (data not shown). The increased degradability of caffeine under oxic conditions has been emphasized by Bradley et al. (2007). Furthermore, the investigated aquifer is known to be affected by wastewater leakage (Hillebrand et al., 2012; Nödler et al., 2012) and the periodical occurrence of overflow events of a wastewater retention basin (Heinz et al., 2009). With the percolating wastewater caffeine is introduced into the aquifer. The regular exposition of the aquifer bacteria to wastewater and therefore to caffeine may result in an adaption of the bacteria to caffeine or rather wastewater related micropollutants in general. This scenario and to a larger extent the sufficiently provided oxygen in the aquifer may explain the effective attenuation of caffeine observed during the tracer experiment. Moreover it is possible that the flow through the unsaturated zone affected the determined attenuation rates.

It has to be emphasized here that the attenuation rate determined for caffeine is an integrated value. No statements with respect to the temporal and spatial distribution can be made. The attenuation along the flow path may have occurred uniformly or at different rates.

Table 4.1. Parameter estimates for a uniaxial, two-region nonequilibrium model to observed tracer breakthrough curves.

Tracer	Uranine	Caffeine
v (m h–1)	34.9	34.9
D (m2 h–1)	135.2	135.2
α (10–3 h–1)	8.91	8.91
β (–)	0.9683	0.9340
R (–)	1	1.046
μ (h–1); T1/2 (h)	0	0.0067; 104
r^2	0.9997	0.9924
RMSE (μg L–1)	0.487	0.027
mm (g)	246	14.8
m (g)	500	30

v= average flow velocity; D= dispersion coefficient; α= mass transfer coefficient; β= partitioning coefficient between mobile and immobile fluid regions; R= retardation coefficient; r^2= coefficient of determination; μ= first order attenuation rate; T1/2=half life; RMSE= root mean square error; mm= tracer injection mass used in the model; m= tracer mass injected into the sinkhole.
Note: values in italics represent fitted values, while underlined values are prescribed values.

A slight shift of the caffeine TBC peak was taken into account by a retardation coefficient of 1.046. This value refers to the best fit of the model and may be affected by the scattering of the measured caffeine concentrations at the peak maximum. If the retardation is due to unpolar or polar interactions with organic carbon or the aquifer material cannot be determined.

From the observed mass loss of caffeine relative to the conservative reference tracer uranine, an attenuation capacity of the aquifer along the flow path of the tracers can be deduced. The high attenuation rate highlights the potential of caffeine as groundwater tracer to indicate the natural attenuation potential even in rapid flowing systems. Due to its low limit of quantification, very little amounts of caffeine can be used while still producing a pronounced TBC. Together with the fluorometrically detectable uranine an inexpensive and environmentally benign method for the indication of the *in-situ* attenuation potential along the tracer flow path is presented. In contrast to laboratory experiments, this method determines the natural attenuation potential and the risk posed by micro-contaminants in aquifers *in-situ*. The complexity of the system is captured and considered by lumped parameters, i. e. spatially averaged values across the length of the whole flow path.

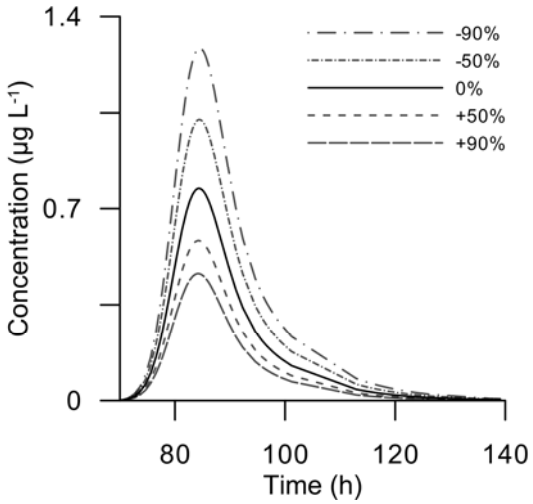

Figure 4.4. Effect of varying the attenuation rate µ in the model for caffeine. The higher the attenuation rate, the lower the peak and vice versa. The shape of the tracer breakthrough curve is not affected by the variation of the attenuation rate.

4.3.2 Sensitivity analysis

By varying single parameters and comparing the root mean square errors (RMSE), the sensitivity of the modelled concentrations to each parameter was assessed. Geyer et al. (2007) discussed the sensitivity of the model concentrations with respect to the parameters v, D, θ_m, α and β. The sensitivity of the model to the parameters µ and R were evaluated for caffeine (Figure 4.3). The parameter R is investigated, varying (R^{-1}) instead of R, since the difference to 1 quantifies the shift of the TBC. The effect of varying the attenuation rate µ rate is illustrated in Figure 4.4. The higher the attenuation rate, the smaller is the peak and vice versa. Except for a shorter tailing for high attenuation rates, the shape of the TBC is not affected by changes in the attenuation rate.

4.3.3 Implication

The determined attenuation rates from the large scale artificial dualtracer test could be used to improve the estimation of wastewater volumes infiltrating the aquifer within the catchment area (Hillebrand et al., 2012). In that study wastewater volumes appearing in the spring water were quantified, employing caffeine concentrations. An attenuation of caffeine between source and spring was neglected as intrinsic attenuation data were missing. The mean rate of infiltrating wastewater was determined to be 2.2 ± 0.5 $m^3\ d^{-1}$. Taking the here presented results into account, the impact of caffeine attenuation should be included in the wastewater impact estimation. Extending the formula stated by Hillebrand et al. (2012) with a first-order attenuation term leads to:

$$WW = \frac{c \cdot e^{\mu \cdot t} \cdot WC \cdot Q}{I} \qquad \text{Eq. 4.6}$$

where WW is the volume of wastewater discharging at the spring per day; c the caffeine concentration at the spring; μ the first-order attenuation rate (0.0067 h^{-1}); the mean residence time of wastewater in the subsurface t (115 ± 20 h); the daily water consumption per capita in the spring catchment WC (134 L d^{-1} $person^{-1}$); spring discharge Q and the load of caffeine in untreated wastewater I (15.8 ± 3.8 mg d^{-1} $person^{-1}$; Buerge et al., 2003). A mean wastewater infiltration rate of 4.7 ± 1.4 m^3 d^{-1} could be calculated. The temporal distribution is shown in Figure 4.5.

The sensitivity of the wastewater estimation method is affected by the effective attenuation of caffeine as well. Considering the method quantification limit of caffeine (4.3 ng L^{-1}) and a mean spring discharge of 0.5 m^3 s^{-1} the minimum volume of wastewater, which can be quantified is 3.4 ± 1.0 m^3 d^{-1}.

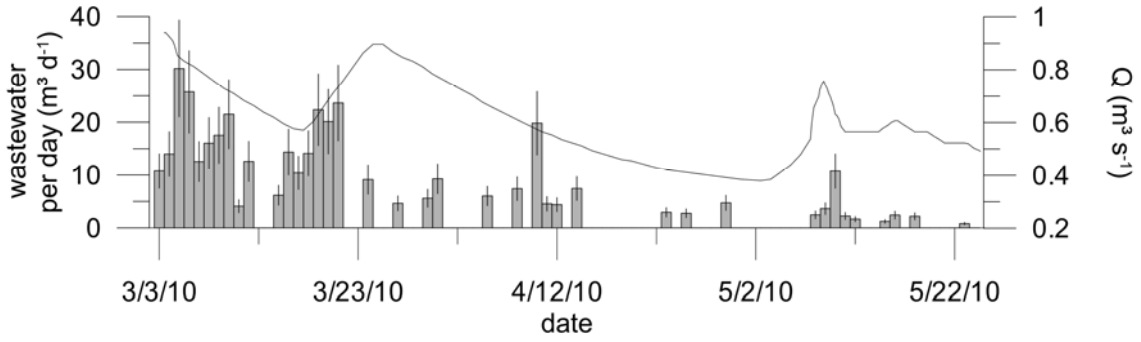

Figure 4.5. Calculated volumes of wastewater at the investigated spring under consideration of the determined first-order attenuation term. Adapted from Hillebrand et al. (2012).

4.4 Conclusion

- A methodology to identify the attenuation potential of a karst aquifer is presented employing a dualtracer test with uranine and the reactive indicator caffeine.
- Surprisingly high attenuation rates for caffeine indicate a higher attenuation potential of the investigated karst aquifer than expected.
- To identify reactive transport and potential attenuation, the use of a conservative reference tracer (e. g. uranine) is a prerequisite.
- The application of uranine and caffeine during a dualtracer experiment is an inexpensive and environmentally benign approach for the assessment of the *in-situ* attenuation potential even in rapidly flowing groundwater systems.

Acknowledgment

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Chapter 5

5 Multitracer experiment to evaluate the attenuation of selected micropollutants in a karst aquifer

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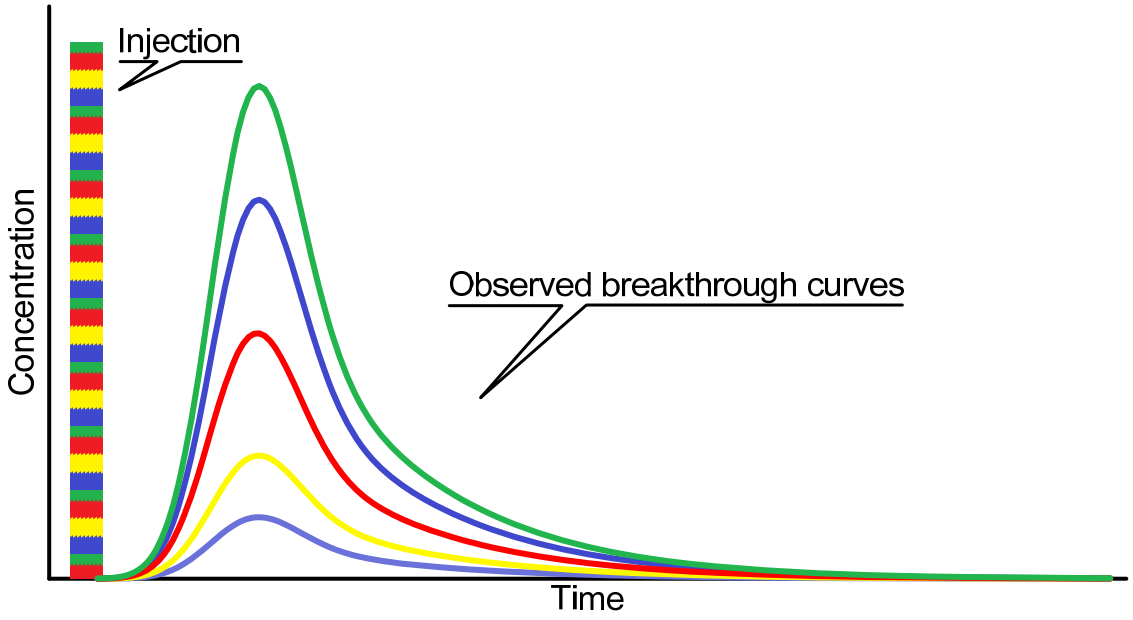

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Abstract

The increasing pressure on drinking water resources urges for the successful management of potential and actual drinking water bodies. Karst aquifers may play a key role supplying the world's population with drinking water. Yet around one quarter of all drinking water is produced from these formations. Unfortunately these rapid systems are prone to contamination. For a successful management, a fundamental understanding concerning the transport and attenuation of possible contaminants is vital. In the here presented study, a multitracer experiment was performed for determining the attenuation potential of a karst environment by assessing the environmental fate of selected relevant micropollutants. Uranine, acesulfame and carbamazepine were injected into a sinkhole as reference tracers together with the reactive compounds atenolol, caffeine, cyclamate, ibuprofen and paracetamol (also known as acetaminophen). The arrival of the tracers was monitored at a karst spring in a distance of 3 km. The breakthrough curves of the reactive compounds were interpreted relative to the reference substances. No significant retardation was found for any of the investigated micropollutants. The determined half-lives of the reactive compounds range from 38 to 1400 h (i. e. persistent within the investigation period) in the following order (from high to no observed attenuation): paracetamol > atenolol ≈ ibuprofen > caffeine >> cyclamate. The attenuation rates are generally in agreement with studies from other environmental compartments. The occurrence of the biotransformation product atenolol acid served as evidence for the occurrence of *in-situ* biotransformation within the aquifer system. The results of this study highlight the yet underestimated attenuation potential of karst aquifers.

5.1 Introduction

The necessity of clean water for mankind and life in general reached the public awareness at least since the UN declared the access to clean water a human right (U.N. General Assembly, 2010). This resolution inhibits not only the need of tapping safe water resources where necessary but to maintain the state of cleanliness of potential and actual drinking water resources as well. In western countries the supply with clean drinking water is not yet a problem. However, river as well as ground water has been registered to be affected by micropollutants (Hughes et al., 2013; Loos et al., 2009; Loos et al., 2010), reaching the aquatic environment over direct input of untreated wastewater (Kuroda et al., 2012; Musolff et al., 2010) or from incomplete elimination in wastewater treatment plants (WWTPs; Loos et al., 2013; Joss et al., 2006).

Sorption and degradation were identified as significant mechanisms for the attenuation of micropollutants in the environment (Huntscha et al., 2013; Kunkel und Radke, 2012; Nakada et al., 2008). Often these parameters are assessed indirectly from empirical correlations (Franco and Trapp, 2008; Sabljic et al., 1995) or from laboratory experiments (Radjenovic et al., 2008; Schaffer et al., 2012a; Scheytt et al., 2005). However, the transfer of the results to natural environments is known to be difficult and defective (Buerge et al., 2011) and the most accurate determination is to be found in the environment of interest itself, i. e. *in-situ* (Hillebrand et al., 2012a; Kunkel and Radke, 2011).

As karst aquifer systems supply up to 25% of the world's population with drinking water (Ford and Williams, 2007), the understanding of contaminant transport and fate in these systems is of vital importance. Due to their specific characteristics and high transport velocities, karst aquifers are particularly prone to contamination. Consequently, the vulnerability of karst aquifers is often estimated from residence time distributions (Einsiedl et al., 2009; Ozyurt, 2008). However, employing a dualtracer experiment with caffeine (CAF), Hillebrand et al. (2012a) demonstrated that even a low residence time within a karst aquifer system can lead to significant attenuation of selected contaminants. Yet, no

statement could be made, if biodegradation or any other process was responsible for the observed attenuation.

The aim of this study was to remedy this shortcoming, verify the reproducibility of the attenuation of caffeine in a karst environment and examine the transferability of the results to other compounds by a multitracer experiment. Furthermore, the attenuation potential of karst aquifers should be evaluated by investigating the attenuation of caffeine, cyclamate (CYC), ibuprofen (IBU) and paracetamol (PAC, acetaminophen), which are encountered in high concentrations in wastewater (Lange et al., 2012; own unpublished results from investigations in the study area). Additionally to these 4 substances, the fate of atenolol (ATE) was investigated to detect possible biotransformation, as it is known to be biologically transformed to atenolol acid (AAC), which is a biologically recalcitrant transformation product (TP) (Radjenovic et al., 2008). For reference purposes, the stable compounds acesulfame (ACE) (Scheurer et al., 2011) and carbamazepine (CBZ) (Clara et al., 2004) as well as uranine were included in the list of tracers.

5.2 Experimental section

5.2.1 Chemicals

Acesulfame potassium, atenolol and carbamazepine were purchased from TCI Europe (Zwijndrecht, Belgium), atenolol acid from LGC Promochem (Wesel, Germany), caffeine, paraxanthine, sodium cyclamate, ibuprofen sodium, theobromine, theophylline, 1-methylxanthine and 3-methylxanthine from Sigma-Aldrich (Steinheim, Germany), paracetamol from Fagron (Barsbüttel, Germany), and uranine from Acros Organics (Geel, Belgium).

The internal standards (IS) paraxanthine-D_6, theobromine-D_6, caffeine-D_9, atenolol-D_7, and ibuprofen-D_3 were purchased from Sigma-Aldrich, (Steinheim, Germany). Acesulfame-D_4 was from Campro Scientific (Berlin, Germany). Carbamazepine-D_{10}, cyclamate-D_{11}, and paracetamol-D_4 were purchased from LGC Promochem (Wesel, Germany). An IS-mix with 25 ng μL^{-1} of each substance was prepared in acetonitrile.

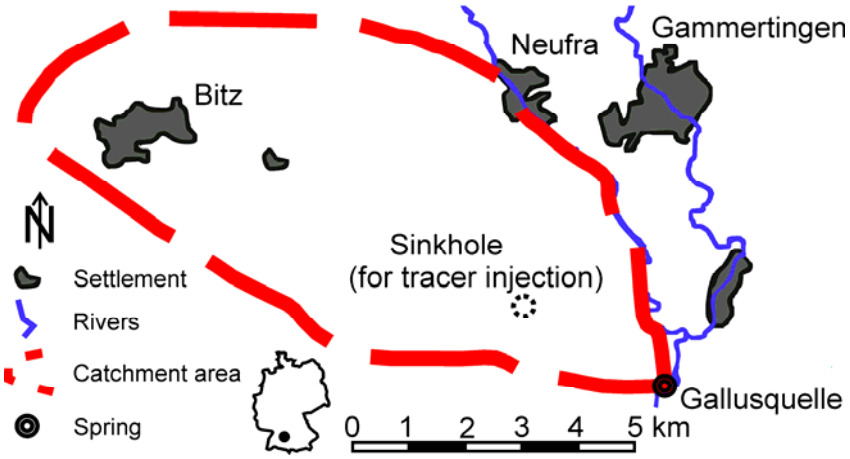

Figure 5.1. Catchment of the Gallusquelle spring. The tracers were injected into a sinkhole in a linear distance of 3 km to the sampling point (Gallusquelle; modified from Hillebrand et al., 2014).

5.2.2 Multitracer experiment

The tracer experiment was carried out at the Gallusquelle, a karst spring in SW-Germany (Figure 5.1). Its average annual discharge is 500 L s^{-1} (100–2500 L s^{-1}), draining a catchment of 45 km². The spring water is used for drinking water production. The aquifer system and spring water quality is known to be affected by irregular leakage of untreated wastewater (Hillebrand et al., 2012b; Nödler et al., 2012).

For tracer injection a sinkhole at a distance of 3 km to the spring was used. The point-to-point connection of the sinkhole with the spring has been demonstrated in previous studies (Geyer et al., 2007; Hillebrand et al., 2012a). As the unsaturated zone has a thickness of 100 m (Geyer et al., 2007), flushing of the sinkhole before and after the injection of the tracers was necessary. For flushing, 88 m³ of tap water were used respectively (~3.5 h). One hour before injection the tracers were dissolved in a container with ~850 L of drinking water. The injected masses of the tracers are shown in Table 5.1. The injection of the tracers took 15 minutes. The container was rinsed thoroughly with drinking water.

Table 5.1. Injected and recovered masses of all analytes.

Tracer	Uranine	ACE	CYC	CBZ	ATE	CAF	IBU	PAC
Injected mass (g)	700.0	24.3	26.7	10.0	30.0	30.0	30.0	30.0
Recovered mass (%)	66.2	67.8	63.8	61.2	30.6	41.1	31.3	17.6
Recovered mass (%)[a]	100	100	94.1	100	50	67.2	51.1	28.8

[a]relative to the respective reference tracer.

Uranine was used as a conservative tracer to detect possible retardation of any of the micropollutants. The concentration of uranine was monitored online with the field spectrofluorometer GGUN-FL30 (excitation: 470 nm, detection: wratten orange filter; limit of quantification: 100 ng L^{-1}). Spring water temperature (T), turbidity and electrical conductivity (EC) were monitored by a multiparameter probe at an interval of 20 minutes. The spring discharge was obtained from water level readings and transformed to discharge applying a rating curve. The spring discharge (275 L s^{-1}), T, and the EC were constant during the experiment. The turbidity exhibited a peak prior to the arrival of the tracers, which was likely caused by the flushing of the sinkhole. However, as the turbidity was very low (0.03–0.12 NTU), there was no impact on the detection of uranine.

For the analysis of the micropollutants, spring water was sampled in 0.5 L bottles (clear glass, screw cap). The sampling frequency was adjusted to the uranine reading of the fluorometer. During the main breakthrough (from the beginning to 94% of the total recovered uranine mass) samples were collected every 0.5 h. Afterwards, the sampling frequency was gradually decreased to 1 sample every 4, 8, and later on 12 h. In total, 76 samples were collected.

5.2.3 Solid phase extraction (SPE) and analysis of micropollutants

For the extraction 500 mL of sample was spiked with 10 µL of the IS-mix and 5 mL of a phosphate buffer concentrate (neutral pH; Nödler et al., 2010). The micropollutants were extracted by using the stacked-cartridges-approach (combination of OASIS HLB and WAX) as described by Nödler et al. (2013). The artificial sweeteners were extracted by the WAX sorbent whereas all other micropollutants were retained on the HLB sorbent material. The extraction was performed within 12 h after sampling and the dried SPE cartridges were frozen (−18 °C) until analysis. This method was proved to be an adequate approach regarding sample stabilisation (Hillebrand et al., 2013).

Prior to analysis, ACE and CYC were eluted according to Nödler et al. (2013). All other analytes were eluted according to Nödler et al. (2010). Detection and quantification of the analytes were conducted by high-performance liquid chromatography combined with electrospray ionisation and tandem mass-spectrometry (HPLC-ESI-MS/MS). Two different

analytical methods were applied: Method 1 for ATE, CAF, CBZ, IBU, PAC, the transformation products (TPs) 1-methylxanthine, 3-methylxanthine, atenolol acid (AAC), paraxanthine, theobromine, and theophylline; method 2 for ACE and CYC. Details regarding instrumentation, chromatographic conditions, and MS/MS-parameters can be found in the appendix (Appendix A.3).

For the calibration artificial samples containing all analytes were prepared in ultrapure water and processed according the spring water samples (i. e. including the extraction process). Seven calibration levels were prepared covering concentration ranges of 10–2000 ng L^{-1} for ACE, CAF, IBU, ATE, CYC, and PAC, 5–1000 ng L^{-1} for CBZ, and 2–400 ng L^{-1} for the TPs. The correlation coefficients for all compounds exceeded 0.99 and the relative standard deviation (RSD) was < 5%.

5.2.4 Quality assurance

A small aliquot (1 mL) of the injected tracer solution was diluted with spring water according to the linear range of the methods. This sample of the initial concentration (c_0) was extracted and analysed as previously described. To determine the exact volume of tracer solution and thus the dilution factor and corresponding concentrations of the injected micropollutants, uranine was analysed directly with the fluorometer. Except for ATE (109%) the recoveries of all injected micropollutants in the c_0 were 98–104%.

5.2.5 Calculations and modeling

The recovered masses of all investigated analytes have been determined by integration applying the trapezoidal rule.

For breakthrough-curve (BTC) interpretation a uniaxial two-region non-equilibrium advection dispersion model has been employed with CXTFit 2.0 (Toride et al., 1995). The analytical equations for this model are given as follow (modified from van Genuchten and Wagenet, 1989):

$$\beta R \frac{\partial c_m}{\partial t} = D \frac{\partial^2 c_m}{\partial x^2} - v \frac{\partial c_m}{\partial x} - \alpha (c_m - c_{im}) - \beta R \mu_1 c_m \quad \text{Eq. 5.1}$$

$$(1-\beta) R \frac{\partial c_{im}}{\partial t} = \alpha (c_m - c_{im}) - (1-\beta) R \mu_2 c_{im} \quad \text{Eq. 5.2}$$

with x being the space coordinate, t is the time, c_m and c_{im} are the solute concentrations in the mobile and immobile fluid region, μ_1 and μ_2 are first order attenuation rates in the two regions respectively. All other parameters are defined in Table 5.2. Details on the model can be found in former studies (Field and Pinsky 2000; Geyer et al., 2007; Hauns et al., 2001; Hillebrand et al., 2012a) or in the appendix (Appendix A.4).

The tracer injection is implemented in the model as a pulse input, i. e. the duration of the injection is assumed to be negligible compared to the duration of the tracer experiment. For the interpretation of the BTCs of the reactive tracers, a step-wise calibration strategy was applied (Geyer et al., 2007; Hillebrand et al., 2012a): conservative transport parameters (e. g. transport velocity or dispersion) were determined from the BTCs of conservative reference tracers. ACE and CBZ are known for their persistency and conservative transport behaviour (Clara et al., 2004; Lam et al., 2004; Scheurer et al., 2011). They were used as reference substances for the remaining analytes of the two analytical methods: ACE served as reference for CYC; CBZ was used as reference for ATE, CAF, IBU, and PAC. These parameters were then kept constant for the interpretation concerning the attenuation rates of the reactive tracers. The injected mass of the reactive tracers was normalised to the recovery of the reference tracer (see Table 5.2). The recovered mass of the reference tracers was then set to be 100% (i. e. processes affecting all analytes, even the conservatives, are excluded from further interpretation). This procedure allows for the interpretation of reactive tracers, relative to conservative reference tracers.

As initial values for the fitting parameters, values from Hillebrand et al. (2012a) were used for uranine. For ACE and CBZ the results from the BTC of uranine were employed. The attenuation rate is considered to be identical for the mobile and immobile fluid region. The determined first order attenuation rate for the reactive tracers comprises all possible

mechanisms (e. g. degradation, hydrolysis, (irreversible) sorption) leading to a lower recovered mass than the respective reference tracer.

5.3 Results and discussion

5.3.1 Mass recoveries of reference tracers

The recovered masses of all analytes are shown in Table 5.1. The mass loss of uranine is likely to be related to discharge occurring below the gauging station (Geyer et al., 2007). From the monitored discharge and the recovered mass of uranine, this fraction can be calculated to be around 140 L s^{-1}, which is in good agreement with previous estimations (Sauter, 1992).

For the two reference tracers ACE and CBZ similar recoveries were observed. Please note, that for both reference substances background concentrations (18 ng L^{-1} for ACE, 3 ng L^{-1} for CBZ) existed. The monitored BTCs were corrected by subtracting the background concentrations. Due to the absence of recharge events, additional background effects can be excluded for any of the analytes (Hillebrand et al., 2012a).

5.3.2 Model results

The results from the modeling are illustrated in Table 5.2. For all BTCs correlation coefficients $R^2 > 0.96$ have been realised.

5.3.2.1 Retardation of the investigated micropollutants

Applying the model, the retardation coefficient R has never exceeded 1.01 for any analyte, although a significant retardation was observed for some of the tracers in experiments with sediment (Schaffer et al., 2012a; 2012b). Consequently, it has been set to 1 and kept constant during all subsequent fitting procedures. With R being kept constant the parameter β was kept constant as well, yet fitted for each analytical method separately.

Although a variety of substances has been used as tracers, the fitting of the BTCs revealed only minor variations (maximum ± 7%) for the investigated transport parameters (v, D, β, α and R). If only the transport behaviour is to be investigated, neglecting the attenuation of a

substance in a karst environment, a conservative reference tracer seems to yield sufficiently accurate estimations.

Table 5.2. Modeling results for all injected tracers.

Tracer	Uranine	ACE	CYC	CBZ	ATE	CAF	IBU	PAC
v (m h^{-1})	49.3	49.5	49.5	48.4	48.4	48.4	48.4	48.4
D (m^2 h^{-1})	218	209	209	233	233	233	233	233
β (–)	0.965	0.967	0.967	0.952	0.952	0.952	0.952	0.952
α (h^{-1})	0.0070	0.0076	0.0076	0.0067	0.0067	0.0067	0.0067	0.0067
$t_{1/2}$ (h)	–	–	1366	–	61.8	89.1	79.9	37.5
R^2 (–)	0.999	0.999	0.997	0.996	0.990	0.974	0.968	0.966
m_m (g)	463.4	16.4	18.0	6.2	18.7	18.7	18.7	18.7

v= average flow velocity; D=dispersion coefficient; β= partitioning coefficient between the mobile and immobile fluid region; α= mass transfer coefficient between the mobile and immobile fluid region; $t_{1/2}$= half-life; R^2= coefficient of determination; m_m= injected tracer mass used in the model (which equals the observed recovery for Uranine, ACE and CBZ).
Note: bold values are fitted, underlined values are prescribed values.

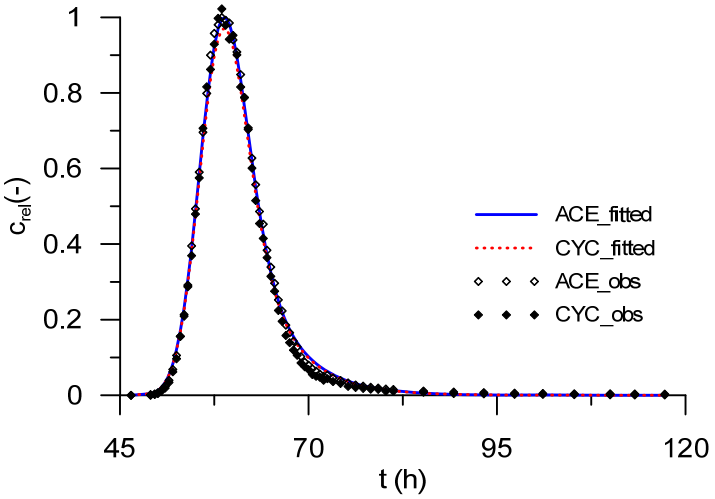

Figure 5.2. Observed and fitted BTCs for the artificial sweeteners ACE and CYC. The concentrations are normalised to the injected mass and the maximum concentration of ACE.

5.3.2.2 Attenuation of atenolol

ATE is known to be readily affected by biodegradation under aerobic (Radjenovic et al., 2008) conditions. In their study, AAC has been shown to be persistent under aerobic conditions for at least 25 days, allowing for a reliable estimation of the role of biotransformation in the attenuation process of ATE in karst. They performed batch experiments with activated sludge and different concentrations of ATE and observed 40–60% (i. e. <100%) of ATE to be metabolised to AAC. The observed half-life of ATE in that study was in the same order of magnitude as in the here presented study. In the investigated karst system, at least parts of the observed attenuation of ATE can clearly be attributed to biotransformation, as the TP AAC has been found simultaneously to the arrival

of ATE (Figure 5.3a). From the modelling results and mass-balance calculations 9.5% of the mass loss of ATE (relative to the reference tracer CBZ) can be directly attributed to biotransformation. The remaining 90.5% of the mass loss must be attributed to other biotransformation pathways or attenuation processes.

Please note that ATE is a chiral substance and the racemate was injected. Kasprzyk-Hordern and Baker (2012) observed stereoselective degradation of ATE leading to an enrichment of the R-form in wastewater treatment plants and the inverse, i. e. an enrichment of S-ATE, with the flow of a river. The observed attenuation rate of ATE in the here presented study must be considered as an average of possibly two independent attenuation rates. If one enantiomer is attenuated at a higher rate than the other, a decreasing attenuation rate is to be expected with time, approximating the lower of the two attenuation rates.

5.3.2.3 Attenuation of caffeine and cyclamate

The half-life of CAF found in this study corresponds well with the determined half-life from a previous study (89 vs. 104 h; Hillebrand et al., 2012a). The remarkable reproducibility of the results underlines the reliability and stability of the determined attenuation rates. Similarly to the study of Hillebrand et al., (2012a), none of the investigated transformation products (mono- and dimethylxanthines) were detected in spring water during the breakthrough of the tracers. This does not imply that biodegradation is not involved, but that a further distinction of the attenuation process cannot be made. Possible explanations for the absence of the selected transformation products involve (i) faster degradation rates of the transformation products than of the parent compound, (ii) transformation pathways that lead to TPs, which are not considered in the list of analytes. However, knowing for the possibility of biotransformation within the investigated karst system, that was inferred from the occurrence of AAC (see section 5.3.2.2), and the biotransformability of CAF (Benotti and Brownawell, 2009), it is likely that biodegradation is an important attenuation process for CAF in the investigated karst system.

Scheurer et al. (2009, 2010) observed rapid degradation of CYC during soil aquifer treatment and in fixed-bed bioreactors after a lag-phase of about one week. For the *in-situ*

attenuation in a karst aquifer generally a lower value should be expected. Considering the short residence time of the tracers within the subsurface during the performed tracer experiment, no attenuation of CYC was expected. In fact, fitting the observed BTC of CYC with the two-region model approach leads to a very high half-life (Table 5.2), which is about three times larger than for sterilised soil (Buerge et al., 2011). The lower recovered mass of CYC in comparison to ACE (Figure 5.2, Table 5.1) is therefore termed insignificant. The determined attenuation rate is likely to be unreliable as the residence time within the karst system was too low. Taking a possible lag-phase into account, an estimated travel time of several weeks would be required to assess the attenuation potential of karst aquifers for CYC by a tracer experiment.

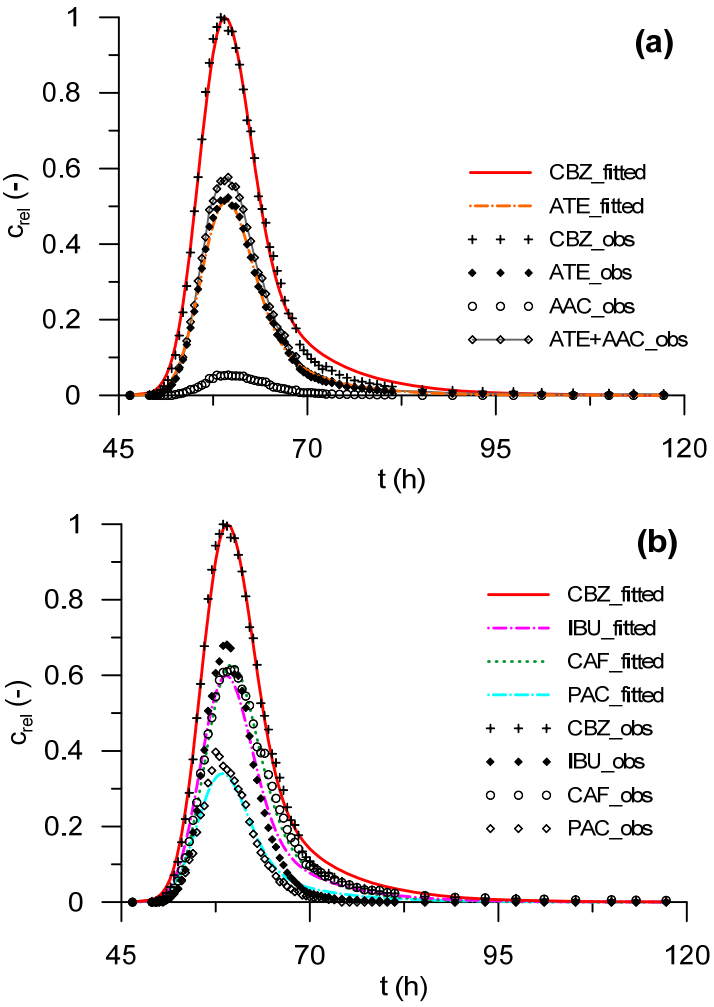

Figure 5.3. Observed and fitted BTCs for (a) CBZ, ATE and AAC and (b) CBZ, IBU, CAF and PAC. The concentrations are normalised to the injected mass of the analyte (for AAC the injected mass of ATE was used) and the maximum concentration of CBZ.

CAF and CYC have both been proposed to be used as indicators for untreated wastewater (Hillebrand et al., 2012b; Tran et al., 2014). However, CYC may be a more sensitive indicator as (i) its concentration in wastewater is similar to the concentration of CAF (Buerge et al., 2003; Lange et al., 2012;), (ii) it is degradable in wastewater treatment plants (Lange et al., 2012), (iii) a long-term stability is not to be expected (Lange et al., 2012) and (iv) on a short time-scale no significant attenuation occurs (this study). Yet, as a consequence of the higher stability of CYC, its detection does not necessarily indicate a recent input of wastewater to the investigated karst system. A combined application of CAF and CYC as indicators is therefore recommended, allowing for an unambiguous and sensitive detection of a contamination with untreated wastewater.

5.3.2.4 Attenuation of ibuprofen and paracetamol

For these two reactive tracers good attenuation rates have been observed. In a comparative study with diclofenac and IBU a lower recovery was observed for IBU than for diclofenac in a karst aquifer, which was explained by occurring but yet ineffective biodegradation (Einsiedl et al., 2010). In the here presented study the half-life of IBU within the investigated karst system was in the same order as of CAF. This low half-life surprised, as for an estuary and incubation laboratory experiments at 25 °C half-lives of 48–96 h have been reported (Nakada et al., 2008). The half-life determined for a karst aquifer in the here presented study is similar, although at a significantly lower ambient T of about 8 °C (Hillebrand et al., 2012b). This emphasises the potentially high attenuation rate that can occur within a karst aquifer system.

Similar to ATE the chirality of IBU must be considered. Poiger et al. (2003) observed stereoselective degradation, S-IBU being faster degraded than R-IBU in WWTPs. Hence, the observed attenuation rate for IBU must be considered as an average of possibly two independent attenuation rates.

The lowest half-life and therefore the highest attenuation rate of all analytes was observed for PAC. However, it appears to be nearly twice as persistent in the observed karst aquifer, than in aquatic outdoor field microcosms (with fish, aquatic plants, plankton and bacteria) (Lam et al., 2004). Whether the difference is related to a difference in the ambient T or to

differing fauna/flora can not be clarified with certainty in this study. Yet, it should be stressed that the low half-life in the observed karst aquifer is higher than in the above cited study, which is in agreement with the commonly expected decreased attenuation potential of karst in comparison to other natural systems.

5.3.2.5 Comparison of attenuation rates

The investigated organic micropollutants can be put in an order concerning their tendency to be attenuated in karst. From high ($t_{1/2}$= 37 h) to no attenuation: PAC > ATE ≈ IBU > CAF >> CYC. Comparing this result to other studies, requires comparative studies, i. e. single studies investigating more than one of the above named micropollutants, as results for single substances from different studies can not necessarily be compared to one another. For karst aquifers no such comparative studies exist to the knowledge of the authors. For other environmental compartments the attenuation/degradation rates are generally high for CAF, IBU and PAC, while the order of these three substances varies. (Benotti and Brownawell, 2009; Castiglioni et al., 2006; Kunkel and Radke, 2011; Lam et al., 2004; Maeng et al., 2011). For ATE very little comparative information can be found concerning its half-life in the environment. In a riverine system a similar recovery could be observed as for IBU (Castiglioni et al., 2006), which is in good agreement with the results of this study. However, data from a river are likely to be affected by photodegradation (e. g. Matamoros et al., 2008; Zeng et al., 2012), which is obviously irrelevant for groundwater systems.

The low half-lives observed in this study for a variety of micropollutants in a karst aquifer are similar to those observed in studies for other systems (Benotti and Brownawell, 2009; Lam et al., 2004). This underlines the finding of Hillebrand et al. (2012a), that a much higher attenuation rate should be expected for karst aquifers, than is done so far. However, in a river stretch a significantly lower half-life for IBU was observed employing a multitracer experiment (Kunkel and Radke, 2011).

From the results of this study it seems legitimate to transfer the attenuation potential of a karst aquifer for one of the reactive tracers to the others. However, possible lag-phases have to be considered for the estimation of the short-term fate of reactive compounds.

5.4 Conclusions

- Retardation seems to be an irrelevant attenuation process for any of the compounds in the investigated karst aquifer.
- Readily degradable substances (ATE, CAF, IBU, PAC) exhibit high attenuation rates (low half-lives).
- Biotransformation was observed for ATE, highlighting the biological activity in the investigated karst system. It is likely, that biodegradation is an important process in the attenuation of CAF, PAC and IBU.
- Insignificant attenuation was observed for the more persistent compound CYC.
- Despite the rapid flow and transport within the karst aquifer, significant mitigation of contaminants can be observed.

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Chapter 6

6 General conclusions and perspective

Owing to their heterogeneity and complexity of flow and transport processes, karst aquifers are difficult to characterise with conventional hydrogeological methods. Employing organic micropollutants as tracers and specific indicators are promising approaches to improve the fundamental understanding of these systems. They can be used as tracers in the context of artificial tracer tests to investigate the attenuation potential of karst aquifers and demonstrate the relative importance of individual attenuation processes, and they can be used as indicators, according to the definition of Licha (2013). As such they allow for the identification of transport paths, flow components and the estimation of the long-term storage potential of karst aquifers. This information provides valuable insights into the fate and transport processes occurring within karst systems and is vital for, e. g., the setup of predictive models and the estimation of the vulnerability of karst systems.

6.1 Stabilisation of water samples

The concentrations of compounds in water samples may change after the sample was collected. If the immediate analysis of water samples is not possible, proper stabilisation and storage of the samples is essential in order to obtain reliable actual concentrations of the investigated compounds. For the preservation and handling of water samples specific guidelines are available for various parameters (DIN EN ISO 5667-3, 2012). However, little information exists on strategies for the stabilisation of organic micropollutants in water samples.

The influence of different storage temperatures and stabilisation approaches for 46 organic micropollutants has been evaluated for river water as well as treated effluent. In non-stabilised water samples 60% of the analytes exhibited unacceptable recoveries (< 80% relative to the frozen reference sample) at the end of the investigation period in both water matrices. This highlights the need for reliable sample stabilisation strategies. For river water generally better recoveries of the analytes were observed than for treated effluent. This is likely an effect related to the larger number of microorganisms in treated effluent and the fact, that microorganisms in treated effluent are already adapted. However, for some

analytes higher losses were observed for the river water than for the treated effluent. Lower storage temperatures and shorter storage durations result in higher recoveries.

Among the three investigated stabilisation approaches, the addition of copper sulphate proved to be unsuitable. Better results could be achieved by the addition of sodium azide, which resulted in higher recoveries for most analytes in comparison to the non-stabilised samples. However, for some analytes worse recoveries were observed. This may be due to interactions with the azide anion that has been described by several authors, or due to further cross-reactions. The best results could be achieved by the immediate processing of the samples by SPE. Storing the cartridges at 28 °C successfully stabilised 33 of the 46 analytes for 10 days. Storing the cartridges at 4 °C stabilised all investigated analytes over the whole investigation period of 20 days. The reduction of the water content and storage at low temperatures seems to mitigate all relevant processes that affect the recovery of the investigated compounds.

Even though the processing of the water samples by SPE involves a higher amount of work than the addition of sodium azide it is the most promising of the investigated approaches and has a further benefit: depending on the sampled volume of water the size and weight of the samples can be reduced significantly. To allow for SPE in remote areas, appropriate technologies have been developed (Moraes et al., 2003). Further research might aim for even more successful stabilisation techniques obviating the need for low storage temperatures.

Although the investigated micropollutants comprise a large variety of readily degradable and highly persistent compounds, the results of the study are not directly transferable to other organic micropollutants without further investigation. Depending on the subject of a study, the selection of analytes and external circumstances, like ambient temperature or access to cooling devices, an investigation of an appropriate stabilisation strategy is required. In any case the necessity for sample stabilisation needs to be considered in order to obtain reliable information on compounds concentrations in water samples.

6.2 Long-term storage potential of karst aquifers

Karst aquifers are often considered as rapid systems, referring to the possibility of fast percolation, flow and transport that can be observed in karst aquifer systems. While this fast flow component is certainly important, especially for the estimation of the microbial vulnerability of a karst system, the long-term storage potential of the aquifer matrix needs consideration as well. Contaminants can be transported into the fissured aquifer matrix with the percolating rainwater or in the course of exchange processes of the conduit system with the aquifer matrix. From here the contaminants can be released slowly into the conduit system and transported to the spring. As a result of the slow flow and transport within the

aquifer matrix, this process may take decades and the contaminants can be detected in spring water for long durations.

To characterise the long-term storage potential of a German karst aquifer two herbicides and one degradation product (atrazine, metazachlor and desethylatrazine) were employed as indicators, investigating the time-series of these compounds. Although for the two herbicides initially a similar input scenario can be assumed (both herbicides are likely to reach the groundwater after application on agricultural areas), different behaviour after recharge events can be observed at the investigated karst spring. Metazachlor is still applied in the catchment and can only be detected in the spring water shortly after recharge events and is therefore likely to be transported to the spring via concentrated recharge and conduit flow. No background concentration could be observed. Contrary, the application of atrazine has been prohibited in Germany more than 20 years ago and it can almost always be detected in spring water of the Gallusquelle. From the correlation with Ca^{2+} and Mg^{2+} it can be concluded that the detected atrazine and its degradation product desethylatrazine nowadays originate from the (fissured) aquifer matrix.

Compounds that are transported through the aquifer matrix are diluted and possibly degraded. To allow for the detection of a compound that is transported along this path, the compound needs to be introduced into the environment in sufficient quantity and degradation processes need to be negligible. In fact, two compounds that are known for their low degradability exhibit a background concentration in the spring water as well. For the artificial sweetener acesulfame and the anticonvulsant carbamazepine background concentrations of 20 and 3 ng L^{-1}, respectively, are observed, suggesting that both compounds are slowly released from the aquifer matrix into the conduit system. For acesulfame a similar behaviour as described for atrazine can be observed (unpublished data). Even if carbamazepine or acesulfame would be banned, these compounds were likely to be detectable in spring water for a considerable time.

The above described phenomenon is only expected for persistent compounds. Hence, a long-term impact of organic micropollutants on spring water quality can be prevented by preventing the release of persistent organic micropollutants to the environment. The European Parliament, the Council of the European Union and the European Commission already agreed, that until 2020 chemicals should be produced that do not have severe impact on the environment (EU, 2002). In the course of the registration procedure of the European Communities for new veterinary and human pharmaceuticals the investigation of their environmental sustainability is required by law. Inacceptable environmental risks are sufficient for a refusal of the compound in the case of veterinary pharmaceuticals (EU, 2001a; EU, 2004a) but not for human pharmaceuticals (EU, 2001b; EU 2004b). For human pharmaceuticals measures, such as ‘ecolabeling’, have been suggested in the scientific literature (Joss et al., 2006; Joss et al., 2008; Kümmerer et al., 2010; Wennmalm and Gunnarsson, 2005). Similar efforts and suggestions are part of ‘green chemistry’.

Considering the environmental fate in the course of compound design apart from its functionality or choosing the environmentally more benign compound for otherwise similarly effective agents are promising approaches on long- and short-term respectively to overcome the above illustrated difficulties related to the storage potential of karst aquifers and environmental pollution with so-called organic micropollutants in general.

6.3 Attenuation potential during conduit transport

The potential for contaminant attenuation during transport in karst conduits is believed to be low. Factors, such as high transport velocity and low surface to volume ratio are considered to reduce the potential for contaminant sorption and degradation significantly. However, studies investigating the actual attenuation potential of karst aquifers are scarce.

A methodology has been developed, to identify the attenuation potential during transport in karst conduits employing a dualtracer experiment with the conservative tracer uranine and the reactive tracer caffeine. The readily biodegradable caffeine allows for the environmentally benign assessment of the *in-situ* attenuation potential even in groundwater systems that are characterised by rapid flow and transport. The result was verified by a multitracer experiment, employing a total of 8 tracers to investigate differentiated transport. Uranine, acesulfame and carbamazepine were used as reference tracers, while atenolol, caffeine, cyclamate, ibuprofen and paracetamol served as reactive tracers. The reactive compounds are usually readily biodegraded in wastewater treatment plants indicating their susceptibility to biodegradation in general. The list of tracers comprises organic anions, cations and uncharged species.

The attenuation rate of caffeine determined in the course of the dualtracer experiment was 0.0067 h^{-1} (corresponding to a half-life of $t_{1/2}$= 104 h). This was surprisingly high and indicates potentially good attenuation of contaminants during transport in karst conduits. The half-lives of the reactive tracers atenolol, caffeine, ibuprofen and paracetamol determined in the course of the multitracer experiment were 38–89 h, confirming the potentially good attenuation during transport in karst conduits. From the simultaneous occurrence of the biotransformation product atenolol acid in the spring water, it can be concluded, that biotransformation is one important degradation process in karst conduits. Contrary to the other reactive tracers, cyclamate was not affected by attenuation. This highlights the existing limits for attenuation during transport in karst conduits. The observed attenuation rates are in agreement with those that have been described for other natural and pseudo-natural systems in the scientific literature. Neither in the dualtracer experiment, nor in the multitracer experiment retardation was observed for any of the tracers.

Despite the rapid transport and low surface areas a significant attenuation was observed for some readily degradable compounds in the conduit system of the investigated karst aquifer. Biotransformation was shown to be an important degradation process for atenolol

and is likely to be a relevant process for the other attenuated tracers as well. It could not be clarified if other processes are involved in the attenuation of the investigated micropollutants. Compounds, that are known to be more recalcitrant to attenuation or that require a certain lag phase, pass the underground passage unaltered. Retardation was shown to be irrelevant even though potentially sorbing compounds have been used as tracers. All these findings refer to a single investigated aquifer and hence require the verification in other karst systems, preferably karst systems that are karstified to a (i) similar degree as the investigated aquifer of the Gallusquelle to confirm the observations and (ii) differing degree to investigate the possible effect of the degree of karstification of a karst aquifer on its attenuation potential. Additionally, the aquifer state of cleanliness may influence the determined attenuation rates and its impact needs further investigation. It is likely, that an aquifer that is heavily affected by wastewater (e. g. urban aquifer) exhibits a higher attenuation potential than a pristine one.

Tracer experiments with reactive tracers allow for a reliable determination of possibly occurring retardation and *in-situ* attenuation rates in karst conduits. To avoid ambiguity the simultaneous use of conservative reference tracers is necessary. Although the observed attenuation rates are similar to other natural or pseudo-natural systems, the low residence time in karst conduits lowers the *de facto* attenuation.

As long as not all possible sources for contaminants are removed, the occurrence of compounds related to wastewater and agriculture or accidentally released contaminants is still likely in karst spring water. Considering the environmental properties of a compound in the course of its design and favouring the environmentally more benign of otherwise similarly effective agents as explained in section 6.2 and proposed by e. g. Papa et al. (2013), may minimize the introduction of wastewater related compounds via discharge of treated effluent and allow for contaminant attenuation within the karst system, if release or leakage of untreated wastewater occurred. To prevent pesticides to affect spring water quality, the characteristics of karst aquifers need to be taken into account when designating groundwater protection zones, also considering the possibility of rapid horizontal transport in the epikarst. The application of pesticides need to be prevented in the vicinity of dolines, dry valleys and other karst features that allow for rapid transport from the ground surface to the conduit system and consequently to the spring.

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A Appendix

Table A.1. List of analytes and their related internal standards (adapted from Nödler et al., 2010)

Analytes	Related internal standard
1H-benzotriazole, 1-methylxantine, 3-methylxanthine, caffeine, paracetamol, paraxanthine, theobromine, theophylline, tolyltriazole	Paraxanthine-D6
Atenolol, atenolol acid, metoprolol, sotalol	Atenolol-D_7
Iohexol, iomeprol, iopamidol, iopromide	Desmethoxyiopromide
Primidone, sulfamethoxazole	Sulfamethoxazole-$^{13}C_6$
Isoproturon	Isoproturon-D_6
Atrazine, desethylatrazine, desisopropylatrazine	Atrazine-D_5
Benzoylecgonine, carbamazepine, cetirizine, metazachlor, phenazone, trimethoprim	Carbamazepine-D_{10}
Erythromycin	Erythromycin-N-methyl-^{13}C-D_3
Clarithromycin, roxithromycin	Ery-methyloxime
Citalopram, diazepam, tetrazepam	Diazepam-D_5
Loratadine	Loratadine-D_4
Bezafibrate, clofibric acid, diclofenac, diuron, gemfibrozil, ibuprofen, naproxen	Ibuprofen-D_3
Mecoprop	Mecoprop-D_3
Fluoxetine, Sertraline	Fluoxethine-D_6
Pantoprazole	Lansoprazole

Table A.2. A list of all spiked compounds and their respective recoveries in non-stabilised water samples of treated effluent stored as liquid at 4 °C.

		Relative recovery (%) after "x" h									
	Time (h)-->	0		95		191		286		404	
Application	Compound	%	+/-	%	+/-	%	+/-	%	+/-	%	+/-
	Diclofenac	100	4	100	1	94	4	91	2	80	0
Analgesics	Ibuprofen	100	1	99	3	84	6	70	2	70	1
and	Naproxen	100	1	104	7	89	2	92	6	102	1
anti-inflammatories	Paracetamol	100	5	61	4	28	1	0	0	0	0
	Phenazone	100	1	108	2	101	0	102	0	100	0
Stimulants	Caffeine	100	1	58	0	25	3	11	1	5	0
and	Paraxanthine	100	2	60	0	26	2	17	5	7	1
caffeine metabolites	Theobromine	100	2	48	3	15	1	5	1	0	0
	Theophylline	100	2	73	1	30	3	20	3	17	3
	1-Methylxanthine	100	6	64	4	41	1	25	0	21	3
	3-Methylxanthine	100	3	47	2	23	2	0	0	0	0
Antihypertensive	Atenolol	100	1	91	0	87	0	87	2	86	2
agents	Metoprolol	100	1	96	1	92	1	89	1	102	1
	Sotalol	100	0	95	1	92	0	91	1	102	0
Contrast	Iohexol	100	2	101	3	91	4	97	2	93	4
media	Iomeprol	100	4	92	1	95	2	95	0	83	0
	Iopamidol	100	2	94	1	87	2	95	4	81	0
	Iopromide	100	1	93	5	85	0	86	4	89	1
Antibiotics	Clarithromycin	100	1	98	1	97	1	94	0	97	0
	Erythromycin	100	1	97	0	93	1	92	1	92	1
	Roxithromycin	100	0	98	0	98	2	93	0	97	1
	Sulfamethoxazole	100	0	98	1	98	1	96	1	96	0
	Trimethoprim	100	5	109	4	108	1	106	0	112	0
Lipid regulators	Bezafibrate	100	5	96	1	95	2	87	7	77	2
	Clofibric acid	100	7	103	4	106	3	96	10	77	3
	Gemfibrozil	100	8	108	2	107	5	102	4	86	0
Antihistamines	Cetirizine	100	3	88	1	86	1	84	2	92	1
	Loratadine	100	0	93	1	90	2	89	1	91	1
Anticonvulsants	Carbamazepine	100	4	104	2	102	1	100	0	106	2
and sedatives	Diazepam	100	2	92	2	91	1	87	1	88	0
	Primidone	100	2	95	4	98	1	97	4	94	0
	Tetrazepam	100	1	93	2	91	0	88	0	92	0
SSRI	Citalopram	100	2	96	2	92	1	50	40	90	4
	Fluoxetine	100	1	99	3	98	1	98	1	98	4
	Sertraline	100	2	98	9	102	1	93	1	68	5
Pesticides	Atrazine	100	1	98	1	96	1	97	0	98	1
and	Desethylatrazine	100	0	102	1	96	2	53	44	93	0
pesticide metabolites	Desisopropylatrazine	100	1	111	0	109	2	107	2	100	2
	Diuron	100	11	101	4	105	2	89	14	64	6
	Isoproturon	100	4	84	1	84	3	83	0	77	0
	Mecoprop	100	2	96	1	95	0	94	1	95	0
	Metazachlor	100	2	99	1	97	1	93	0	98	2
Corrosion inhibitors	1H-Benzotriazol	100	2	93	2	95	4	86	2	97	0
	Tolyltriazol	100	1	95	2	96	3	89	1	92	2
Cocaine metabolite	Benzoylecgonin	100	4	107	4	102	0	100	1	110	0
Proton pump inhibitor	Pantoprazole	100	3	97	5	87	1	84	2	92	2

Table A.3. A list of all spiked compounds and their respective recoveries in non-stabilised water samples of treated effluent stored as liquid at 28 °C.

		Relative recovery (%) after "x" h									
	Time (h)-->	0		46		91		187		283	
Application	Compound	%	+/-	%	+/-	%	+/-	%	+/-	%	+/-
	Diclofenac	100	3	110	15	96	1	77	6	80	1
Analgesics	Ibuprofen	100	4	87	5	71	2	11	11	0	0
and	Naproxen	100	2	95	4	91	4	70	9	66	3
anti-inflammatories	Paracetamol	100	1	79	2	55	0	13	1	7	1
	Phenazone	100	2	108	8	101	9	93	0	79	15
Stimulants	Caffeine	100	0	48	3	19	1	1	1	0	0
and	Paraxanthine	100	1	60	4	36	2	13	7	5	0
caffeine metabolites	Theobromine	100	3	46	1	16	1	0	0	0	0
	Theophylline	100	0	58	2	26	2	10	6	5	3
	1-Methylxanthine	100	4	61	0	4	0	10	7	5	0
	3-Methylxanthine	100	2	64	4	30	0	0	0	0	0
Antihypertensive	Atenolol	100	2	103	6	99	4	73	11	78	1
agents	Metoprolol	100	0	98	4	102	5	91	1	94	1
	Sotalol	100	1	93	5	96	4	92	1	95	1
Contrast	Iohexol	100	1	99	4	103	6	97	3	95	1
media	Iomeprol	100	0	102	7	100	5	89	0	87	1
	Iopamidol	100	2	108	11	104	9	89	1	86	0
	Iopromide	100	2	102	8	100	8	88	2	83	1
Antibiotics	Clarithromycin	100	2	103	4	102	5	82	11	85	1
	Erythromycin	100	1	100	8	94	7	83	0	76	0
	Roxithromycin	100	1	106	10	102	7	88	4	88	3
	Sulfamethoxazole	100	2	100	2	99	5	90	5	75	6
	Trimethoprim	100	2	112	10	102	6	93	1	92	1
Lipid regulators	Bezafibrate	100	1	107	10	101	9	70	2	65	2
	Clofibric acid	100	1	114	8	109	13	93	2	84	2
	Gemfibrozil	100	4	106	11	103	7	75	6	59	1
Antihistamines	Cetirizine	100	2	94	7	91	3	89	3	89	2
	Loratadine	100	1	101	7	101	7	96	0	90	0
Anticonvulsants	Carbamazepine	100	0	105	4	103	7	99	2	96	1
and sedatives	Diazepam	100	0	104	2	101	5	94	1	95	0
	Primidone	100	0	99	1	109	5	107	1	103	3
	Tetrazepam	100	0	98	3	92	7	89	1	83	0
SSRI	Citalopram	100	2	103	17	80	4	79	3	89	2
	Fluoxetine	100	3	109	7	105	8	93	2	92	4
	Sertraline	100	4	99	4	114	11	91	0	102	1
Pesticides	Atrazine	100	1	105	7	101	8	97	2	94	1
and	Desethylatrazine	100	3	107	9	101	8	96	0	95	2
pesticide metabolites	Desisopropylatrazine	100	2	109	10	101	7	92	4	92	0
	Diuron	100	4	107	7	103	14	83	1	81	4
	Isoproturon	100	2	111	5	111	10	98	0	97	1
	Mecoprop	100	1	106	4	104	4	97	1	95	1
	Metazachlor	100	0	105	5	104	7	94	2	90	1
Corrosion inhibitors	1H-Benzotriazol	100	1	110	2	91	8	80	0	69	14
	Tolyltriazol	100	2	103	3	85	5	64	3	64	1
Cocaine metabolite	Benzoylecgonin	100	0	110	8	101	5	86	0	57	15
Proton pump inhibitor	Pantoprazole	100	6	85	10	47	4	25	5	8	1

Table A.4. A list of all spiked compounds and their respective recoveries in non-stabilised river water samples stored as liquid at 4 °C.

		Relative recovery (%) after "x" h									
	Time (h)-->	0		95		191		286		404	
Application	Compound	%	+/-	%	+/-	%	+/-	%	+/-	%	+/-
	Diclofenac	100	2	108	1	81	6	76	6	96	0
Analgesics	Ibuprofen	100	2	98	0	91	0	89	4	75	0
and	Naproxen	100	1	128	3	88	17	85	9	94	2
anti-inflammatories	Paracetamol	100	0	89	3	86	1	87	5	77	2
	Phenazone	100	1	102	1	102	4	100	3	101	7
Stimulants	Caffeine	100	0	94	3	97	1	94	2	91	1
and	Paraxanthine	100	0	97	2	97	1	95	0	93	0
caffeine metabolites	Theobromine	100	0	96	0	96	4	89	0	86	1
	Theophylline	100	1	98	2	95	6	91	4	90	3
	1-Methylxanthine	100	2	91	5	92	2	99	0	101	2
	3-Methylxanthine	100	1	90	1	94	1	103	4	105	2
Antihypertensive	Atenolol	100	0	94	1	92	1	89	1	89	1
agents	Metoprolol	100	2	93	2	92	2	95	0	98	2
	Sotalol	100	2	91	1	89	2	96	3	90	2
Contrast	Iohexol	100	4	99	2	99	1	97	1	97	2
media	Iomeprol	100	5	98	11	95	6	85	3	85	4
	Iopamidol	100	2	103	2	99	2	88	4	88	8
	Iopromide	100	2	99	3	98	1	95	0	92	2
Antibiotics	Clarithromycin	100	1	103	1	101	0	99	1	98	1
	Erythromycin	100	0	93	0	94	0	91	1	91	0
	Roxithromycin	100	1	101	0	102	0	97	1	98	1
	Sulfamethoxazole	100	0	98	3	92	3	92	1	81	3
	Trimethoprim	100	1	104	0	103	1	98	1	99	3
Lipid regulators	Bezafibrate	100	1	109	2	94	9	96	4	99	4
	Clofibric acid	100	2	108	3	95	9	98	6	97	4
	Gemfibrozil	100	2	111	2	87	10	86	5	92	1
Antihistamines	Cetirizine	100	5	112	2	100	13	98	8	105	6
	Loratadine	100	1	95	2	95	3	93	1	92	0
Anticonvulsants	Carbamazepine	100	0	103	0	104	1	100	0	101	2
and sedatives	Diazepam	100	1	98	3	97	0	96	1	96	1
	Primidone	100	5	96	2	95	1	97	4	93	4
	Tetrazepam	100	1	95	1	98	1	94	0	92	2
SSRI	Citalopram	100	1	94	0	101	5	92	1	100	5
	Fluoxetine	100	1	94	1	85	3	87	3	85	1
	Sertraline	100	6	109	5	81	8	59	4	71	10
Pesticides	Atrazine	100	1	98	1	93	1	92	0	95	1
and	Desethylatrazine	100	1	111	3	97	2	95	4	94	3
pesticide metabolites	Desisopropylatrazine	100	2	114	1	94	4	91	7	94	3
	Diuron	100	5	105	4	91	12	97	11	88	9
	Isoproturon	100	3	98	1	92	1	91	0	93	2
	Mecoprop	100	1	95	1	96	3	93	1	94	2
	Metazachlor	100	1	96	1	97	0	96	3	95	5
Corrosion inhibitors	1H-Benzotriazol	100	1	63	5	69	11	79	8	64	7
	Tolyltriazol	100	1	62	4	65	17	76	6	61	10
Cocaine metabolite	Benzoylecgonin	100	1	105	0	98	4	96	1	95	1
Proton pump inhibitor	Pantoprazole	100	2	99	4	62	34	59	10	50	20

Table A.5. A list of all spiked compounds and their respective recoveries in non-stabilised river water samples stored as liquid at 28 °C.

		Relative recovery (%) after "x" h									
	Time (h)-->	0		46		91		187		283	
Application	Compound	%	+/-	%	+/-	%	+/-	%	+/-	%	+/-
	Diclofenac	100	1	111	8	117	4	86	2	90	4
Analgesics	Ibuprofen	100	0	94	0	88	3	37	7	15	5
and	Naproxen	100	1	95	2	89	2	77	2	75	5
anti-inflammatories	Paracetamol	100	2	94	1	83	3	66	4	43	7
	Phenazone	100	1	103	1	108	3	102	1	88	5
Stimulants	Caffeine	100	1	99	3	100	1	55	4	27	8
and	Paraxanthine	100	0	97	1	93	2	65	3	37	9
caffeine metabolites	Theobromine	100	1	93	2	90	5	53	6	26	8
	Theophylline	100	1	100	2	98	1	63	5	35	7
	1-Methylxanthine	100	0	99	3	61	4	16	1	10	2
	3-Methylxanthine	100	0	95	4	99	4	60	2	37	6
Antihypertensive	Atenolol	100	0	91	1	90	2	67	3	34	6
agents	Metoprolol	100	2	96	3	100	2	83	2	58	0
	Sotalol	100	1	93	4	93	2	96	0	93	3
Contrast	Iohexol	100	2	100	5	90	2	92	4	91	4
media	Iomeprol	100	6	84	2	83	0	75	0	78	2
	Iopamidol	100	3	102	3	102	2	91	6	86	3
	Iopromide	100	4	98	1	97	2	92	1	91	1
Antibiotics	Clarithromycin	100	1	101	2	99	2	96	0	83	7
	Erythromycin	100	1	97	0	93	0	88	1	80	0
	Roxithromycin	100	1	100	0	97	1	92	2	86	4
	Sulfamethoxazole	100	2	90	3	77	1	72	1	64	2
	Trimethoprim	100	1	101	2	103	4	98	1	94	3
Lipid regulators	Bezafibrate	100	1	96	0	98	4	28	6	0	0
	Clofibric acid	100	1	91	1	101	9	90	3	89	1
	Gemfibrozil	100	0	94	0	98	2	94	2	91	2
Antihistamines	Cetirizine	100	4	113	2	115	1	103	5	97	2
	Loratadine	100	0	98	0	97	1	96	1	94	1
Anticonvulsants	Carbamazepine	100	1	100	3	100	1	98	1	95	0
and sedatives	Diazepam	100	1	97	2	99	0	96	1	95	0
	Primidone	100	1	98	1	82	14	101	1	101	4
	Tetrazepam	100	1	97	3	97	1	92	1	91	0
SSRI	Citalopram	100	2	100	1	103	2	87	0	86	4
	Fluoxetine	100	0	91	2	83	0	64	2	48	6
	Sertraline	100	4	64	6	74	15	52	1	54	9
Pesticides	Atrazine	100	1	96	2	95	1	95	1	94	1
and	Desethylatrazine	100	1	98	4	91	1	94	1	94	2
pesticide metabolites	Desisopropylatrazine	100	0	94	3	88	0	88	3	50	29
	Diuron	100	4	80	4	81	4	81	6	85	2
	Isoproturon	100	0	92	2	92	1	90	1	88	0
	Mecoprop	100	2	101	3	100	1	100	1	98	1
	Metazachlor	100	1	102	1	109	4	101	1	94	1
Corrosion inhibitors	1H-Benzotriazol	100	1	100	2	97	7	89	3	55	12
	Tolyltriazol	100	0	95	4	87	11	83	3	83	4
Cocaine metabolite	Benzoylecgonin	100	1	101	2	102	2	91	1	44	12
Proton pump inhibitor	Pantoprazole	100	1	85	1	58	5	49	1	38	1

Table A.6. A list of all spiked compounds and their respective recoveries in water samples of treated effluent stored as liquid at 4 °C; stabilised with NaN_3.

		Relative recovery (%) after "x" h									
	Time (h)-->	0		95		191		286		404	
Application	Compound	%	+/-	%	+/-	%	+/-	%	+/-	%	+/-
	Diclofenac	100	8	99	0	98	4	86	4	87	4
Analgesics	Ibuprofen	100	2	94	1	95	3	89	1	88	3
and	Naproxen	100	3	98	3	91	0	81	2	83	4
anti-inflammatories	Paracetamol	100	4	94	4	97	5	95	1	95	2
	Phenazone	100	2	103	1	96	1	100	0	99	1
Stimulants	Caffeine	100	2	92	2	89	3	85	4	85	1
and	Paraxanthine	100	2	100	0	96	2	97	1	97	1
caffeine metabolites	Theobromine	100	1	98	3	99	0	91	2	93	1
	Theophylline	100	3	96	4	101	3	93	2	104	12
	1-Methylxanthine	100	6	96	3	95	0	98	3	108	4
	3-Methylxanthine	100	7	99	4	98	2	107	5	111	2
Antihypertensive	Atenolol	100	4	86	1	82	0	80	0	81	0
agents	Metoprolol	100	2	102	2	101	1	98	0	102	2
	Sotalol	100	1	95	1	91	1	87	1	92	3
Contrast	Iohexol	100	1	100	0	101	2	99	1	96	1
media	Iomeprol	100	4	99	5	97	4	97	2	100	1
	Iopamidol	100	5	113	9	101	1	107	3	109	7
	Iopromide	100	2	97	2	96	2	96	1	94	4
Antibiotics	Clarithromycin	100	1	94	0	96	0	93	0	95	2
	Erythromycin	100	1	98	0	96	0	95	0	95	1
	Roxithromycin	100	1	91	1	93	0	92	1	92	0
	Sulfamethoxazole	100	2	105	1	105	0	106	1	106	2
	Trimethoprim	100	6	111	1	108	2	110	1	112	0
Lipid regulators	Bezafibrate	100	2	96	0	97	3	93	4	93	4
	Clofibric acid	100	2	105	2	108	5	104	3	102	3
	Gemfibrozil	100	5	112	7	110	3	104	5	104	5
Antihistamines	Cetirizine	100	5	94	2	92	0	90	0	94	0
	Loratadine	100	5	88	1	90	1	86	1	87	2
Anticonvulsants	Carbamazepine	100	3	101	1	99	1	101	1	99	0
and sedatives	Diazepam	100	1	95	1	94	0	91	0	92	1
	Primidone	100	3	103	1	104	1	100	2	101	3
	Tetrazepam	100	2	94	0	96	1	93	2	91	1
SSRI	Citalopram	100	1	103	2	94	1	95	0	98	1
	Fluoxetine	100	6	88	1	85	1	90	2	87	0
	Sertraline	100	0	84	6	89	2	96	0	91	4
Pesticides	Atrazine	100	2	101	0	100	1	100	1	100	0
and	Desethylatrazine	100	1	103	0	97	0	99	0	98	0
pesticide metabolites	Desisopropylatrazine	100	5	116	1	111	1	114	1	111	0
	Diuron	100	3	111	4	117	3	114	2	113	5
	Isoproturon	100	5	91	0	90	0	87	2	86	2
	Mecoprop	100	2	94	1	95	0	94	1	94	0
	Metazachlor	100	0	93	1	94	0	94	0	95	2
Corrosion inhibitors	1H-Benzotriazol	100	6	86	24	102	7	96	11	109	1
	Tolyltriazol	100	6	93	19	102	7	100	7	107	1
Cocaine metabolite	Benzoylecgonin	100	4	109	1	103	0	106	0	104	1
Proton pump inhibitor	Pantoprazole	100	3	92	9	88	2	80	9	88	3

Table A.7. A list of all spiked compounds and their respective recoveries in water samples of treated effluent stored as liquid at 28 °C; stabilised with NaN_3.

		Relative recovery (%) after "x" h									
	Time (h)-->	0		46		91		187		283	
Application	Compound	%	+/-	%	+/-	%	+/-	%	+/-	%	+/-
	Diclofenac	100	2	95	5	93	2	87	1	86	3
Analgesics	Ibuprofen	100	3	95	2	92	1	96	0	99	1
and	Naproxen	100	9	91	5	103	0	99	2	96	4
anti-inflammatories	Paracetamol	100	2	81	1	75	6	67	1	65	4
	Phenazone	100	1	104	1	100	1	91	3	79	16
Stimulants	Caffeine	100	2	80	2	84	3	85	2	89	0
and	Paraxanthine	100	0	93	2	96	5	96	1	94	3
caffeine metabolites	Theobromine	100	7	90	2	104	10	103	0	103	3
	Theophylline	100	4	93	1	96	2	97	2	100	3
	1-Methylxanthine	100	4	96	1	83	10	56	13	32	4
	3-Methylxanthine	100	1	97	0	99	6	97	2	103	3
Antihypertensive	Atenolol	100	1	99	1	100	1	92	1	93	1
agents	Metoprolol	100	1	90	1	94	1	95	1	99	1
	Sotalol	100	2	88	1	95	1	95	2	97	1
Contrast	Iohexol	100	3	107	4	106	1	98	2	99	1
media	Iomeprol	100	1	99	1	93	2	89	3	87	3
	Iopamidol	100	6	120	0	109	6	88	5	96	3
	Iopromide	100	0	94	1	102	0	91	0	91	4
Antibiotics	Clarithromycin	100	1	98	1	97	3	97	0	92	0
	Erythromycin	100	1	96	4	91	0	85	2	82	0
	Roxithromycin	100	2	99	5	99	1	95	1	93	0
	Sulfamethoxazole	100	1	108	1	105	1	112	3	110	0
	Trimethoprim	100	0	102	1	95	1	93	0	97	3
Lipid regulators	Bezafibrate	100	3	112	1	100	4	95	0	96	0
	Clofibric acid	100	7	114	1	97	2	93	1	87	2
	Gemfibrozil	100	4	110	3	101	2	95	1	93	1
Antihistamines	Cetirizine	100	3	92	0	90	2	92	0	94	5
	Loratadine	100	2	96	2	98	1	96	0	96	1
Anticonvulsants	Carbamazepine	100	1	102	1	97	0	99	0	100	3
and sedatives	Diazepam	100	1	101	1	100	1	99	1	96	2
	Primidone	100	1	80	35	107	2	104	2	105	1
	Tetrazepam	100	2	99	0	102	3	97	1	91	1
SSRI	Citalopram	100	1	98	4	95	0	94	1	95	9
	Fluoxetine	100	0	90	3	87	2	93	5	97	2
	Sertraline	100	4	105	2	104	4	99	3	87	1
Pesticides	Atrazine	100	0	98	2	97	2	93	1	95	0
and	Desethylatrazine	100	3	99	2	97	1	98	1	96	2
pesticide metabolites	Desisopropylatrazine	100	5	100	2	93	1	92	2	87	1
	Diuron	100	5	125	2	104	7	95	1	90	3
	Isoproturon	100	2	106	2	103	4	100	4	97	3
	Mecoprop	100	0	101	1	98	0	98	0	98	0
	Metazachlor	100	0	98	1	93	1	89	1	89	3
Corrosion inhibitors	1H-Benzotriazol	100	4	100	4	94	7	92	2	97	0
	Tolyltriazol	100	3	102	4	98	2	93	0	96	1
Cocaine metabolite	Benzoylecgonin	100	0	94	0	88	2	86	1	83	1
Proton pump inhibitor	Pantoprazole	100	2	78	3	64	2	40	6	26	0

Table A.8. A list of all spiked compounds and their respective recoveries in river water samples stored as liquid at 4 °C; stabilised with NaN_3.

		Relative recovery (%) after "x" h									
	Time (h)-->	0		95		191		286		404	
Application	Compound	%	+/-	%	+/-	%	+/-	%	+/-	%	+/-
	Diclofenac	100	2	97	4	88	1	81	2	77	7
Analgesics	Ibuprofen	100	3	98	2	96	1	96	2	96	1
and	Naproxen	100	1	90	4	72	1	65	1	60	7
anti-inflammatories	Paracetamol	100	2	93	1	99	3	101	2	98	1
	Phenazone	100	1	95	0	101	1	95	2	94	2
Stimulants	Caffeine	100	2	96	1	97	3	94	3	100	0
and	Paraxanthine	100	3	98	1	96	1	96	1	91	5
caffeine metabolites	Theobromine	100	2	95	0	95	0	97	3	103	4
	Theophylline	100	2	101	3	98	4	100	0	85	17
	1-Methylxanthine	100	4	94	8	104	2	107	6	81	24
	3-Methylxanthine	100	7	93	7	108	4	112	6	103	8
Antihypertensive	Atenolol	100	2	96	0	94	0	93	0	93	0
agents	Metoprolol	100	0	96	0	104	2	104	1	103	1
	Sotalol	100	1	101	4	108	2	109	0	99	10
Contrast	Iohexol	100	6	105	0	97	1	94	2	93	0
media	Iomeprol	100	6	108	4	82	10	68	1	71	1
	Iopamidol	100	6	126	2	88	2	77	6	77	4
	Iopromide	100	0	90	3	86	4	85	1	86	0
Antibiotics	Clarithromycin	100	0	99	1	97	1	95	2	93	0
	Erythromycin	100	0	96	1	94	0	89	1	90	0
	Roxithromycin	100	1	96	0	95	0	91	1	92	1
	Sulfamethoxazole	100	5	96	0	97	1	93	4	93	1
	Trimethoprim	100	0	98	1	103	2	98	0	95	0
Lipid regulators	Bezafibrate	100	3	87	6	81	2	72	0	70	1
	Clofibric acid	100	2	88	6	82	1	72	1	71	1
	Gemfibrozil	100	3	92	4	84	1	74	2	73	4
Antihistamines	Cetirizine	100	8	107	7	105	13	112	0	68	37
	Loratadine	100	0	101	3	101	1	100	1	101	1
Anticonvulsants	Carbamazepine	100	1	96	0	97	1	94	1	94	1
and sedatives	Diazepam	100	1	96	0	95	1	94	1	95	1
	Primidone	100	7	89	4	91	1	86	1	89	1
	Tetrazepam	100	1	94	1	97	2	96	1	94	1
SSRI	Citalopram	100	5	96	6	107	6	98	0	105	2
	Fluoxetine	100	1	91	4	92	1	88	0	90	2
	Sertraline	100	6	93	11	81	10	70	1	67	6
Pesticides	Atrazine	100	1	99	0	95	1	93	0	95	1
and	Desethylatrazine	100	6	109	1	96	2	95	1	89	0
pesticide metabolites	Desisopropylatrazine	100	5	109	5	89	0	87	2	81	0
	Diuron	100	6	82	11	74	2	62	1	64	1
	Isoproturon	100	0	94	2	91	1	88	0	89	1
	Mecoprop	100	1	99	1	97	0	94	1	95	1
	Metazachlor	100	0	92	1	97	0	90	3	95	0
Corrosion inhibitors	1H-Benzotriazol	100	15	84	19	90	16	93	2	49	49
	Tolyltriazol	100	21	83	20	85	20	90	3	51	41
Cocaine metabolite	Benzoylecgonin	100	0	102	4	102	4	100	1	80	16
Proton pump inhibitor	Pantoprazole	100	62	133	46	73	47	102	10	56	34

Table A.9. A list of all spiked compounds and their respective recoveries in river water samples stored as liquid at 28 °C; stabilised with NaN_3.

		Relative recovery (%) after "x" h									
	Time (h)-->	0		46		91		187		283	
Application	Compound	%	+/-	%	+/-	%	+/-	%	+/-	%	+/-
	Diclofenac	100	4	92	3	100	10	86	3	99	3
Analgesics	Ibuprofen	100	1	96	0	97	0	93	2	94	0
and	Naproxen	100	3	97	10	109	15	101	2	110	7
anti-inflammatories	Paracetamol	100	4	97	4	90	1	88	2	88	2
	Phenazone	100	0	100	0	101	1	96	2	93	2
Stimulants	Caffeine	100	3	106	5	103	0	103	1	100	0
and	Paraxanthine	100	3	97	0	96	2	98	1	95	0
caffeine metabolites	Theobromine	100	3	100	4	97	3	94	2	93	0
	Theophylline	100	2	91	9	93	6	103	1	98	1
	1-Methylxanthine	100	5	87	11	76	7	83	0	70	1
	3-Methylxanthine	100	4	82	5	73	6	85	2	67	1
Antihypertensive	Atenolol	100	2	95	1	92	1	88	2	81	1
agents	Metoprolol	100	1	102	0	98	2	89	0	89	2
	Sotalol	100	3	93	1	89	2	83	1	76	0
Contrast	Iohexol	100	3	90	3	90	1	84	3	85	3
media	Iomeprol	100	2	118	1	120	0	102	3	128	8
	Iopamidol	100	5	133	3	117	5	103	3	121	7
	Iopromide	100	0	99	1	101	1	94	1	101	1
Antibiotics	Clarithromycin	100	2	93	4	92	1	91	1	88	0
	Erythromycin	100	0	93	2	89	2	82	2	73	3
	Roxithromycin	100	0	93	4	89	3	93	1	89	0
	Sulfamethoxazole	100	1	101	1	98	1	100	1	98	2
	Trimethoprim	100	1	97	2	101	1	97	2	99	0
Lipid regulators	Bezafibrate	100	1	100	2	105	6	107	1	105	7
	Clofibric acid	100	2	100	1	105	3	109	0	107	5
	Gemfibrozil	100	0	98	3	104	9	106	2	104	6
Antihistamines	Cetirizine	100	7	80	27	83	21	90	4	87	2
	Loratadine	100	0	92	3	89	3	90	1	87	1
Anticonvulsants	Carbamazepine	100	2	97	0	94	0	91	0	90	0
and sedatives	Diazepam	100	1	99	2	97	1	95	0	92	0
	Primidone	100	3	97	2	97	2	99	1	103	1
	Tetrazepam	100	0	96	1	95	2	91	2	88	0
SSRI	Citalopram	100	3	103	3	101	1	91	2	86	2
	Fluoxetine	100	5	89	8	87	5	87	2	72	3
	Sertraline	100	1	94	7	99	1	94	4	81	3
Pesticides	Atrazine	100	0	97	0	95	2	93	0	92	0
and	Desethylatrazine	100	2	95	0	95	3	98	1	95	0
pesticide metabolites	Desisopropylatrazine	100	2	90	0	98	7	98	0	97	3
	Diuron	100	2	106	2	118	3	136	4	134	9
	Isoproturon	100	2	98	1	101	1	98	1	98	2
	Mecoprop	100	0	99	1	96	1	98	0	97	0
	Metazachlor	100	1	100	0	96	1	89	3	86	0
Corrosion inhibitors	1H-Benzotriazol	100	14	78	46	74	24	114	7	112	8
	Tolyltriazol	100	19	72	43	69	24	115	14	111	11
Cocaine metabolite	Benzoylecgonin	100	1	86	9	85	6	81	2	70	2
Proton pump inhibitor	Pantoprazole	100	63	85	67	64	35	87	25	68	27

Table A.10. A list of all spiked compounds and their respective recoveries after stabilisation of spiked river water by SPE and storage at 4 °C.

		Relative recovery (%) after "x" h					
	Time (h)-->	0		239		480	
Application	Compound	%	+/-	%	+/-	%	+/-
	Diclofenac	100	5	97	0	100	1
Analgesics	Ibuprofen	100	2	98	0	103	2
and	Naproxen	100	6	101	0	95	1
anti-inflammatories	Paracetamol	100	4	104	1	105	6
	Phenazone	100	2	106	1	102	4
Stimulants	Caffeine	100	3	98	1	102	4
And	Paraxanthine	100	1	98	1	100	3
caffeine metabolites	Theobromine	100	2	98	0	100	3
	Theophylline	100	4	103	2	106	0
	1-Methylxanthine	100	2	97	1	96	4
	3-Methylxanthine	100	2	95	1	95	2
Antihypertensive	Atenolol	100	5	97	1	92	1
agents	Metoprolol	100	1	91	1	87	0
	Sotalol	100	6	102	2	99	5
Contrast	Iohexol	100	6	97	7	95	11
media	Iomeprol	100	9	96	3	96	0
	Iopamidol	100	5	92	1	88	2
	Iopromide	100	7	95	2	89	8
Antibiotics							
	Clarithromycin	100	1	101	2	101	2
	Erythromycin	100	3	98	1	99	0
	Roxithromycin	100	3	101	1	99	2
	Sulfamethoxazole	100	3	101	0	100	2
	Trimethoprim	100	1	106	0	100	3
Lipid regulators							
	Bezafibrate	100	3	98	1	102	1
	Clofibric acid	100	2	96	1	101	1
	Gemfibrozil	100	1	96	3	101	0
Antihistamines	Cetirizine	100	3	104	1	97	1
	Loratadine	100	3	95	1	93	0
Anticonvulsants	Carbamazepine	100	2	100	0	98	3
and sedatives	Diazepam	100	4	98	1	95	1
	Primidone	100	6	102	1	102	0
	Tetrazepam	100	6	101	0	96	2
SSRI	Citalopram	100	4	100	1	92	3
	Fluoxetine	100	3	92	0	84	3
	Sertraline	100	3	100	1	106	2
Pesticides	Atrazine	100	1	98	1	100	1
and	Desethylatrazine	100	3	102	2	103	0
pesticide metabolites	Desisopropylatrazine	100	4	101	1	102	2
	Diuron	100	4	89	6	95	6
	Isoproturon	100	2	95	2	98	1
	Mecoprop	100	2	98	1	100	1
	Metazachlor	100	2	96	3	95	1
Corrosion inhibitors							
	1H-Benzotriazol	100	4	101	2	97	4
	Tolyltriazol	100	1	96	3	100	3
Cocaine metabolite	Benzoylecgonine	100	2	109	1	101	5
Proton pump inhibitor	Pantoprazole	100	3	98	0	97	1

Table A.11. A list of all spiked compounds and their respective recoveries after stabilisation of spiked river water by SPE and storage at 20 °C.

		Relative recovery (%) after "x" h							
	Time (h)-->	0		120		240		358	
Application	Compound	%	+/-	%	+/-	%	+/-	%	+/-
	Diclofenac	100	6	101	2	101	13	96	4
Analgesics	Ibuprofen	100	4	101	1	97	2	96	0
and	Naproxen	100	1	99	8	85	12	81	3
anti-inflammatories	Paracetamol	100	4	106	3	103	4	108	0
	Phenazone	100	8	106	9	105	5	109	3
Stimulants	Caffeine	100	8	97	0	101	6	97	1
and	Paraxanthine	100	4	99	2	97	1	96	2
caffeine metabolites	Theobromine	100	6	97	2	94	4	96	7
	Theophylline	100	4	102	5	98	4	100	7
	1-Methylxanthine	100	7	93	8	101	1	104	3
	3-Methylxanthine	100	6	91	5	97	3	110	1
Antihypertensive	Atenolol	100	3	90	4	83	1	81	3
agents	Metoprolol	100	2	84	1	77	2	75	1
	Sotalol	100	2	100	1	91	3	87	6
	Iohexol	100	6	90	7	104	0	85	9
Contrast	Iomeprol	100	4	81	3	92	12	82	4
media	Iopamidol	100	3	92	7	104	5	111	7
	Iopromide	100	4	100	3	103	3	103	2
Antibiotics	Clarithromycin	100	4	100	2	98	4	95	1
	Erythromycin	100	2	97	0	97	3	98	1
	Roxithromycin	100	3	103	0	100	0	97	2
	Sulfamethoxazole	100	1	100	2	100	1	101	3
	Trimethoprim	100	6	97	0	102	1	104	1
Lipid regulators	Bezafibrate	100	14	105	3	108	1	107	0
	Clofibric acid	100	9	110	1	106	2	103	4
	Gemfibrozil	100	3	87	5	84	3	83	3
Antihistamines	Cetirizine	100	9	100	4	106	0	105	2
	Loratadine	100	1	79	1	80	4	80	2
	Carbamazepine	100	5	92	2	92	0	91	3
Anticonvulsants	Diazepam	100	2	101	0	100	1	106	4
and sedatives	Primidone	100	6	96	6	97	2	96	4
	Tetrazepam	100	0	101	2	105	2	111	6
	Citalopram	100	7	91	5	85	3	90	4
SSRI	Fluoxetine	100	2	77	4	64	0	63	3
	Sertraline	100	8	87	3	82	2	78	3
	Atrazine	100	2	103	1	102	1	100	1
	Desethylatrazine	100	6	103	2	100	3	97	3
Pesticides	Desisopropylatrazine	100	8	101	1	95	3	91	5
and	Diuron	100	7	108	8	99	1	109	8
pesticide metabolites	Isoproturon	100	2	96	2	95	1	94	2
	Mecoprop	100	1	98	1	98	0	94	1
	Metazachlor	100	5	94	4	93	0	95	1
Corrosion inhibitors	1H-Benzotriazol	100	4	99	1	93	5	85	2
	Tolyltriazol	100	3	93	1	94	6	100	6
Cocaine metabolite	Benzoylecgonine	100	7	101	1	105	3	100	0
Proton pump inhibitor	Pantoprazole	100	4	97	2	94	1	97	3

Table A.12. A list of all spiked compounds and their respective recoveries after stabilisation of spiked river water by SPE and storage at 40 °C.

		Relative recovery (%) after "x" h							
	Time (h)-->	0		47		120		240	
Application	Compound	%	+/-	%	+/-	%	+/-	%	+/-
	Diclofenac	100	5	82	2	90	0	86	9
Analgesics	Ibuprofen	100	2	95	1	98	1	95	4
and	Naproxen	100	6	92	4	96	2	83	13
anti-inflammatories	Paracetamol	100	4	106	3	108	6	109	9
	Phenazone	100	2	90	5	93	4	100	2
Stimulants	Caffeine	100	3	97	2	97	3	97	0
and	Paraxanthine	100	1	100	1	98	0	96	8
caffeine metabolites	Theobromine	100	2	107	1	111	6	110	8
	Theophylline	100	4	99	2	106	13	105	10
	1-Methylxanthine	100	2	85	2	88	14	83	4
	3-Methylxanthine	100	2	89	1	93	11	94	10
Antihypertensive	Atenolol	100	5	81	2	66	4	55	4
agents	Metoprolol	100	1	76	1	74	9	72	4
	Sotalol	100	6	89	4	81	4	73	5
	Iohexol	100	6	97	4	100	1	85	5
Contrast	Iomeprol	100	9	97	5	92	13	90	1
media	Iopamidol	100	5	100	1	101	3	99	3
	Iopromide	100	7	94	4	90	2	80	3
Antibiotics	Clarithromycin	100	1	102	5	96	1	91	1
	Erythromycin	100	3	101	0	98	1	97	2
	Roxithromycin	100	3	102	3	96	3	90	3
	Sulfamethoxazole	100	3	99	0	99	1	93	1
	Trimethoprim	100	1	91	2	90	2	91	5
Lipid regulators	Bezafibrate	100	3	96	1	102	4	99	4
	Clofibric acid	100	2	96	1	99	1	94	1
	Gemfibrozil	100	1	94	2	95	1	95	4
Antihistamines	Cetirizine	100	3	97	1	92	0	93	4
	Loratadine	100	3	81	0	85	14	71	10
	Carbamazepine	100	2	100	1	100	1	95	3
Anticonvulsants	Diazepam	100	4	98	4	92	1	93	0
and sedatives	Primidone	100	6	103	4	102	1	98	0
	Tetrazepam	100	6	104	3	95	3	92	3
	Citalopram	100	4	85	0	80	10	85	7
SSRI	Fluoxetine	100	3	75	1	56	6	28	2
	Sertraline	100	3	91	4	82	8	51	6
	Atrazine	100	1	101	4	101	3	96	1
	Desethylatrazine	100	3	106	6	109	0	106	1
Pesticides	Desisopropylatrazine	100	4	105	3	112	2	102	1
and	Diuron	100	4	88	3	99	2	86	2
pesticide metabolites	Isoproturon	100	2	97	2	88	1	68	1
	Mecoprop	100	2	95	0	97	0	93	2
	Metazachlor	100	2	98	2	98	2	91	1
Corrosion inhibitors	1H-Benzotriazol	100	4	82	3	93	9	87	10
	Tolyltriazol	100	1	83	3	97	7	95	11
Cocaine metabolite	Benzoylecgonine	100	2	92	1	90	3	87	5
Proton pump inhibitor	Pantoprazole	100	3	92	1	95	0	90	2

Table A.13. A list of all spiked compounds and their respective recoveries after stabilisation of spiked treated effluent by SPE and storage at 4 °C.

		Relative recovery (%) after "x" h					
	Time (h)-->	0		242		480	
Application	Compound	%	+/-	%	+/-	%	+/-
	Diclofenac	100	5	106	0	105	0
Analgesics	Ibuprofen	100	2	98	1	100	1
and	Naproxen	100	6	93	3	97	2
anti-inflammatories	Paracetamol	100	5	102	4	101	1
	Phenazone	100	6	97	1	99	0
Stimulants	Caffeine	100	2	99	2	92	4
and	Paraxanthine	100	7	106	1	101	3
caffeine metabolites	Theobromine	100	4	101	3	103	2
	Theophylline	100	2	99	3	96	2
	1-Methylxanthine	100	6	103	6	102	2
	3-Methylxanthine	100	4	106	6	97	2
Antihypertensive	Atenolol	100	3	97	4	95	1
agents	Metoprolol	100	2	97	4	96	1
	Sotalol	100	1	97	2	98	5
	Iohexol	100	1	93	10	95	10
Contrast	Iomeprol	100	5	102	2	96	1
media	Iopamidol	100	7	105	1	104	3
	Iopromide	100	4	86	2	94	2
Antibiotics	Clarithromycin	100	2	98	1	97	2
	Erythromycin	100	1	97	0	97	1
	Roxithromycin	100	5	95	2	95	3
	Sulfamethoxazole	100	2	98	0	99	1
	Trimethoprim	100	7	100	4	101	2
Lipid regulators	Bezafibrate	100	9	102	1	103	3
	Clofibric acid	100	6	106	1	105	2
	Gemfibrozil	100	5	105	2	104	0
Antihistamines	Cetirizine	100	6	96	4	97	1
	Loratadine	100	4	99	2	96	1
	Carbamazepine	100	5	97	1	99	1
Anticonvulsants	Diazepam	100	2	99	4	99	1
and sedatives	Primidone	100	2	96	2	97	4
	Tetrazepam	100	3	103	10	99	1
	Citalopram	100	7	96	1	94	2
SSRI	Fluoxetine	100	2	91	4	90	1
	Sertraline	100	3	100	6	98	7
	Atrazine	100	3	101	1	100	0
	Desethylatrazine	100	3	102	1	101	1
Pesticides	Desisopropylatrazine	100	3	103	2	100	2
and	Diuron	100	10	96	2	100	2
pesticide metabolites	Isoproturon	100	4	103	2	102	0
	Mecoprop	100	2	99	0	99	1
	Metazachlor	100	2	97	1	97	1
Corrosion inhibitors	1H-Benzotriazol	100	4	101	4	93	1
	Tolyltriazol	100	6	102	4	97	1
Cocaine metabolite	Benzoylecgonine	100	7	99	1	99	0
Proton pump inhibitor	Pantoprazole	100	3	102	2	99	1

Table A.14. A list of all spiked compounds and their respective recoveries after stabilisation of spiked treated effluent by SPE and storage at 20 °C.

		Relative recovery (%) after "x" h							
	Time (h)-->	0		119		242		360	
Application	Compound	%	+/-	%	+/-	%	+/-	%	+/-
	Diclofenac	100	3	105	1	105	3	87	7
Analgesics	Ibuprofen	100	2	99	0	99	3	87	8
and	Naproxen	100	7	100	3	102	1	89	8
anti-inflammatories	Paracetamol	100	8	97	9	103	6	89	6
	Phenazone	100	1	102	2	101	3	94	8
Stimulants	Caffeine	100	7	99	0	102	5	85	6
and	Paraxanthine	100	3	95	0	98	1	88	6
caffeine metabolites	Theobromine	100	2	100	2	95	0	92	4
	Theophylline	100	9	94	4	98	0	89	5
	1-Methylxanthine	100	8	87	6	79	2	61	1
	3-Methylxanthine	100	3	87	1	80	4	69	1
Antihypertensive	Atenolol	100	4	93	0	92	1	76	7
agents	Metoprolol	100	2	95	0	94	1	83	5
	Sotalol	100	9	100	10	98	3	80	3
	Iohexol	100	7	88	0	95	7	89	1
Contrast	Iomeprol	100	1	96	2	93	4	90	7
media	Iopamidol	100	6	99	4	96	2	87	7
	Iopromide	100	6	104	2	102	2	92	8
Antibiotics	Clarithromycin	100	1	96	2	102	1	91	7
	Erythromycin	100	1	96	0	100	0	91	9
	Roxithromycin	100	12	104	2	106	0	94	9
	Sulfamethoxazole	100	3	96	1	97	1	90	9
	Trimethoprim	100	4	101	2	102	2	90	3
Lipid regulators	Bezafibrate	100	7	102	5	108	5	94	7
	Clofibric acid	100	2	97	2	104	6	92	10
	Gemfibrozil	100	3	93	3	103	6	91	6
Antihistamines	Cetirizine	100	8	105	2	107	3	94	4
	Loratadine	100	3	93	3	97	0	80	9
	Carbamazepine	100	1	102	2	105	1	97	7
Anticonvulsants	Diazepam	100	1	99	2	98	1	89	6
and sedatives	Primidone	100	1	99	1	99	1	91	3
	Tetrazepam	100	1	95	0	96	2	86	6
	Citalopram	100	2	93	1	95	2	82	7
SSRI	Fluoxetine	100	24	96	3	97	1	75	7
	Sertraline	100	2	75	1	84	3	64	10
	Atrazine	100	2	99	0	102	3	94	8
	Desethylatrazine	100	2	102	0	104	4	94	7
Pesticides	Desisopropylatrazine	100	3	99	2	102	4	91	15
and	Diuron	100	4	98	3	108	10	93	13
pesticide metabolites	Isoproturon	100	4	101	1	103	2	92	9
	Mecoprop	100	2	97	1	98	1	90	7
	Metazachlor	100	2	101	2	102	3	93	6
Corrosion inhibitors	1H-Benzotriazol	100	1	91	3	88	2	77	9
	Tolyltriazol	100	3	92	3	94	4	83	9
Cocaine metabolite	Benzoylecgonine	100	3	101	3	104	1	91	5
Proton pump inhibitor	Pantoprazole	100	1	98	2	99	1	90	7

Table A.15. A list of all spiked compounds and their respective recoveries after stabilisation of spiked treated effluent by SPE and storage at 40 °C.

		Relative recovery (%) after "x" h							
	Time (h)-->	0		47		122		242	
Application	Compound	%	+/-	%	+/-	%	+/-	%	+/-
	Diclofenac	100	3	102	2	99	5	96	3
Analgesics	Ibuprofen	100	2	98	1	99	1	101	4
and	Naproxen	100	7	95	2	92	1	84	3
anti-inflammatories	Paracetamol	100	2	98	4	97	7	90	0
	Phenazone	100	1	98	3	99	1	100	2
Stimulants	Caffeine	100	5	95	2	96	3	101	4
and	Paraxanthine	100	3	96	2	94	3	91	1
caffeine metabolites	Theobromine	100	2	90	1	95	1	98	4
	Theophylline	100	9	97	2	93	5	92	5
	1-Methylxanthine	100	8	85	5	77	1	76	2
	3-Methylxanthine	100	3	86	1	80	2	80	2
Antihypertensive	Atenolol	100	4	84	2	74	2	64	3
agents	Metoprolol	100	2	93	2	82	0	77	2
	Sotalol	100	9	86	3	83	9	64	2
	Iohexol	100	7	98	5	87	5	85	7
Contrast	Iomeprol	100	1	99	2	97	3	96	1
media	Iopamidol	100	6	104	4	93	1	86	5
	Iopromide	100	6	100	4	95	1	96	1
Antibiotics	Clarithromycin	100	1	99	1	98	2	100	0
	Erythromycin	100	1	99	0	98	2	95	1
	Roxithromycin	100	12	101	0	100	2	98	3
	Sulfamethoxazole	100	3	97	0	98	1	97	1
	Trimethoprim	100	4	95	1	95	0	92	2
Lipid regulators	Bezafibrate	100	7	102	0	100	3	103	1
	Clofibric acid	100	2	98	0	97	1	98	2
	Gemfibrozil	100	3	101	0	103	1	100	1
Antihistamines	Cetirizine	100	8	104	2	93	11	98	1
	Loratadine	100	3	87	5	84	2	86	2
	Carbamazepine	100	1	102	0	103	1	103	2
Anticonvulsants	Diazepam	100	1	98	3	96	1	92	0
and sedatives	Primidone	100	1	95	4	101	2	100	2
	Tetrazepam	100	1	98	7	93	5	90	1
	Citalopram	100	2	93	0	86	1	79	3
SSRI	Fluoxetine	100	2	75	2	51	4	31	2
	Sertraline	100	2	68	4	57	2	49	5
	Atrazine	100	2	101	1	101	3	98	3
	Desethylatrazine	100	2	103	2	102	2	101	1
Pesticides	Desisopropylatrazine	100	3	101	7	96	1	98	4
and	Diuron	100	4	92	4	82	0	77	1
pesticide metabolites	Isoproturon	100	4	96	0	78	6	54	2
	Mecoprop	100	2	95	1	96	1	94	0
	Metazachlor	100	2	98	2	98	2	92	3
Corrosion inhibitors	1H-Benzotriazol	100	1	89	0	80	4	71	0
	Tolyltriazol	100	3	89	3	85	2	78	1
Cocaine metabolite	Benzoylecgonine	100	3	96	0	96	0	96	2
Proton pump inhibitor	Pantoprazole	100	1	97	2	94	2	92	0

Appendix A.1. Calculation procedure for hydrograph separation

The fraction of rapidly transported rain water at the spring is determined as follows:

$$c = fr \cdot c_p + (1 - fr) \cdot c_{bg} \qquad \textbf{Eq. A.1}$$

resulting in

$$fr = \frac{c - c_{bg}}{c_p - c_{bg}} \qquad \textbf{Eq. A.2}$$

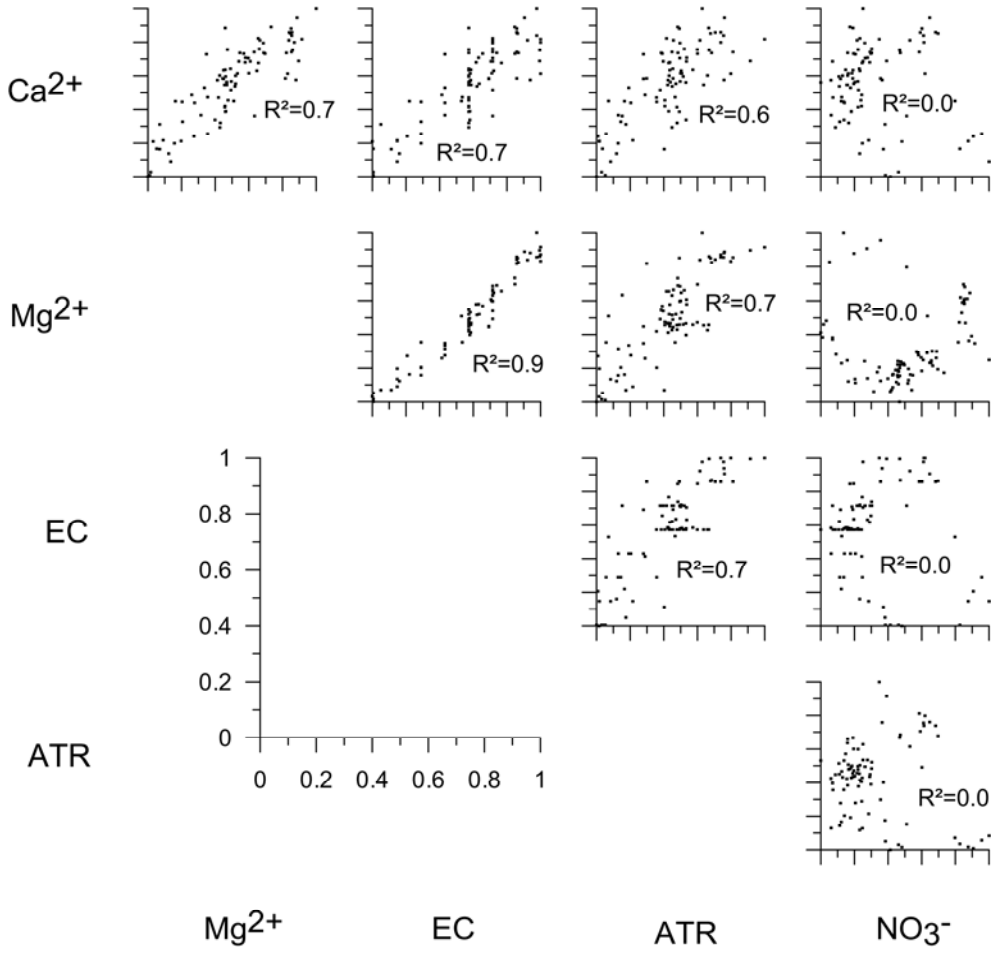
with c the observed parameter concentration in spring water at the time of sampling, c_p the parameter concentration in the precipitation sample, c_{bg} the background concentration of the parameter in spring water and *fr* the fraction of rapidly transported rain water in spring water.

The discharge of rapidly transported rainwater as part of the total spring discharge can be calculated according to:

$$Q_{fr} = Q \cdot fr = Q \cdot \frac{c - c_{bg}}{c_p - c_{bg}} \qquad \textbf{Eq. A. 3}$$

with Q_{fr} being the discharge of rapidly transported rainwater at the time of sampling, Q the total spring discharge at the time of sampling

Appendix A.2. Correlation of atrazine (ATR) with inorganic ions and the electrical conductivity (EC).



The parameters are displayed in normalised form to attain values between 0 and 1 for their minimum and maximum values respectively.

Table A.16. Estimated mean concentrations of atrazine (ATR) and desethylatrazine (DEA) in spring water as well as atrazine and desethylatrazine loads. While the values for the year 2010 are observed values, all others are estimated according to the trend of the data in Tappe et al. (2002), i. e. declining concentrations following an exponential decline with a decline-rate of 0.26 a^{-1} for atrazine and 0.22 a^{-1} for desethylatrazine. For the years 1965–1991 a constant discharge of atrazine and desethylatrazine is assumed which is equal to the estimated value for 1992.

Year	Estimated mean concentration of ATR in spring water (ng L^{-1})	Estimated ATR load in spring water (g a^{-1})	Estimated mean concentration of DEA in spring water (ng L^{-1})	Estimated DEA load in spring water (g a^{-1})
2010	2.4	37.8	2.8	44.2
2009	3.1	48.9	3.5	55.1
2008	4.0	63.2	4.4	68.8
2007	5.2	81.7	5.5	86.0
2006	6.7	105.7	6.8	107.4
2005	8.7	136.6	8.5	134.1
2004	11.2	176.6	10.6	167.4
2003	14.5	228.3	13.3	209.1
2002	18.7	295.1	16.6	261.1
2001	24.2	381.4	20.7	326.1
2000	31.3	493.1	25.8	407.2
1999	40.4	637.4	32.2	508.5
1998	52.3	823.9	40.3	635.0
1997	67.5	1065.1	50.3	792.9
1996	87.3	1376.8	62.8	990.2
1995	112.9	1779.8	78.4	1236.5
1994	145.9	2300.7	97.9	1544.1
1993	188.6	2974.1	122.3	1928.2
1992	243.8	3844.6	152.7	2407.9
1991	243.8	3844.6	152.7	2407.9
1990	243.8	3844.6	152.7	2407.9
1989	243.8	3844.6	152.7	2407.9
1988	243.8	3844.6	152.7	2407.9
1987	243.8	3844.6	152.7	2407.9
1986	243.8	3844.6	152.7	2407.9
1985	243.8	3844.6	152.7	2407.9
1984	243.8	3844.6	152.7	2407.9
1983	243.8	3844.6	152.7	2407.9
1982	243.8	3844.6	152.7	2407.9
1981	243.8	3844.6	152.7	2407.9
1980	243.8	3844.6	152.7	2407.9
1979	243.8	3844.6	152.7	2407.9
1978	243.8	3844.6	152.7	2407.9
1977	243.8	3844.6	152.7	2407.9
1976	243.8	3844.6	152.7	2407.9
1975	243.8	3844.6	152.7	2407.9
1974	243.8	3844.6	152.7	2407.9
1973	243.8	3844.6	152.7	2407.9
1972	243.8	3844.6	152.7	2407.9
1971	243.8	3844.6	152.7	2407.9
1970	243.8	3844.6	152.7	2407.9
1969	243.8	3844.6	152.7	2407.9
1968	243.8	3844.6	152.7	2407.9
1967	243.8	3844.6	152.7	2407.9
1966	243.8	3844.6	152.7	2407.9
1965	243.8	3844.6	152.7	2407.9

Appendix A.3. Detection and quantification of the micro-contaminants

The HPLC system consisted of a Varian ProStar 410 autosampler and a high-pressure gradient system of two Varian ProStar 210 pumps. A Varian 1200 L triple quadrupole mass spectrometer with electrospray interface (ESI) was used for detection and quantification. The MS/MS transitions of all injected micro-contaminants, their transformation products 1-methylxanthine, 3-methylxanthine, paraxanthine, theobromine, theophylline, and atenolol acid are presented in Table SI1. The transitions of the assigned internal standards are presented in Table SI2.

Method 1. For the chromatographic separation of atenolol, caffeine, carbamazepine, ibuprofen, paracetamol, 1-methylxanthine, 3-methylxanthine, atenolol acid, paraxanthine, theobromine, and theophylline, the Polaris C18-Ether HPLC column (150×2 mm, 3 µm particle size; Varian, Darmstadt, Germany) was used. The flow was 200 µL min^{-1}, the column was operated at 30 °C, and the injection volume was 100 µL. Eluent A was 0.015% formic acid + 1% methanol in ultrapure water, eluent B was methanol. The elution started with 5% B, followed by a linear gradient of 15 min to 35% B. This step was followed by a 5-min linear gradient to 95% B. This composition was held for 5 min followed by a 1-min linear gradient to 5% B, which was maintained for 9 min to equilibrate the system.

Method 2. For the analysis of acesulfame the ASCENTIS® Express OH5 (100×2.1 mm, 2.7 µm particle size; Sigma-Aldrich, Steinheim, Germany) HPLC column was used. The flow was 300 µL min^{-1}, the column was operated at 25 °C, and the injection volume was 5 µL. The separation was conducted isocratically by using 5% of eluent A (aqueous buffer of 20 mM ammonium acetate and 1% acetonitrile, adjusted to pH 3.5 with glacial acetic acid) and 95% eluent B (acetonitrile).

Table A.17. ESI-MS/MS conditions of the analytes.

Method	Compound	Quantifier	[a]Cap U [V]	[b]CE [V]	Qualifier	[a]Cap U [V]	[b]CE [V]	[c]Assigned IS No.
1	Acesulfame	162 > 82	−30	12.5	162 > 78	−30	28.5	1
	Cyclamate	178 > 80	−55	23.5				2
2	1-methylxanthine	165 > 108	−55	19.0	165 > 80	−55	25.0	9
	3-methylxanthine	165 > 122	−55	19.0	165 > 150	−55	18.0	9
	Atenolol	267 > 145	55	−20.0	267 > 190	55	−11.0	3
	Atenolol acid	268 > 145	60	−17.5	268 > 191	60	−12.0	3
	Caffeine	195 > 138	55	−9.5	195 > 110	55	−9.0	4
	Carbamazepine	237 > 194	45	−11.0	237 > 179	45	−27.0	5
	Ibuprofen	205 > 161	−25	5.5				6
	Paracetamol	152 > 110	40	−20.0	152 > 93	40	−18.5	7
	Paraxanthine	181 > 124	60	−8.0	181 > 96	60	−10.5	8
	Theobromine	181 > 138	55	−9.5	181 > 110	55	−13.0	9
	Theophylline	181 > 124	60	−8.0	181 > 96	60	−10.5	9

[a] Capillary voltage.

[b] Collision energy.

[C] Corresponding to Table SI2

Table A.18. ESI-MS/MS conditions of the internal standards.

Method	Compound	Quantifier	[a]Cap U [V]	[b]CE [V]	IS No.
1	Acesulfame-D_4	166 > 86	−30	12.5	1
	Cyclamate-D_{11}	189 > 80	−55	23.5	2
2	Atenolol-D_5	274 > 145	55	−17.5	3
	Caffeine-D_9	204 > 144	60	−8.5	4
	Carbamazepine-D_{10}	247 > 204	45	−13.0	5
	Ibuprofen-D_3	208 > 164	−25	6.0	6
	Paracetamol-D_4	156 > 116	40	−20.0	7
	Paraxanthine-D_6	187 > 127	60	−9.0	8
	Theobromine-D_6	187 > 144	55	−13.5	9

a Capillary voltage.

b Collision energy.

Appendix A.4. Modelling details.

The following remarks are based on Hillebrand et al. (2012). For breakthrough-curve interpretation a uniaxial two-region non-equilibrium advection dispersion model has been employed with CXTFit 2.0 (Toride et al., 1995). This approach considers two different regions within a karst conduit: (i) a mobile region, where water is displaced by plug flow and (ii) an immobile region, where water is retained in e. g. eddies or vortices. These regions have been described earlier in Field and Pinsky (2000), Hauns et al. (2001) or Geyer et al. (2007).

For solute transport the following processes are considered: advection, dispersion, mass transfer between the two fluid regions (mobile and immobile), reversible sorption and tracer attenuation (summarizing all possible processes that lead to a higher mass loss, than for the reference tracers, e. g. degradation). The analytical equations for the model are given as follows (modified from van Genuchten and Wagenet, 1989):

$$\beta R \frac{\partial c_m}{\partial t} = D \frac{\partial^2 c_m}{\partial x^2} - v \frac{\partial c_m}{\partial x} - \alpha(c_m - c_{im}) - \beta R \mu_1 c_m \quad \text{Eq. A.4}$$

$$(1-\beta) R \frac{\partial c_{im}}{\partial t} = \alpha(c_m - c_{im}) - (1-\beta) R \mu_2 c_{im} \quad \text{Eq. A.5}$$

with the retardation coefficient, defined as:

$$R = 1 + \frac{A}{V} K_a \quad \text{Eq. A.6}$$

for non-porous matrix blocks. The solute partitioning coefficient between the two fluid regions β is given as:

$$\beta = \frac{\theta_m + f(R-1)}{R} \quad \text{Eq. A.7}$$

t is time, x is the space coordinate, D is the dispersion coefficient, v is the average flow velocity, α is a first-order mass transfer coefficient between mobile and immobile fluid regions. c_m and c_{im} are the solute concentrations in, μ_1 and μ_2 are first-order attenuation rates within the mobile and immobile fluid region respectively. Comparable to Hillebrand et al. (2012), a uniform attenuation rate in the mobile and immobile region was considered ($\mu_1=\mu_2=\mu$). θ_m and θ_{im} are the volumetric fraction of the mobile and immobile fluid region respectively, while $\theta_m + \theta_{im}=\theta=1$ for a fully saturated conduit. A/V is the surface to volume ratio of a karst conduit, K_a is the linear distribution coefficient defined as the ratio of tracer mass per unit surface area of the solid phase to the unit concentration of the tracer within the conduit. The parameter f refers to the fraction of reversible adsorption sites that equilibrates with the mobile liquid phase. The retardation coefficient R captures the retardation of unpolar sorption as well as from reversible polar interactions as shown by Geyer et al. (2007). Rearranging eq. 4 and inserting physically reasonable values for f (between 0 and 1) allows constraining β (Geyer et al., 2007):

$$\frac{\theta_m}{R} \leq \beta \leq 1 - \frac{\theta_{im}}{R} \quad \text{Eq. A.8}$$

Fitting the breakthrough-curve of a conservative tracer yields estimates for the parameters v, D, α and θ_m. The application of uranine as conservative tracer in karst hydrology has been shown in several large scale field studies (Birk et al., 2005, Geyer et al., 2007, Hillebrand et al., 2012). Conservative transport parameters can be assumed to be equal for conservative and reactive solute tracers (Geyer et al., 2007). Consequently, the calibration of the reactive transport model is reduced to the transport parameters R, β and the attenuation coefficient μ if a conservative reference tracer is applied simultaneously. As the retardation coefficient R has never exceeded 1.01 for any analyte, it has been set to 1 and kept constant during all fitting procedures

As transport distance, the linear distance of 3000 m between the injection-point and the Gallusquelle spring was used. The initial values for v, D and θ_m for the calibration of the model were taken from Hillebrand et al. (2012). Estimates for α are not generally possible (Field and Pinsky, 2000).

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van Genuchten MT, Wagenet R, 1989. Two-site/two-region models for pesticide transport and degradation: theoretical development and analytical solutions. Soil Science Society of America Journal 53 (5), 1303–1310.

B Appendix

List of all journal articles, conference abstracts, and miscellaneous publications authored or co-authored by me and directly related to the presented work (latest update 08/2014).

Journals (Peer-Reviewed)

Hillebrand O, Nödler K, Licha T, Sauter M, Geyer T, 2012. Caffeine as an indicator for the quantification of untreated wastewater in karst systems. Water Research 46 (2), 395–402.

Hillebrand O, Nödler K, Licha T, Sauter M, Geyer T, 2012, Identification of the attenuation potential of a karst aquifer by an artificial dualtracer experiment with caffeine. Water Research 46 (16), 5381–5388.

Hillebrand O, Musallam S, Scherer L, Nödler K, Licha T, 2013. The challenge of sample-stabilisation in the era of multi-residue analytical methods: a practical guideline for the stabilisation of 46 organic micropollutants in aqueous samples. Science of the Total Environment 454–455, 289–298.

Nödler K, **Hillebrand O**, Idzik K, Strathmann M, Schiperski F, Zirlewagen J, Licha T, 2013. Occurrence and fate of the angiotensin II receptor antagonist transformation product valsartan acid in the water cycle – A comparative study with selected β-blockers and the persistent anthropogenic wastewater indicators carbamazepine and acesulfame. Water Research 47 (17), 6650–6659.

Hillebrand O, Nödler K, Geyer T, Licha T, 2014. Investigating the dynamics of two herbicides at a karst spring in Germany - consequences for sustainable raw water management. Science of the Total Environment 482–483, 193–200.

Reh R, **Hillebrand O**, Geyer T, Nödler K, Licha T, Sauter M, 2014. Charakterisierung zweier Karstsysteme mit Hilfe organischer Spurenstoffe. Grundwasser (accepted manuscript), doi: 10.1007/s00767-014-0264-6.

Conference Abstracts

Hillebrand O, Nödler K, Licha T, Geyer T, Sauter M. 2011. Application of caffeine as indicator for the quantification of recharging wastewater in karst systems - A case study. Geophysical Research Abstracts 13, EGU2011-3377, EGU General Assembly 2011, Vienna, Austria.

Hillebrand O, Nödler K, Licha T, Geyer T, 2012. Multitracer-Test zur Bestimmung von Transport und *in-situ* Abbau organischer Spurenstoffe am Beispiel von Coffein. Schriftenreihe der Deutschen Gesellschaft für Geowissenschaften Heft 78 anlässlich der Jahrestagung 2012 der Fachsektion Hydrogeologie in der Deutschen Gesellschaft für Geowissenschaften (FH-DGG), p. 105, Dresden, Germany.

Hillebrand O, Nödler K, Licha T, Sauter M, Geyer T, 2012. Anwendung von Coffein als Indikator zur Quantifizierung von versickerndem Abwasser in Karstsystemen – Ein Fallbeispiel. Schriftenreihe der Deutschen Gesellschaft für Geowissenschaften Heft 78 anlässlich der Jahrestagung 2012 der Fachsektion Hydrogeologie in der Deutschen Gesellschaft für Geowissenschaften (FH-DGG), p. 145, Dresden, Germany.

Hillebrand O, Nödler K, Licha T, Geyer T, 2012. Abschätzungen des Transportverhaltens und der *in-situ* Degradation von Coffein in einem Karstgrundwasserleiter mithilfe eines Multitracer-Versuchs.

Referate zur Jahrestagung 2012 der Wasserchemischen Gesellschaft, pp. 365–369, Neu-Ulm, Germany.

Hillebrand O, Nödler K, Licha T, Geyer T, 2012. Multitracer test for the determination of transport and *in-situ* degradation of organic micro-contaminants in karst aquifers on the example of caffeine. Geophysical Research Abstracts 14, EGU2012-17-1, EGU General Assembly 2012, Vienna, Austria.

Hillebrand O, Nödler K, Licha T, Sauter M, Geyer T, 2013. Chemograph analysis of two herbicides in a German karst aquifer. Geophysical Research Abstracts 15, EGU2013-1503, EGU General Assembly 2013, Vienna, Austria.

Hillebrand O, Nödler K, Licha T, Geyer T, 2013. Auftreten und Varianz von Spurenstoffen an einer Karstquelle – Ergebnisse der Jahresmessung an der Gallusquelle. Wasser 2103 – Tagung der Wasserchemischen Gesellschaft, pp. 373–377, Goslar, Germany.

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The challenge of sample-stabilisation in the era of multi-residue analytical methods: A practical guideline for the stabilisation of 46 organic micropollutants in aqueous samples

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HIGHLIGHTS

- Stabilisation strategies for 46 micro-pollutants in water samples are evaluated.
- Influence of temperature, water matrix, two common biocides and SPE is investigated.
- Sodium azide is found to stabilise some, but not all analytes.
- Copper sulphate interferes with caffeine and other azole and imidazole structures.
- Solid phase extraction is determined to be the most promising stabilisation strategy.

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ABSTRACT

Water sample storage and stabilisation may affect data quality, if samples are managed improperly. In this study three stabilising strategies are evaluated for 46 relevant organic micro-pollutants: addition of the biocides (i) copper sulphate and (ii) sodium azide to water samples directly after sampling with subsequent sample storage as liquid phase and (iii) direct solid phase extraction (SPE), stabilising the samples by reducing the activity of water. River water and treated effluent were chosen as commonly investigated matrices with a high potential of biotransformation activity. Analyses were carried out for sample storage temperatures of 4 and 28 °C for water samples stored as liquid phase and for sample storage temperatures of 4, 20 and 40 °C for SPE cartridges. Cooling of water samples alone was not sufficient for longer storage times (>24 h). While copper sulphate caused detrimental interferences with nitrogen containing heterocyclic compounds, sodium azide proved to be a suitable stabilising agent. The best results could be obtained for SPE cartridges stored cool. Recommendations for samples preservation are provided.

1. Introduction

Within the last 20 years, researchers increasingly investigated the occurrence and fate of organic compounds in trace concentrations (μg L^{-1} to ng L^{-1}). These so-called micro-contaminants or micro-pollutants, such as pharmaceuticals and personal care products, endocrine disrupting compounds, pesticides and/or industrial chemicals at low concentrations were detected in virtually all parts of the water cycle (Focazio et al., 2008; Heberer, 2002; Schwarzenbach et al., 2006; Ternes, 2007; Weigel et al., 2001). Due to the diversity of these compounds, analytical methods focussing on only one class of compounds do not meet the requirements of current research undertaken in environmental sciences (Estévez et al., 2012; Nödler et al., 2011; Reh et al., 2013). However, thanks to significant progress in the field of analytical science several multi-residue analytical methods were developed (e.g. Huntscha et al., 2012; Nödler et al., 2010; Nurmi and Pellinen, 2011; Wode et al., 2012).

Although the diversity of compounds can nowadays be handled analytically by multi-residue analysis, the wide spectrum of compounds with various stabilities and reactivities (e.g. Nödler et al., 2010; Wode et al., 2012) results in a challenge for sample preservation. In cases when the immediate sample analysis is difficult or impossible (e.g. remote areas) or the sampling is intended to be realised over longer periods (e.g. weekly-integrated sampling; Kylin, 2013), the storage conditions become highly relevant (Barceló and Alpendurada, 1996; U.S. EPA, 2010; Vanderford et al., 2011). Especially for easily degradable compounds, their reliable determination largely depends on proper sample storage conditions. Various processes such as microbial degradation, chemical reactions, volatilisation or adsorption may occur even during relatively short sample storage times resulting in low analyte recoveries. For example, caffeine, ibuprofen and paracetamol (acetaminophen) are

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Table 1
Investigated analytes and their application/origin.

Application or origin	Compound	Application or origin	Compound
Analgesics/anti-inflammatories	Diclofenac	Lipid regulators	Bezafibrate
	Ibuprofen		Clofibric acid
	Naproxen		Gemfibrozil
	Paracetamol	Antihistamines	Cetirizine
	Phenazone		Loratadine
Stimulants/caffeine metabolites	Caffeine	Anticonvulsants/sedatives	Carbamazepine
	Paraxanthine		Diazepam
	Theobromine		Primidone
	Theophylline		Tetrazepam
	1-Methylxanthine	Selective serotonin reuptake inhibitors	Citalopram
	3-Methylxanthine		Fluoxetine
Antihypertensive agents	Atenolol		Sertraline
	Metoprolol	Herbicides/herbicide metabolites	Atrazine
	Sotalol		Desethylatrazine
Iodinated contrast media	Iohexol		Desisopropylatrazine
	Iomeprol		Diuron
	Iopamidol		Isoproturon
	Iopromide		Mecoprop
Antibiotics	Clarithromycin		Metazachlor
	Erythromycin	Corrosion inhibitors	1H-benzotriazole
	Roxithromycin		Tolyltriazole
	Sulfamethoxazole	Cocaine metabolite	Benzoylecgonine
	Trimethoprim	Gastric acid regulator	Pantoprazole

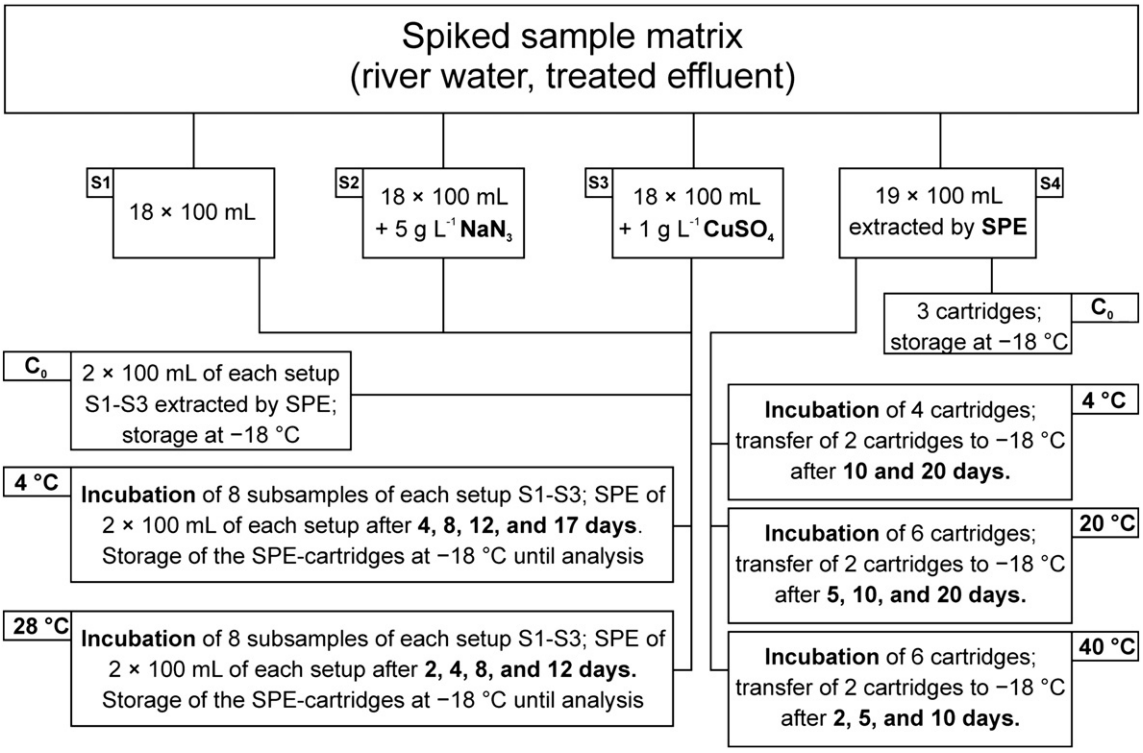
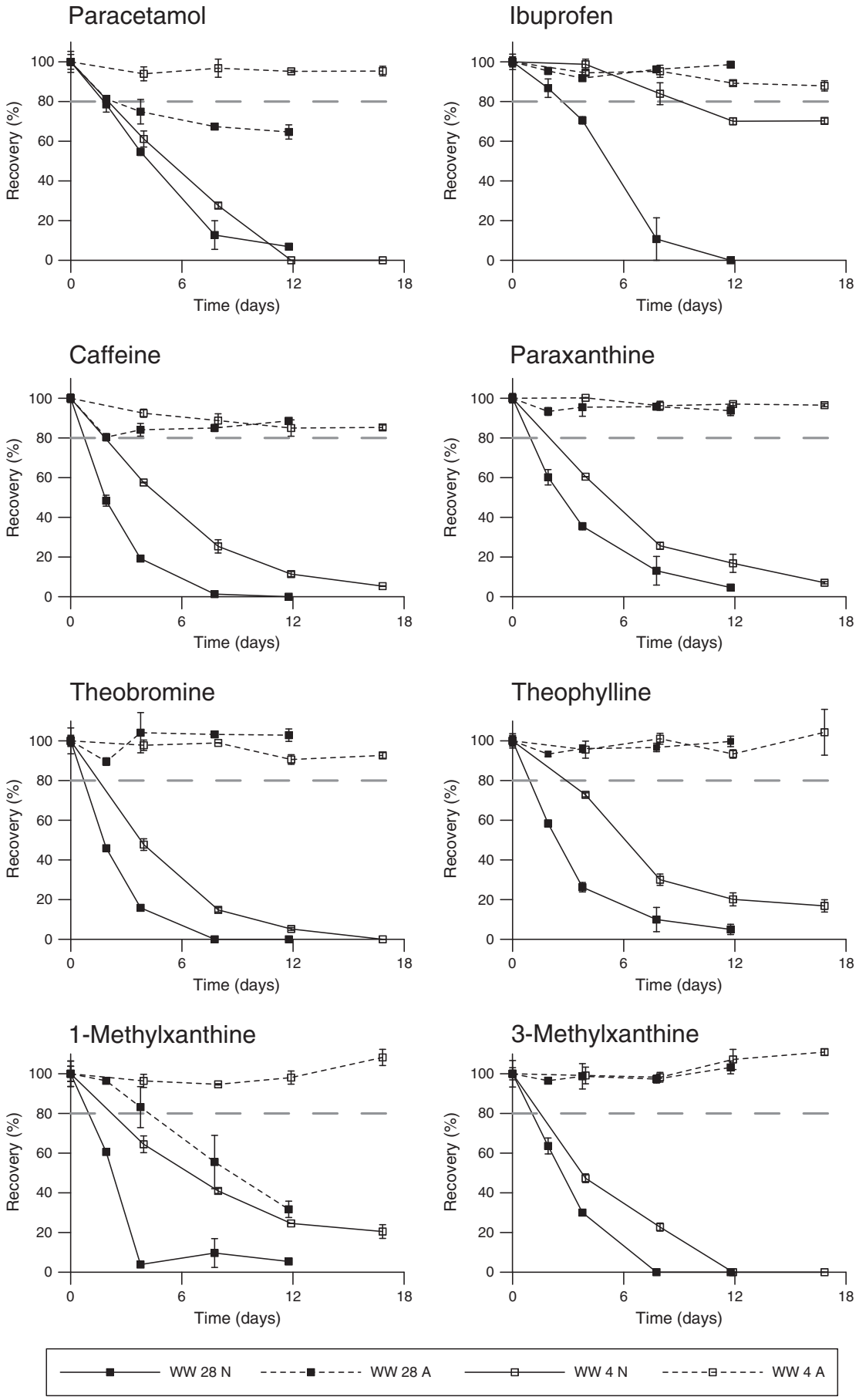


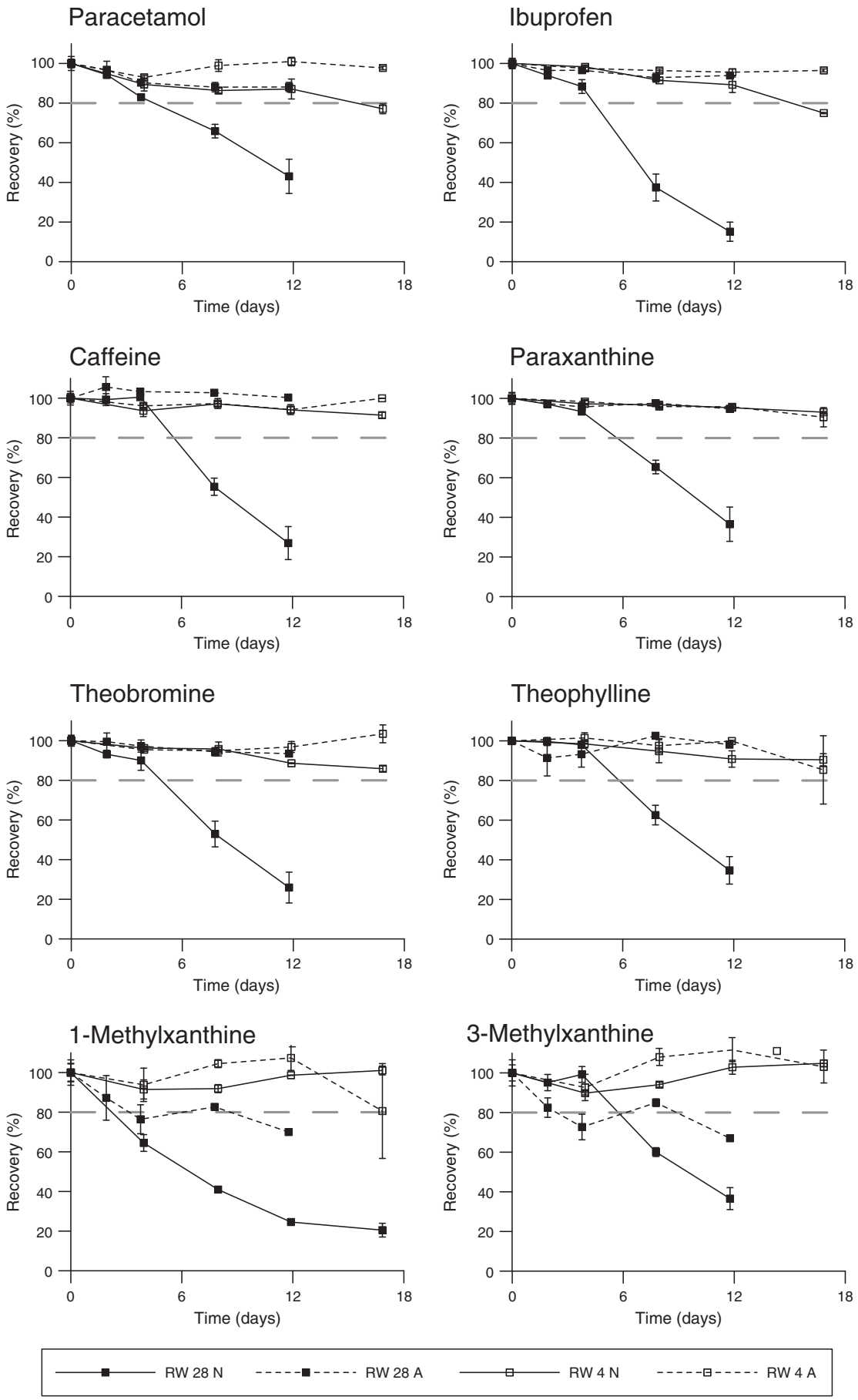
Fig. 1. Schematic overview of the experiments to investigate the influence of different stabilisation techniques (c_0 = initial concentration).

commonly investigated micro-contaminants and known to be easily degradable in wastewater treatment plants (WWTPs) and in the environment (e.g. Halling-Sørensen et al., 1998; Joss et al., 2006) while carbamazepine is known to be a very stable compound (Clara et al., 2004; Gasser et al., 2010). Acknowledging the large range of stability encountered for compounds in multi-residue analysis, it is obvious that a proper sample pre-treatment and storage is essential to obtain reliable results. Thus, sample stabilisation methods should be applied to minimise concentration changes between sampling and analysis. These methods are most common in inorganic analysis and include addition of chemicals, cooling, pH-modifications and choice of storage container.

For micro-contaminants the influence of storage temperatures, the material of the storage container and different quenching agents have been investigated for water samples, stored as liquid phase (U.S. EPA, 2010; Vanderford et al., 2011). As stabilising agents sodium

Fig. 2. Recoveries of selected analytes in WWTP treated effluent with respect to storage time; stored as liquid (WW 28 N = non-stabilised wastewater sample stored at 28 °C; WW 28 A = wastewater sample stored at 28 °C, stabilised with NaN_3; WW 4 N = non-stabilised wastewater sample stored at 4 °C; WW 4 A = wastewater sample stored at 4 °C, stabilised with NaN_3; the dashed grey line at 80% indicates the significance threshold).


Paracetamol
Ibuprofen
Caffeine
Paraxanthine
Theobromine
Theophylline
1-Methylxanthine
3-Methylxanthine
Recovery (%)
Time (days)
0
20
40
60
80
100
0
6
12
18
WW 28 N
WW 28 A
WW 4 N
WW 4 A


Paracetamol
Ibuprofen
Caffeine
Paraxanthine
Theobromine
Theophylline
1-Methylxanthine
3-Methylxanthine
Recovery (%)
Time (days)
0
20
40
60
80
100
0
6
12
18
RW 28 N
RW 28 A
RW 4 N
RW 4 A

azide and sulphuric acid have been tested (Vanderford et al., 2011). However, these recent investigations focussed on the sample treatment of water samples stored as liquid phase, although the advantages of using SPE-cartridges for sample stabilisation have been recognised several years ago (Barceló and Alpendurada, 1996).

To inhibit biological degradation in water samples, two biocidal additives which can be used are sodium azide and copper sulphate. Sodium azide is frequently used in laboratory studies (e.g. Vanderford and Snyder, 2006), especially to produce abiotic reference samples in degradation experiments (e.g. Margesin et al., 2000; Ying et al., 2008) and has been described by Vanderford et al. (2011) as the most benign of the investigated preservatives for sample stabilisation. Copper sulphate is particularly applied for the stabilisation of phenols and phenolics (DIN 38409–16, 1984; Hossain and Salehuddin, 2009). A common non-chemical stabilisation technique is solid phase extraction (SPE). By reducing the water activity, the microbial growth can be controlled (Madigan et al., 2003).

The aim of this study was to evaluate the influence of the water sample matrix, the storage temperature, the addition of two selected chemical preservatives and the direct application of SPE on the recovery of 46 analytes. The here investigated micro-contaminants comprise of a large variety of different compound-classes including readily degradable and highly persistent compounds.

2. Methods and materials

2.1. Chemicals

Sodium azide (NaN_3) and copper sulphate pentahydrate ($CuSO_4 \cdot 5 H_2O$) were purchased from Fisher Scientific (Schwerte, Germany). The suppliers of all target analytes, the internal standards (IS), the SPE cartridges, and all other reagents were published previously (Nödler et al., 2010). The investigated trace organic compounds are presented in Table 1.

2.2. Sample preparation

A schematic overview of the experiments is presented in Fig. 1. Water samples were collected by using 1 L and 2 L clear-glass bottles, pre-rinsed with the respective water sample. The samples were taken from the effluent of the wastewater treatment plant (WWTP) Göttingen (Germany, ~120,000 inhabitants) and the Leine River (Göttingen, Germany). Under dry weather discharge conditions, the mean hydraulic residence time within the WWTP was 20-24 h. The treatment processes consisted of a mechanical treatment for the separation of solid material followed by activated sludge treatment, including nitrification and denitrification. Additionally, chemical P-removal was performed. During a previously published study, the treated effluent was analysed on a daily basis for 27 days and easily degradable compounds such as ibuprofen, caffeine and its degradation products were not detected (Nödler et al., 2011). Therefore, the presence of highly adapted micro-organisms can be assumed, which underlines the big challenge of stabilising these compounds in this sample matrix. The presence of anthropogenic micro-pollutants in the Leine River was also demonstrated in previous studies (Nödler et al., 2010, 2011). Therefore, adapted micro-organisms were expected in both matrices.

2.2.1. Sample subsets S1–S3

Composite samples of 6.5 L river water and treated effluent, respectively, were prepared and spiked with 650 μL stock solution containing all analytes. The stock solution was prepared in 50/50 water/methanol (v/v); the final methanol concentration in the water samples was therefore 0.005% (v/v). Spike levels of 2 μg L^{-1} of each individual iodinated contrast media and the individual concentration of 1 μg L^{-1} of all other compounds were applied. The spiked composite sample was stirred for 30 min by a magnetic stirrer. Aliquots of 100 mL sample were taken by a 100 mL glass pipette and transferred into 100 mL clear-glass and screw cap bottles. As the samples were not filtered, stirring was applied to enable the transfer of representative aliquots including dispersed particles. Fifty-four 100 mL sub-samples were prepared. Of each sample matrix 18 sub-samples were spiked with 1 mL of an aqueous NaN_3 stock solution resulting in a final concentration of 5 g L^{-1} NaN_3 (Wender et al., 2000; Ying et al., 2008). Another 18 aliquots were spiked with 1 mL of an aqueous $CuSO_4 \cdot 5 H_2O$ solution resulting in a concentration of 1 g L^{-1} $CuSO_4$ (DIN 38409–16, 1984). However, acidification of the Cu-stabilised samples as recommended by the DIN standard (DIN 38409–16, 1984) was not applied, as some of the analytes are sensitive to low pH-values. To the remaining 18 aliquots 1 mL ultrapure water was added to keep the sample volumes comparable to the stabilised samples. Because some of the analytes were already present in the native samples (Nödler et al., 2010, 2011), duplicates of each spiked sample matrix were immediately extracted by SPE to determine the here applied initial concentration of the analytes (c_0).

Atenolol acid was identified by Radjenovic et al. (2008) and Barbieri et al. (2012) as a microbial transformation product (TP) of atenolol, generated by hydrolysis of its amide bond. Therefore, the compound was monitored to evaluate the fate of atenolol in the prepared subsets. The analysis was performed according to Reh et al. (2013).

To simulate the impact of the preservatives depending on the storage temperature, samples were stored in a refrigerator (4 °C) and in an incubator (28 °C), respectively. All samples were covered to prevent photodegradation. The incubation of the samples was terminated according to the schedule presented in Fig. 1 and samples were immediately extracted. For the extraction, the sample (100 mL) was spiked with 10 μL of an IS-mix (for details on the used internal standards please refer to Nödler et al., 2010; Reh et al., 2013 or SI-Table 1) and 1 mL of a phosphate buffer concentrate (pH 7) and extracted by SPE (OASIS® HLB) according to Nödler et al. (2010). After extraction the cartridges were sealed with parafilm, covered in alumina foil, and stored in a freezer at −18 °C until elution and analysis. It is assumed that storing the SPE cartridges at −18 °C stabilises all analytes. Alterations of the samples during this storage phase are not part of this manuscript.

2.2.2. Sample subset S4

River and treated effluent matrix were spiked and 19 100 mL subsamples of each matrix were extracted by SPE similar to the other subsets. However, the samples were not spiked with the above mentioned IS-mix prior to the SPE. The loaded cartridges were incubated according to Fig. 1 in a GC-oven (40 °C; Chrompack CP 9001), in a temperature-controlled laboratory (20 °C; protected from light) and in a refrigerator (4 °C). The minimum and maximum temperatures were monitored and the deviation did not exceed 1 °C. In comparison to the native water sample the SPE process reduces the water activity. As this is a well-known strategy in microbial growth control (Madigan et al., 2003), the effect of biotransformation on the analytes was suspected to be significantly lower than in the subsets S1–S3. Therefore, in comparison with S1–S3 a higher maximum incubation temperature (40 °C) was chosen.

Fig. 3. Recoveries of selected analytes in river water with respect to storage time; stored as liquid (RW 28 N = non-stabilised river water sample stored at 28 °C; RW 28 A = river water sample stored at 28 °C, stabilised with NaN_3; RW 4 N = non-stabilised river water sample stored at 4 °C; RW 4 A = river water sample stored at 4 °C, stabilised with NaN_3; the dashed grey line at 80% indicates the significance threshold).

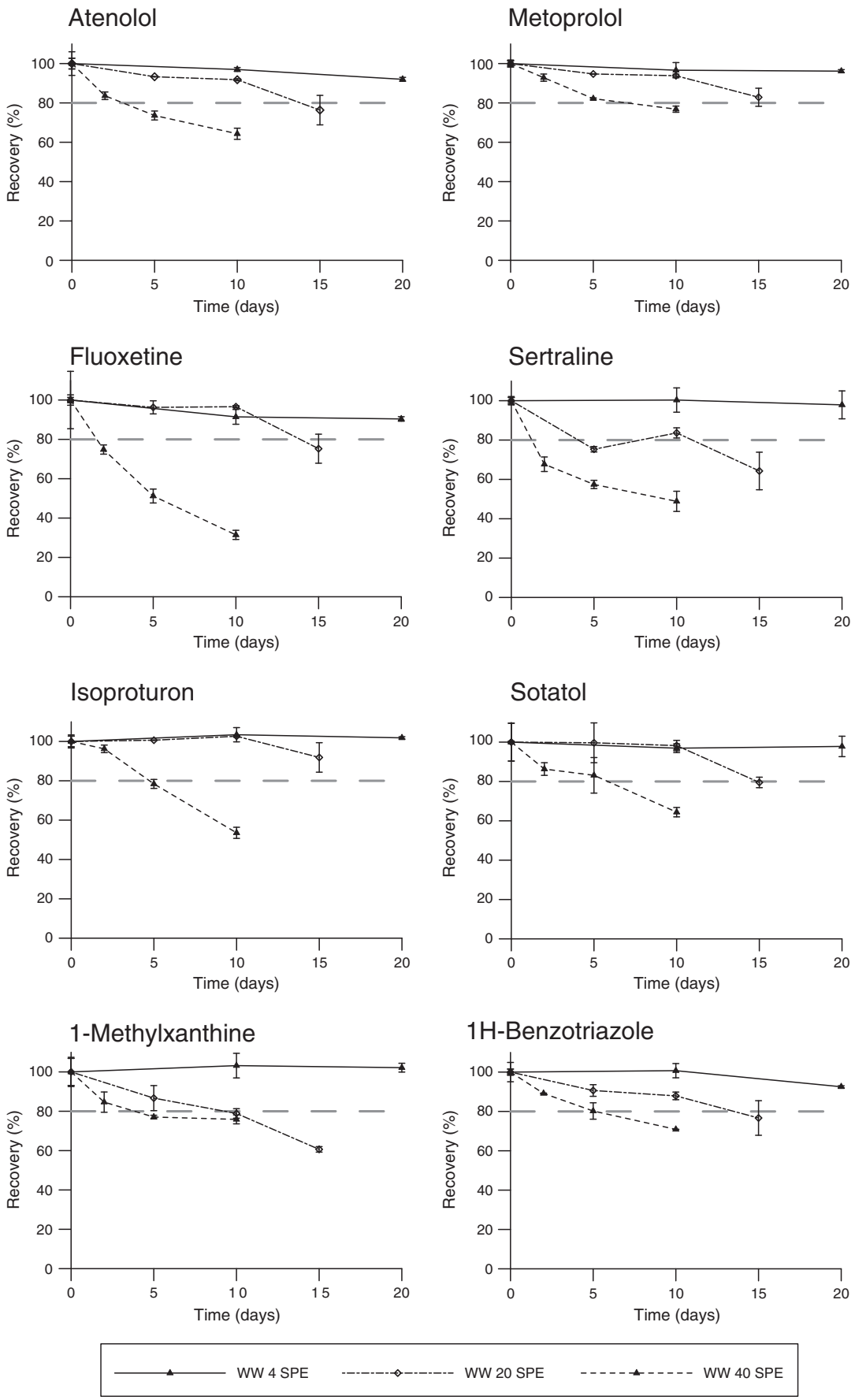


Fig. 4. Observed recoveries of selected analytes after solid phase extraction of spiked wastewater samples (WW 4 SPE = cartridges stored at 4 °C; WW 20 SPE = cartridges stored at 20 °C; WW 40 SPE = cartridges stored at 40 °C; the dashed grey line at 80% indicates the significance threshold).

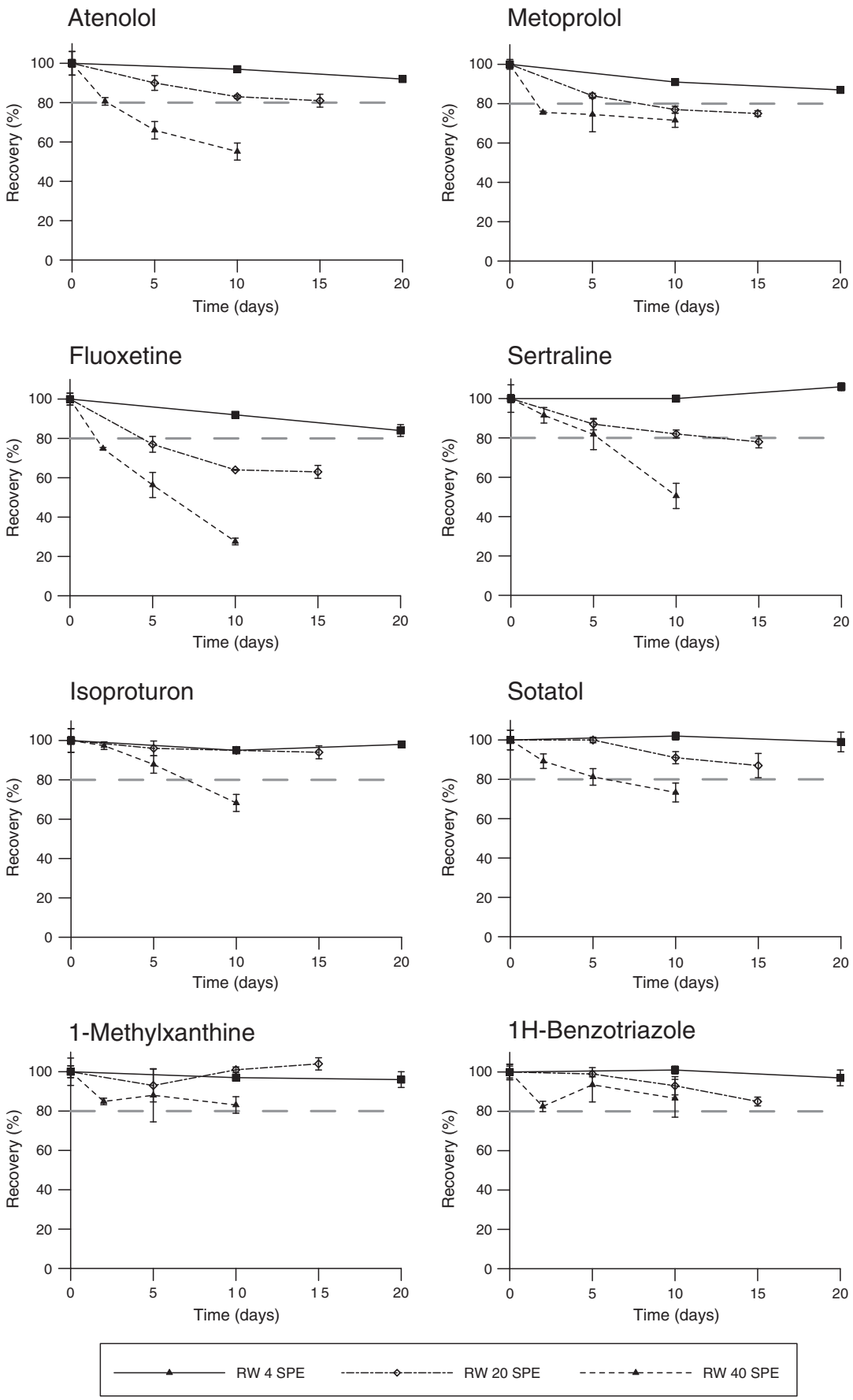


Fig. 5. Observed recoveries of selected analytes after solid phase extraction of spiked river water samples (RW 4 SPE = cartridges stored at 4 °C; RW 20 SPE = cartridges stored at 20 °C; RW 40 SPE = cartridges stored at 40 °C; the dashed grey line at 80% indicates the significance threshold).

2.3. Elution from the SPE-cartridge and analysis of the analytes

The analytes (subsets S1–S3) were eluted with methanol and ethyl acetate under vacuum (flow rate ~1 mL min^{-1}). The solvents were evaporated to dryness at 40 °C with a gentle stream of nitrogen and re-dissolved in 1 mL of aqueous 5 mM ammonium acetate solution, containing 4% methanol. The extract was transferred into an autosampler vial and centrifuged for 10 min (4000 rpm). The compounds were analysed with a multi-residue analytical method based on high performance liquid chromatographic separation coupled to an electrospray ionisation with tandem mass spectrometric detection (HPLC/MS–MS; Nödler et al., 2010). The extracts of subset 4 were spiked with 10 μL of the above mentioned IS-mix before the evaporation step of the solvents. The further procedure and analysis was according to the subsets S1–S3.

3. Results and discussion

A significance level of 80% is assumed in all experiments i.e. if the recovery of the analyte is reduced by less than 20% over the period of observation, it is declared to be insignificant and acceptable.

3.1. Water samples stored as liquid phase

3.1.1. Stability of compounds in non-stabilised water samples (subset 1)

Pantoprazole exhibited incomparable duplicates for river water (RW) and was therefore discarded from further analysis. For the treated effluent matrix (WW) a significantly low recovery could be observed at 28 °C for the stabilised as well as the non-stabilised samples. Out of the remaining 45 micro-pollutants, 18 proved to be stable (recovery ≥80%) in both water matrices (river and WWTP effluent) at 4 and 28 °C although non-stabilised. The stable substances are, among others, all but one investigated contrast media, both antihistamines and all anticonvulsants and sedatives (cf. Table 1). This was expected since their stability is well known. The persistence of carbamazepine, for example, was demonstrated in previous studies (Castiglioni et al., 2006; Clara et al., 2004). For a table with all spiked compounds and their respective recoveries, see SI-Table 2 and 3.

The analytes, for which unacceptable recoveries have been observed at the end of the investigation period, were generally the same for both water matrices.

It can be assumed that, in sewage, more micro-organisms are present and they readily cause a more efficient transformation, whereas in natural water (e.g. river water) the bacteria require a longer lag phase to adapt to changed conditions (Madigan et al., 2003). Thus, it can be expected that recoveries from WW are generally lower than from RW. This is partially confirmed by the presented study. However, for the compounds atenolol, metoprolol, iomeprol, sulfamethoxazole, bezafibrate, fluoxetine, sertraline, desisopropylatrazine, 1H-benzotriazole and benzoylecgonine a lower recovery could be observed in the RW samples.

The concentration of atenolol acid in the RW stored at 28 °C increased from 25 ng L^{-1} (present in the native sample) to 250 ng L^{-1} at the end of the incubation period. Assuming the TP being stable within the investigated period, ~30% of the atenolol loss can be attributed to the formation of atenolol acid.

Typically, higher temperatures (within the physical range of micro-organisms) promote the microbial growth and activity, whereas lower temperatures are inhibitory (Castiglioni et al., 2006; Kang and Kondo, 2002; Vieno et al., 2005). Accordingly, except for clofibric acid, sertraline, diuron and isoproturon in WW as well as tolyltriazole in RW, all compounds demonstrated higher recoveries in the cooled samples. The substances with the lowest recovery were methylxanthines (caffeine, paraxanthine, theobromine, theophylline, 1-methylxanthine, 3-methylxanthine), ibuprofen and paracetamol; rapidly decreasing recoveries were observed in the WW samples for both temperatures (Fig. 2). In the RW samples ibuprofen and paracetamol exhibited unacceptably low recoveries at both temperature levels at the end of the observation period. For the methylxanthines, this holds only true for the higher temperature level of 28 °C (Fig. 3).

3.1.2. Stabilisation with sodium azide (subset 2)

Sodium azide inhibits microbial activity and growth (e.g. Margesin et al., 2000; Ying et al., 2008). After two days of incubation at 28 °C, the non-stabilised samples exhibited a clearly visible turbidity. In contrast, the stabilised samples hardly manifested any turbidity. This may be interpreted as an indication for the reduced microbial activity of the stabilised samples.

The addition of sodium azide generally led to higher recoveries of the analytes in the samples, relative to non-stabilised samples. This was observed for all analytes in the WW samples. However, anomalies were observed in the RW samples for naproxen, iomeprol, iopamidol, bezafibrate, clofibric acid, gemfibrozil, sertraline and diuron at 4 °C and for tolyltriazole at 28 °C. Several authors describe interferences of sodium azide with some analytes resulting in a transformation (Chefetz et al., 2006; Lichtenstein et al., 1968; Sharom et al., 1980), which may explain the observations. Chefetz et al. (2006) observed a nucleophilic aromatic substitution reaction: the chlorine atom of the atrazine was replaced by the azide group. This may explain the low recoveries of bezafibrate, clofibric acid, sertraline and diuron in the stabilised samples. However, it does not explain the low recoveries of the other analytes and why it was observed for 4 °C but not for 28 °C. Grenni et al. (2013) found gemfibrozil and naproxen to be biodegradable in river water, while these compounds were observed to be stable in sterilised river water samples. One may read this as an indication that micro-organisms, responsible for the degradation of gemfibrozil and naproxen, are either affected to a lower extend or not affected at all by sodium azide.

It is noteworthy, that Vanderford et al. (2011) found recoveries to be unacceptable for atenolol and fluoxetine in water samples, stabilised with sodium azide and stored at 4 °C. This cannot be confirmed from the observations of this study. However Vanderford et al. (2011) used higher storage times and a slightly more strict level of significance.

While the easily degradable compounds from the methylxanthines group, ibuprofen and paracetamol demonstrated rapidly decreasing recoveries in the non-stabilised samples, they could be successfully stabilised with sodium azide in both water matrices between 2 and 17 days, depending on the analyte (Figs. 2 and 3). In the stabilised and cooled samples all these compounds exhibit acceptably high recoveries over the entire investigated period. For paracetamol and 1-methylxanthine a higher, but still unacceptably low recovery, was observed over the observation period in the WW samples at 28 °C. Thus, it can be assumed that biodegradation is not the only effect, which influences the stability of these compounds in the aqueous phase.

For a table with all spiked compounds and their respective recoveries, see SI-Table 4 and 5.

3.1.3. Stabilisation with copper sulphate (subset 3)

The stabilisation of the samples with copper sulphate led to significant analytical problems. On the one hand, the addition of the stabilising additive led to a milky-blue precipitate which is assumed to be copper-(II)-hydroxide ($Cu(OH)_2$) given its low solubility ($K_{SP} = 2.20 \times 10^{-20}$; Patnaik, 2003). Due to this precipitate SPE was difficult without prior filtration. On the other hand, the methylxanthines exhibited poor recoveries in all samples. Tolyltriazole could hardly and pantoprazole and 1H-benzotriazole could not be detected at all. Probably this is caused by complexation with copper: all the above mentioned compounds comprise an azole structure. 1H-benzotriazole and tolyltriazole are used as corrosion inhibitors for metals including copper. After adsorption of the inhibitor on the copper surface a copper–azole complex is formed (Subramanian and

Lakshminarayanan, 2002). In a third step, polymerisation can occur (Antonijevic and Petrovic, 2008). Imidazole and its derivatives are also efficient copper corrosion inhibitors in various media (Stupnisek-Lisac et al., 2002; Subramanian and Lakshminarayanan, 2002).

In conclusion, it can be assumed that the methylxanthines and pantoprazole containing imidazole-like structures can also form complexes with copper resulting in the observed low to very low recoveries. Due to these problems a further discussion of the results from these samples is excluded.

3.2. Stabilisation by SPE (subset 4)

By SPE of the water samples, the water activity and the concentration of inorganic nutrients were reduced. Although for the high storage temperature (at 40 °C) low recoveries were expected, 33 of the 46 analytes could be stabilised over the complete observation period of 10 days. Compounds that showed recoveries lower than the significance level of 80% include all antihypertensive agents, all SSRIs, both corrosion inhibitors, 1-methylxanthine, 3-methylxanthine, loratadine, diuron and isoproturon. The most labile compounds in the non-stabilised samples stored as liquid phase (methylxanthines, paracetamol and ibuprofen) exhibit much higher (1-methylxanthine and 3-methylxanthine) or acceptable recoveries after SPE, whereas fluoxetine, sertraline and atenolol show the lowest recovery of all analytes at the end of the investigation period (28, 49 and 55% respectively; Figs. 4 and 5). Despite the relatively low recovery of atenolol, increasing concentrations of atenolol acid were not observed.

At 4 °C and over the entire investigation period of 20 days no significant decrease in recovery could be observed for any of the investigated compounds. Comparing the water matrices with one another, in general no differences can be observed. In fact, samples from WW and RW showed rather similar recoveries for some of the compounds (e.g. fluoxetine and atenolol). For a table with all spiked compounds and their respective recoveries after SPE, see SI - Table 6 and 7.

4. Conclusions

In the water samples stored as liquid phase the methylxanthines, ibuprofen and paracetamol were ascertained to be among the lowest recovered micro-contaminants in non-stabilised samples of both investigated water matrices, RW and WW. These compounds are valuable indicators for untreated sewage (Bound and Voulvoulis, 2006; Buerge et al., 2006; Hillebrand et al., 2012) and immediate sample preparation and analysis would be the best option to prevent low recoveries due to storage. However, depending on the infrastructure this option may not be feasible and the transport of the samples to the laboratory may take a considerable time.

Stabilising the samples with sodium azide led to significantly higher recoveries in both water matrices. Nevertheless, for some analytes unacceptable recoveries were observed.

The stabilisation of the water samples with copper sulphate caused detrimental interferences with all the methylxanthines, the corrosion inhibitors and pantoprazole, most likely due to the formation of copper–azole complexes. It can be concluded that copper sulphate is an unsuitable stabilising additive for micro-pollutants in water samples when stored as liquid phase especially, if azole- or imidazole-like compounds are to be included in the list of analytes.

Processing the water samples by SPE showed the best results of all stabilising strategies. While for some analytes recoveries ≤80% could be observed at 20 and 40 °C, storing the SPE cartridges at 4 °C led to acceptable recoveries over the whole observation period of 20 days for all investigated analytes.

Concluding from our presented results, the following recommendations for sample preparation and storage can be derived (from best, to worst alternative):

1) Immediate analysis of the samples
2) SPE directly after sampling with SPE cartridge, store as cool as possible
3) Stabilisation of the samples with sodium azide and store as cool as possible
4) Storage of non-stabilised samples as cool as possible

If no immediate analysis is possible, the storage time should be minimised. Depending on the water matrix sampled a ranking can be set up, reflecting its need for sample stabilisation. First, WW samples need to be stabilised. Due to their high number of adapted micro-organisms, the stabilisation of these samples is most urgent. Second, RW samples need to be analysed or otherwise stabilised.

Although groundwater and drinking water have not been investigated in the course of this article, the following can be assumed: for groundwater, which is known to be less loaded with micro-organisms (Schijven et al., 2003; Toze, 2004) as well as drinking water, much less alteration of the analytes is expected. Hence, the analysis or stabilisation of respective samples need to be performed the least urgent.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2013.03.028.

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Investigating the dynamics of two herbicides at a karst spring in Germany: Consequences for sustainable raw water management

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HIGHLIGHTS

- Atrazine can almost always be detected in spring water.
- Metazachlor is only detectable after recharge events and not in winter.
- Atrazine, inorganic cations (Ca^{2+} and Mg^{2+}) and the electrical conductivity correlate.
- The long-term storage potential of karst aquifers may not be ignored.
- Persistent substances are prone to cause long-term contamination in karst systems.

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ABSTRACT

While karst aquifers are considered as rapid flow and transport systems, their high potential for long-term storage is often ignored. However, to achieve a sustainable raw water quality for drinking water production, the understanding of this potential is highly essential. In this study, the transport dynamics of the two herbicides metazachlor and atrazine as well as a degradation product of the latter (desethylatrazine) were investigated at a karst spring over 1 year. Even 20 years after its ban in Germany, atrazine and its degradation product were almost always detectable in the spring water in the low ng L^{-1} range (up to 5.2 ng L^{-1}). Metazachlor could only be detected after precipitation events, and the observed concentrations (up to 82.9 ng L^{-1}) are significantly higher than atrazine or desethylatrazine. Comparing the dynamics of the herbicides with the inorganic ions Ca^{2+}, Mg^{2+} and electrical conductivity, a positive correlation of atrazine with these parameters could be observed. From this observation, atrazine is concluded to be located within the aquifer matrix. To achieve a sustainable raw water management at karst springs, the rapidness of these systems needs to be highlighted as well as their long-term storage potential. Persistent substances or transformation products are prone to deteriorate the raw water quality for decades.

1. Introduction

In the *Guidelines for Drinking-Water Quality*, the World Health Organisation emphasises the advantages and necessities of effective catchment management, i.e., understanding an aquifer and identifying possible water pollution scenarios affecting the raw water quality (WHO, 2011). The understanding of karst aquifers is particularly challenging due to their specific characteristics (e.g., dolines, conduit flow). Still, these highly dynamic and heterogeneous aquifer systems are important drinking water sources all over the world. The complex interaction between developed karst conduits including the related rapid flow and transport processes in them (residence time of a few days, e.g., Pronk et al., 2009; Hillebrand et al., 2012a) and the high-volume porous rock matrix (characterised by slow matrix flow and long residence times of several years, e.g., Einsiedl, 2005) is not yet fully understood and thus still subject to research. Investigating the recharge mechanisms at a shallow karst system rapid preferential flow and diffuse matrix flow (which is characterised by much slower flow rates) were observed (Atkinson, 1977; Haria et al., 2003). However, for some deep aquifers, only slow matrix flow could be identified (Haria et al., 2003; Chilton et al., 2005).

It is a long established fact that recharge events in karst systems lead to strong variations in spring water quality (Jakucs, 1959). Monitoring these spring signals in terms of physical or chemical parameters allows for the integral characterisation of the total catchment area. This feature has been used to, e.g., determine the mean residence time of water within aquifer systems based on tritium data (Maloszewski et al.,

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2002) or to estimate the total amount of wastewater infiltrating such systems by employing caffeine as a semi-quantitative indicator (Hillebrand et al., 2012a). Stueber and Criss (2005) derived the primary immediate sources for water quality components depending on their covariance with the electrical conductivity (EC) or the turbidity. A positive covariance of components time series with the EC implies diffuse (matrix) flow being its primary source, while a positive covariance with turbidity suggests that the components immediate source was from agricultural fields.

In the presented work, two herbicides (atrazine and metazachlor) and the degradation product desethylatrazine are employed, in order to improve the understanding of spring water signals after precipitation events and consequently the understanding of the investigated karst aquifer system, which are vital for providing measures for sustainable raw water quality. Atrazine is one of the most widely used soil and weed herbicides, whereas its use has been prohibited in Germany since 1992. However, it is well dispersed and can still be found in the environment even after more than 20 years (Jablonowski et al., 2011; Nödler et al., 2013; Reh et al., 2013). The potential of atrazine to be degraded in karst aquifers is stated to be very little to non-existent (Johnson et al., 2000; Chilton et al., 2005). One of its degradation products is desethylatrazine (Kolpin et al., 1998). However, desethylatrazine is also formed from other triazine herbicides like propazine (Behki and Khan, 1994). Atrazine is affected by sorption, exhibiting a low desorption rate, which may take several days or even weeks (Dehghani et al., 2005). In contrast to the banned substance atrazine, the weed control agent metazachlor is approved in Germany. Its tendency to adsorb onto soil material is known to be low (Mamy and Barriuso, 2005), while being readily degradable (Allen and Walker, 1987; Beulke and Malkomes, 2001). In the investigated karst system, transport is known to be rapid and an appearance of metazachlor in spring water can still be expected. For reference purposes and to locate the origin of atrazine, desethylatrazine and metazachlor, the time series of these three compounds are compared to the time series of the inorganic ions nitrate (NO_3^-), calcium (Ca^{2+}) and magnesium (Mg^{2+}) as well as the EC of the spring water.

The aims of the study are (i) to improve the understanding of contaminant migration in karst aquifers under consideration of recent and former herbicide applications, (ii) to highlight the long-term storage potential of karst aquifers and (iii) to draw attention to the consequences of unsustainable herbicide application for the raw water quality. The authors hypothesise that the characteristic residence time distribution of water in karst aquifer systems (days to several decades) is reflected in the occurrence and dynamics of the investigated herbicides.

2. Materials and methods

2.1. Field work

2.1.1. Study area

The investigated karst spring is the Gallusquelle, which is located in Southwest Germany (Fig. 1). It is used as a drinking water source for 40,000 people. Its average discharge is 500 L s^{-1}, draining a rural catchment (4,000 inhabitants) of approximately 45 km^2. Around 40% of the catchment is used for agriculture. These areas are used as grasslands and for the cultivation of crops (approximately 14% of the total catchment area; Sauter, 1992). Despite the thick unsaturated zone (~100 m, Fig. 1) within the investigated system, precipitation can quickly reach the groundwater through dolines and dry valleys as concentrated recharge. Through these preferential flow paths, the transport of solutes including contaminants from the ground surface toward the spring is enhanced. The occurrence of contaminants only days after precipitation events has been shown in former investigations (Heinz et al., 2009; Hillebrand et al., 2012b). In contrast, a mean groundwater residence time of more than 20 years was determined by Geyer et al. (2011) employing lumped parameter modelling of tritium in spring water.

2.1.2. Sampling

Over the period of nearly 1 year, a total of 263 spring water samples were collected and analysed for herbicides from March 6, 2010, until February 16, 2011. The sampling rate varied between weekly, daily and multiple daily depending on the spring discharge and the occurrence of recharge events. For one recharge event, a highly increased sampling rate of up to 8 samples per day was realised. Selected major ions concentrations were determined over a period of 3 months, including the mentioned recharge event ($n = 153$). Additionally, a rain water sample was collected during that recharge event with a precipitation-totalisator (accumulative precipitation gauge) for the hydrograph separation. To ensure the stability of the analytes, the samples were stored at 4 °C. For herbicides, samples were preconcentrated by solid phase extraction (SPE) within 48 h and the SPE cartridges were stored at −18 °C until analysis (Hillebrand et al., 2013).

2.2. Chemicals

Methanol (LC/MS grade) was purchased from Fisher Scientific (Schwerte, Germany), and ammonium acetate, ethyl acetate, formic acid, potassium dihydrogen phosphate and disodium hydrogen phosphate dihydrate (all analytical grade) were purchased from VWR (Darmstadt, Germany). Atrazine, atrazine-D_5, desethylatrazine and metazachlor were purchased from Dr. Ehrenstorfer (Augsburg, Germany), and carbamazepine-D_{10} was purchased from Promochem (Wesel, Germany).

2.3. Laboratory and on-site analyses

2.3.1. On-site analysis

Hourly data for electrical conductivity (reference temperature: 20 °C) and turbidity of the spring water as well as the spring water levels were gauged with an installed continuous monitoring system. The water levels were transferred into spring discharge data, applying a rating curve.

2.3.2. Inorganic ions

The samples for cation analysis were acidified with methane sulfonic acid (2.5 μL mL^{-1}). The analysis for the inorganic ions was performed by ion chromatography (IC) as described in Nödler et al. (2011).

2.3.3. Herbicides

The analytical method for the determination of the herbicides metazachlor and atrazine as well as its degradation product desethylatrazine is based on SPE and high-performance liquid chromatographic separation coupled with tandem mass spectrometric detection (HPLC/MS-MS). The details of the method have been published earlier (Nödler et al., 2010). Briefly, a sample volume of 500 mL was buffered at neutral pH (phosphate buffer), spiked with 100 ng atrazine-D_5 and carbamazepine-D_{10} and extracted by SPE (500 mg Oasis HLB, Waters, Eschborn, Germany). After extraction, the cartridges were rinsed with ultrapure water, dried, wrapped in aluminium foil and kept frozen (−18 °C) until analysis. Prior to analysis, the herbicides were successively eluted from the sorbent with methanol and ethyl acetate. The eluate was evaporated and re-dissolved in a 5 mM ammonium acetate aqueous solution, containing 4% methanol. Unlike Nödler et al. (2010), only 0.8 mL was used to re-dissolve the analytes. Thus, a higher enrichment factor and consequently lower method detection and quantification limits were achieved: the method detection limits (MDL) of atrazine, desethylatrazine and metazachlor were 0.3, 0.4 and 0.5 ng L^{-1}, respectively. The method quantification limits (MQL) were 1.1 ng L^{-1} for atrazine and 1.4 ng L^{-1} for desethylatrazine and

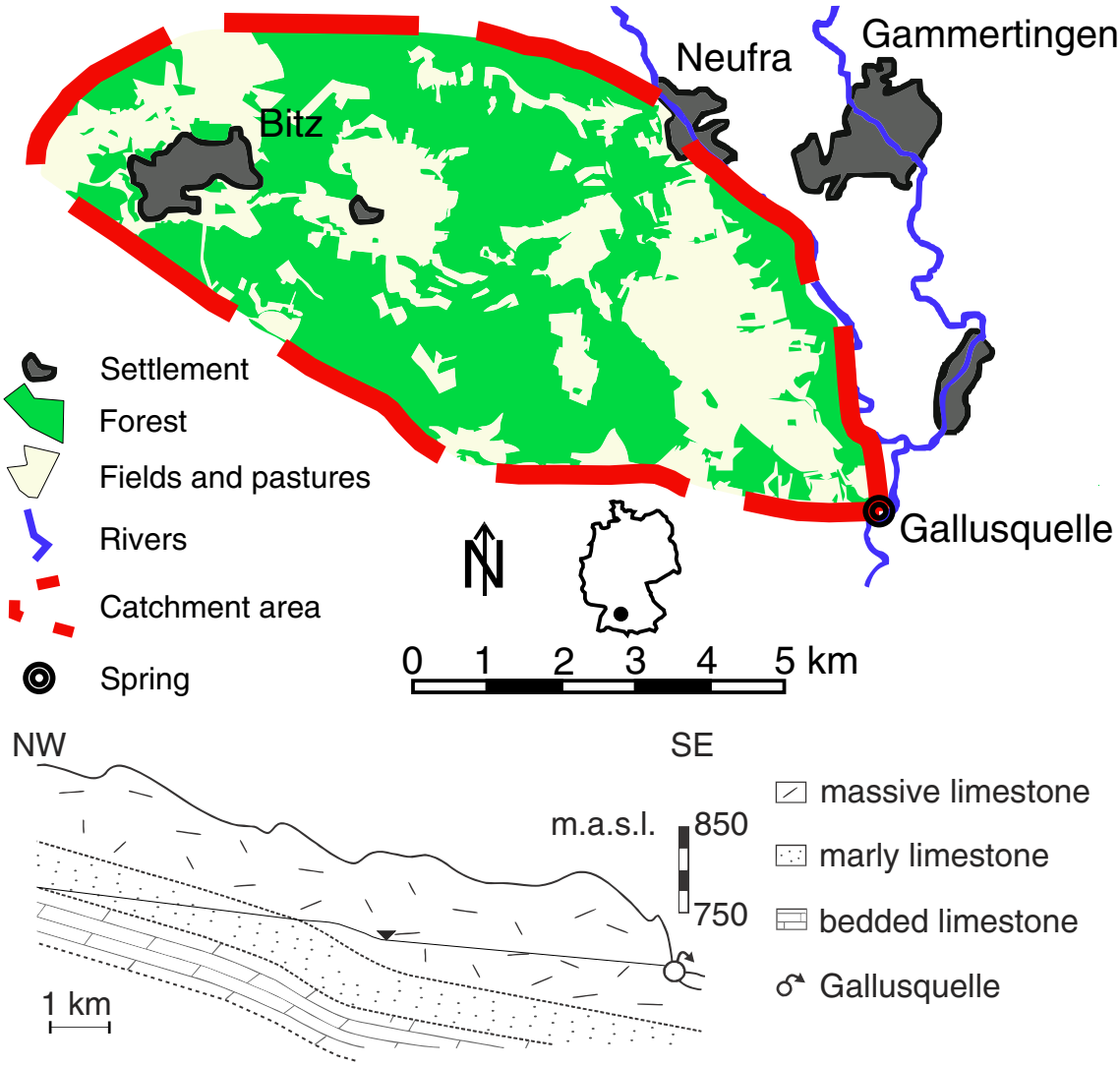

Fig. 1. The catchment area of the Gallusquelle and its geological cross section (delineation of the catchment and cross-section according to Sauter, 1992). (For interpretation of the references to colour in this figure, the reader is referred to the web version of this article.)

metazachlor. The MDLs and MQLs were determined according to DIN 32645 (2008).

2.4. Hydrograph separation

The hydrograph separation technique was employed for estimating the amount of rainwater reaching the spring over rapid recharge. Typically, isotopic data (e.g., Malík and Michalko, 2010), inorganic ions (e.g., Dreiss, 1989) or the EC (e.g., Laudon and Slaymaker, 1997) are used. On the basis of end-member mixing, the variations of the rainwater tracers are utilised in estimating the fraction of rapidly transported rainwater to the spring. The calculation procedure is shown in the supporting information (S1). Please note that the application of end-member mixing is discussed controversially (e.g., Nakamura, 1971; Pilgrim et al., 1979). The approach assumes the conservative behaviour of both end-members, i.e., the investigated components do not change. This is obviously not true for any of the investigated parameters here. On the example of EC, one can assume that the amount of dissolved solids in the rainwater and consequently the EC increases the moment it comes into contact with the earth's surface and with increasing contact time with soil or aquifer material. Taking these uncertainties into account, the hydrograph separation based on end-member mixing of EC can be understood as a lower boundary estimation of the true fraction of rainwater at the spring. The actual amount of rainwater is likely to be higher. Comparable uncertainties must be considered to some extend when performing hydrograph separation by end-member mixing with Ca^{2+}, Mg^{2+}, atrazine and desethylatrazine.

3. Results and discussion

3.1. Variations of investigated parameters

The concentration range of Ca^{2+}, Mg^{2+}, NO_3^-, atrazine, desethylatrazine and metazachlor in spring water are presented in Table 1.

Although NO_3^- may also originate from urban sources, such as leaky sewers and landfills (Wakida and Lerner, 2005), the agricultural application of fertilisers is its main source, especially in sparsely populated and rural areas such as the catchment under investigation. After precipitation events, the concentration of NO_3^- increases before returning to a background concentration within a few days, only slightly affected by dilution (Fig. 2). This is expected for NO_3^- and other substances/ions,

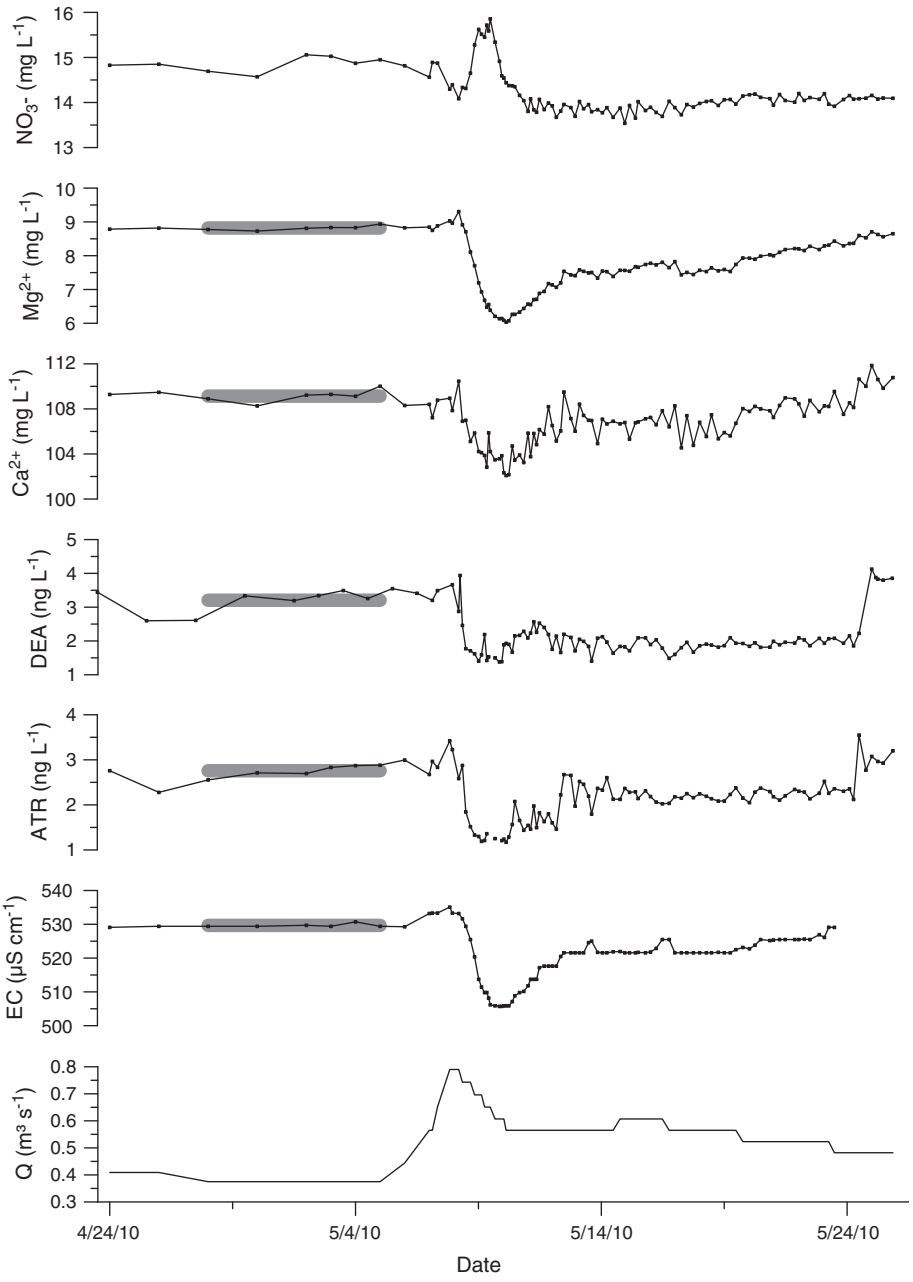

Fig. 2. Behaviour of the inorganic ions NO_3^-, Mg^{2+} and Ca^{2+}, electrical conductivity (EC), desethylatrazine (DEA) and atrazine (ATR) after a precipitation event (Q = spring discharge). The grey bars highlight the background concentration, used for the discharge separation calculations. The discontinuity at the DEA and ATR curves' minimum refers to single samples, where the observed concentrations were below the limit of quantification (LOQ).

which are introduced into the karst system together with the infiltrating rainwater or snow-melt (i.e., recharge events). The same behaviour can be observed for the herbicide metazachlor (Fig. 3). It does not occur evenly distributed over time, but only after precipitation events at comparatively high concentration (Table 1). However, while for NO_3^- a background concentration exists in the spring water, the concentration of metazachlor decreases below the limit of detection (LOD) within a short period of time. The irregular occurrence only after precipitation events indicates the transport of metazachlor with the percolating rainwater through the unsaturated zone to the local karst spring. As metazachlor was not detected in spring water during the winter months, it is unlikely that the occurrence of metazachlor in the spring to autumn months is related to metazachlor sources within the subsurface but originates from recent application (metazachlor is applied as a post-emergence herbicide few weeks after the sowing in spring or at the end of august for winter oilseed rape). This is supported by the low half-life of metazachlor in the environment (Allen and Walker, 1987; Beulke and Malkomes, 2001).

In contrast, for the parameter 'hardness,' the opposite effect (i.e., decreasing concentrations) has been described (Ashton, 1966; Williams, 1983): (i) initial expulsion of phreatic and subcutaneous water; (ii) arrival of flood water, diluting the spring water; and (iii) return to pre-event conditions. This pattern occurs for parameters originating from within the aquifer system which are affected by dilution, i.e., hardness, EC and the inorganic ions dissolved from the rock matrix. At the Gallusquelle spring, this behaviour can be observed for the EC, Ca^{2+}

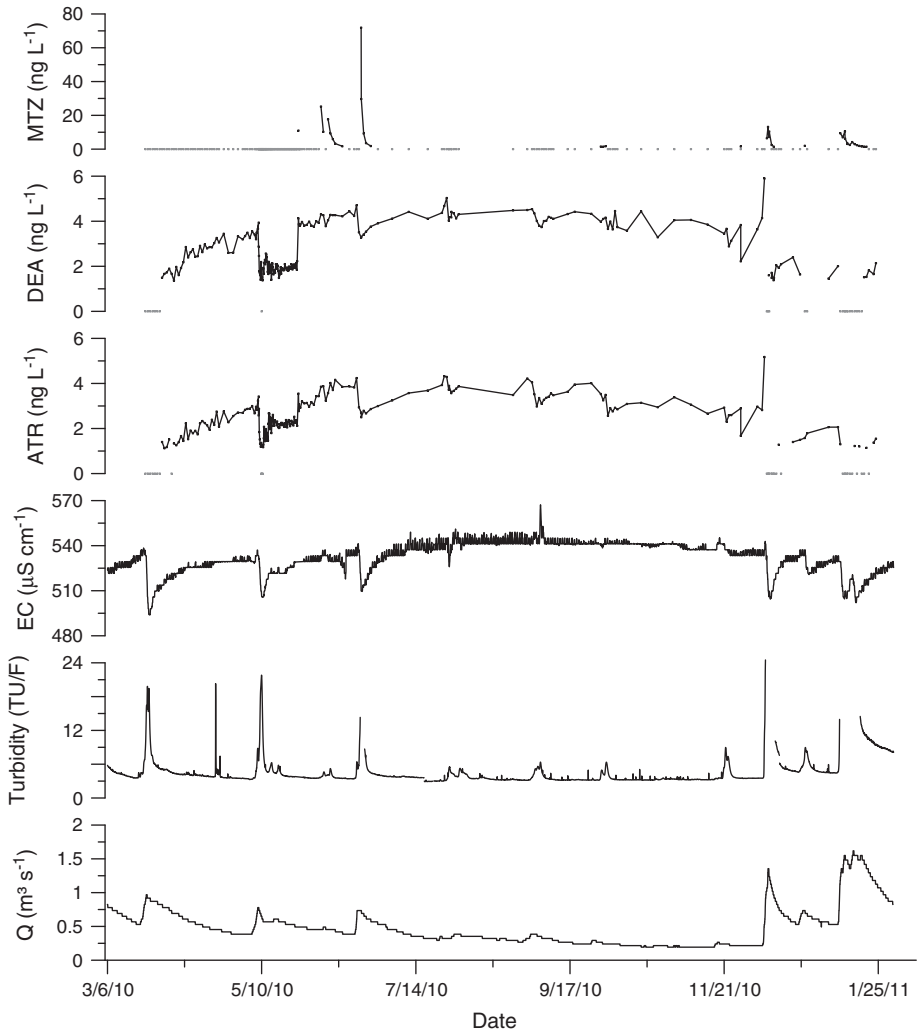

Fig. 3. Variations of metazachlor (MTZ), desethylatrazine (DEA), atrazine (ATR), electrical conductivity (EC), turbidity and daily average spring discharge (Q) at the Gallusquelle spring over the period of investigation. The grey squares at 0 ng L^{-1} for MTZ, DEA and ATR indicate samples for which the observed concentration was below the LOQ. Interruptions in the turbidity line are caused by data loss.

and Mg^{2+} similar to findings of Stueber and Criss (2005). If there were additional sources for Ca^{2+} and Mg^{2+} beside the subsurface/aquifer material, its influence was negligible.

Unlike the irregular occurrence of metazachlor at the spring, atrazine and desethylatrazine were detected in nearly all samples throughout the investigation period (Fig. 3). Their observed concentrations were generally low (Table 1), but comparable to values from Switzerland (Morasch, 2013). Storm pulses (i.e., increasing concentrations with increasing discharge) were reported for atrazine after precipitation events in the U.S. (Vesper et al., 2001), only occurring after application of atrazine (Stueber and Criss, 2005). Similarly, in southwest England, a positive correlation was observed between increased water levels and atrazine concentrations, which was explained by the remobilisation of historic pollution incidents (Lapworth and Gooddy, 2006). At the Gallusquelle spring, a different behaviour can be observed, comparable to that of the EC, Ca^{2+} and Mg^{2+}. In fact, the correlation of normalised (concentrations attain values between 1 and 0 for their maximum and minimum value, respectively) atrazine concentrations with these parameters in normalised form yields values for R^2 of 0.6–0.7 (performing the same correlation calculations with NO_3^- leads to values for R^2 of 0; scatter diagrams for all parameters are provided in the supporting information S2). From the correlation of the time series of Ca^{2+}, Mg^{2+}, EC and atrazine, a similar origin can be deduced (Stueber and Criss, 2005). As Ca^{2+} and Mg^{2+} originate from the aquifer matrix, atrazine is inferred to be located within the aquifer material, i.e., inside the rock matrix. From here, it is released slowly into the groundwater. This is in agreement with findings reported by Morasch (2013), who assigned the observed continuous low atrazine concentrations in a Swiss (where atrazine is still applied) karst aquifer system to its slow but steady release from the aquifer matrix. Please note that

Table 1
Concentration range of the investigated inorganic ions, herbicides and herbicide degradation products in the spring water of the Gallusquelle during the period of investigation. Concentrations of the inorganic ions are expressed in mg L^{-1}, and concentrations of the herbicides (degradation products) are expressed in ng L^{-1}.

	Min[a]	Max[b]	Median	DF[c]
Ca^{2+}[d]	102	114	108	100
Mg^{2+}[d]	6.0	9.3	7.0	100
NO_3^-[d]	11.6	17.5	14.4	100
Atrazine[e]	<LOD[f]	5.2	2.3	99.6
Desethylatrazine[e]	<LOD[f]	5.9	2.3	99.6
Metazachlor[e]	<LOD[f]	82.9	<LOD[f]	30.7

[a] Minimum concentration.
[b] Maximum concentration.
[c] Detection frequency in %.
[d] $n = 153$.
[e] $n = 263$.
[f] Limit of detection.

sorption may partly affect the long-term fate of atrazine, but that the appearance of atrazine even after more than 20 years in the investigated aquifer is more likely related to the slow groundwater flow rates inside the karst matrix and the resulting long residence times, while hardly or not affected by degradation (Johnson et al., 2000; Chilton et al., 2005).

For desethylatrazine, a time series similar to atrazine, Ca^{2+}, Mg^{2+} and the EC could be observed (Fig. 2). However, it is different; a correlation of desethylatrazine with these parameters was significantly worse (R^2 between 0.2 and 0.4, details can be found in the supporting information S2). The arrival of rapid recharge after a precipitation event leads to decreasing concentrations of desethylatrazine, and hence, its origin can be concluded to be situated within the fissured rock matrix, too. However, the time series seems to evolve a plateau before returning to its background concentration rapidly some time after the recharge event, instead of a slow and steady concentration increase. The slightly different behaviour of desethylatrazine to atrazine is difficult to assess and beyond the scope of this study.

3.2. Hydrograph separation

For one event, a hydrograph separation was performed, based on the dilution of the concentrations of atrazine, desethylatrazine, Ca^{2+} and Mg^{2+} and the EC. As the end member for the pre-storm water, the background concentration/value of each parameter was used (illustrated in Fig. 2). A sample of the precipitation contained 1.7 mg L^{-1} of Ca^{2+} and 0.07 mg L^{-1} of Mg^{2+}. As the end member for the rainwater, a concentration/value of 0 is assumed for all parameters. This assumption seems legitimate as the observed concentrations are very low (<1% of the background concentration), and it does not alter the determined fractions of the rainwater at the spring significantly.

The results are illustrated in Fig. 4. The calculated amount of rainwater reaching the spring over direct recharge is similar for the EC (maximum: 4.5%) and Ca^{2+} (maximum: 6.5%). These values are in agreement with previously published results from Sauter (1997), who stated the fraction of rapid recharge to be in the order of 5–10% based on $\delta^{18}O$-data. Using Mg^{2+}, the determined amount of freshly introduced recharge is much larger (maximum: 32%). This observation may originate either from (i) unevenly distributed Mg^{2+} minerals (e.g., dolomite) in the subsurface, i.e., disproportional dilution of the Mg^{2+} concentration or (ii) slower dissolution of dolomite relative to calcite (Liu and Dreybrodt, 2001). It is likely that the extent of the dilution of the Mg^{2+} concentration is affected by both factors. For atrazine and desethylatrazine, even higher dilutions can be observed. Consequently, a higher amount of rainfall reaching the spring over rapid recharge was calculated (maximum: 58% and 57% for atrazine and desethylatrazine, respectively). As atrazine was applied as herbicide on agriculturally used areas, the area of application can be estimated to be 14% of the catchment area at maximum (Sauter, 1992). Considering this fraction, it becomes evident that a larger dilution for atrazine is to be expected than for Ca^{2+} and the EC. Furthermore, the rain component for atrazine is likely to be less influenced by equilibration with the subsurface as a consequence of the restricted areal distribution.

Assuming the maximum dilution of the investigated parameters to be representative for their areal extend, one can estimate the latter (i.e., the magnitude of the maximum dilution is inversely proportional to the fraction of the catchment area over which the parameter is introduced into the system). As Ca^{2+} is believed to occur all over the catchment (i.e., 100%), the area over which atrazine is introduced into the system can be estimated to 11% of the catchment area. This is in good agreement with the reported land use pattern at the time of the application of atrazine (Sauter, 1992). The same applies to desethylatrazine.

3.3. Mass balance for atrazine

Atrazine use has been permitted in Germany from 1958 until its ban in 1992. The total amount of atrazine applied in the investigated area

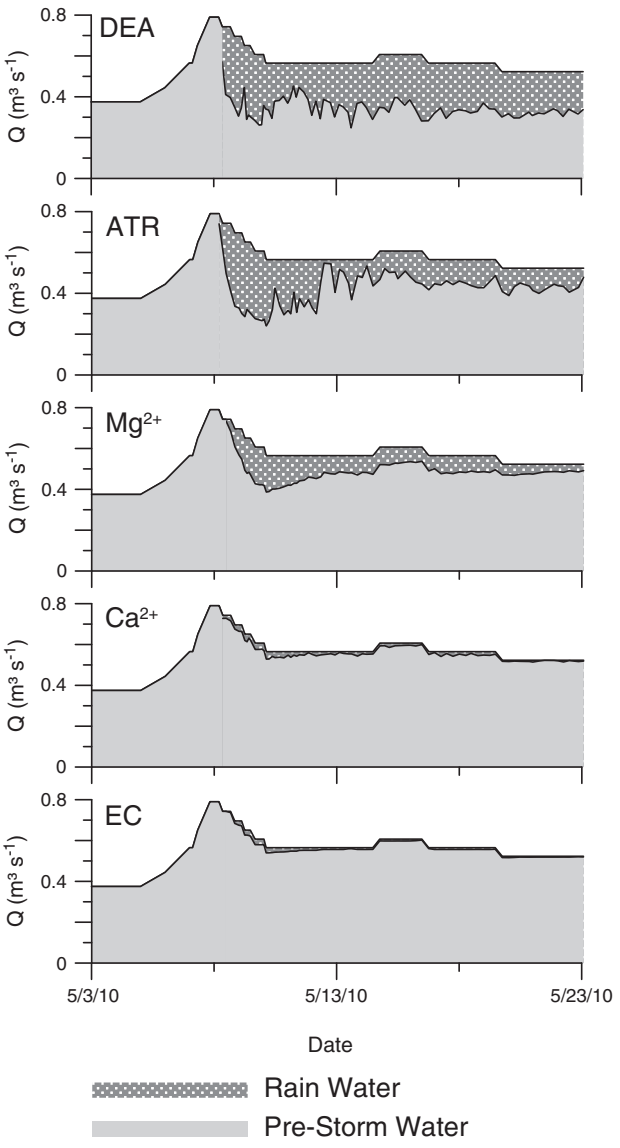

Fig. 4. Calculated discharge (Q) separation based on end member mixing for desethylatrazine (DEA), atrazine (ATR), the inorganic ions Mg^{2+} and Ca^{2+} and the electrical conductivity (EC).

was estimated according to a report from the European Commission (Henriet et al., 1994). The following assumptions were made therein: 11.1% of the agriculturally used area (which is 14% for the investigated catchment; Sauter, 1992) was used for maize, 90% of the fields were treated with atrazine at a mean dose rate of 1.5 kg ha^{-1}, an additional 10% of atrazine is considered for other uses. Presuming a certain time lag until atrazine was applied as herbicide after its permission, 27 years (1965–1992) were assumed as the duration of application. The resulting total mass of atrazine, being applied in the investigated area, was calculated to be approximately 2,800 kg.

To assess the discharge of atrazine, the mean concentration of atrazine in the investigation period was used as well as the mean spring discharge of 500 L s^{-1}. Accordingly, 37.8 g of atrazine was discharged in the course of the investigated year via the Gallusquelle spring. For an estimation of the total mass of discharged atrazine, the following assumptions were made: the concentrations of atrazine were higher in the years 1992–2009 following the same trend as in the data of Tappe et al. (2002), i.e., declining concentrations following an exponential decline with a decline rate of 0.26 a^{-1}. For the years 1965–1991, a constant discharge of atrazine is assumed, which is equal to the estimated value

for 1992 (3.8 kg a^{-1}; a table with the estimated loads for the whole period is provided in the supporting information S3). Over the years 1965–2010, a total discharge of 120 kg (around 4% of the applied mass) of non-metabolised atrazine can be estimated. The remaining 96% of the applied atrazine was either metabolised (e.g., Jablonowski et al., 2009) or still within the aquifer rock matrix. Both possibilities hold true since (i) it could be clearly demonstrated that even after 20 years without application within the catchment, the original compound is still detected in spring water and (ii) the metabolite desethylatrazine has been found in spring water, exhibiting a similar behaviour as atrazine. The estimation of the discharge of atrazine can be refined, taking a quantification of the discharge of desethylatrazine into account. For the calculation, all assumptions were made, as stated above for atrazine. However, the concentration of desethylatrazine declines at a different rate than the concentration of atrazine. The trend has been determined from the results of Tappe et al. (2002) to be 0.22 a^{-1}. The total discharge of desethylatrazine in the years 1965–2010 can be calculated to be 77 kg, corresponding to 88 kg of atrazine. Please note that desethylatrazine is not an unambiguous degradation product and that the calculation is hence unambiguous as well. It must be understood as upper boundary estimation. Thus, a total of 120–208 kg of atrazine could be estimated to discharge at the Gallusquelle in the course of 45 years. This corresponds to 4–7.5% of the estimated total atrazine applied. These low values do no surprise, when taking the low to non-existent degradation rates (Johnson et al., 2000; Chilton et al., 2005) and the low leaching rates (Haria et al., 2003; Baran et al., 2008) of atrazine into account. Please note that further degradation products may occur (Krutz et al., 2003), which have not been considered in the above estimation.

4. Conclusions

The concentration of metazachlor in spring water increases after precipitation events and decreases below the LOD within a short period as expected for herbicides. In contrast, the atrazine and desethylatrazine concentrations are diluted after precipitation events and return to their pre-event level. From the correlation with Ca^{2+} and Mg^{2+}, it can be concluded that atrazine is likely to be located within the aquifer matrix. This duality of transport in karst aquifers needs to be considered carefully, in order to achieve a successful and sustainable raw water management of karst springs. On the one hand, drinking water suppliers need to be aware of rapid recharge and the associated strong variations of raw water quality, which may arise from heavy precipitation or snowmelt events. On the other hand, special attention must be drawn to the high potential of karst aquifers for long-term storage. Potentially persistent substances or transformation products are prone to cause long-term contamination. Due to the high residence time within the rock matrix, persistent contaminants may influence the raw water quality for decades. Although atrazine was prohibited in Germany more than 20 years ago, its impact on the investigated karst aquifer is still detectable. A similarly long after-effect should be expected in any other region, where atrazine (or any other persistent contaminant) was or is still applied.

Conflict of interest

To the authors' knowledge, no financial, personal or other relationships exist to any other people or organisations within 3 years of beginning the submitted work that could inappropriately influence or be perceived to influence this work.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2014.02.117.

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Identification of the attenuation potential of a karst aquifer by an artificial dualtracer experiment with caffeine

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ABSTRACT

Little is known with respect to the attenuation capacity of karst aquifers. Even less is known about the risk posed by emerging micropollutants in these systems. In order to identify the attenuation potential of karst aquifers *in-situ* and to estimate the risk posed by micropollutants, a dualtracer test was conducted in this study in order to investigate differential transport in the subsurface: The reactive compound caffeine was used as a tracer to indicate the attenuation capacity within the aquifer *in-situ*. Due to the low limit of quantification, only small amounts of caffeine needed to be injected. To calibrate a model and to visualize the attenuation of caffeine a conservative reference tracer (uranine) is injected simultaneously. The methodology is tested in a well-characterised karst system in southwest Germany. The results indicate a significantly higher attenuation rate than was expected for karst aquifers. The attenuation is decribed as a first-order process. The corresponding half-life is 104 h. This low half-life suggests that a generally assumed low natural attenuation capacity of karst aquifers is unjustified. The observed mass loss of caffeine illustrates the potential of caffeine to be used as reactive tracer for indicating *in-situ* attenuation capacity within highly hydraulically conductive systems, such as karst aquifers. Due to the high attenuation rate of caffeine it does not pose a threat as a long-time contaminant. In combination with a conservative reference tracer an economical and environmentally benign method is presented in this manuscript for the *in-situ* determination of the attenuation capacity of highly conductive aquifer systems.

1. Introduction

Karst aquifers supply up to one quarter of the world's population with drinking water (Ford and Williams, 2007). Karst springs are referred to as relatively unsafe drinking water sources: Along solutionally widened flow paths contaminants can be transported rapidly from the land surface to a karst spring through the subsurface. In these conduits, flow velocities of several km d^{-1} were reported (e.g. Seiler et al., 1989). The resulting low residence times of the rapidly transported water reduces the potential of contaminant attenuation in case of a contamination. Einsiedl et al. (2009) estimated the vulnerability of a karst aquifer based on the residence time distribution.

The biological activity of karst aquifers is believed to be little, as the nutrient offer is low, i.e. karst aquifers are oligotrophic environments (Gibert et al., 1994; Hirsch, 1986). However, very little is known with respect to the natural attenuation capacity of karst aquifers. As important drinking water sources a successful management and an estimation of the risk posed by (potential) contamination of karst aquifers is of public interest.

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Within the last decades micropollutants have been ubiquitously registered in all compartments of the environment (Schwarzenbach et al., 2006; Ternes, 2007). Several micropollutants have been used as indicators for contamination (Gasser et al., 2010; Buerge et al., 2006), but so far their fate in karst systems has rarely been addressed (Einsiedl et al., 2010). The lack of knowledge with respect to the fate of micropollutants and the known vulnerability of karst aquifers result in an unknown risk posed by emerging pollutants.

To reliably assess the natural attenuation potential of a karst system, tracer experiments with reactive compounds can be employed. Haggerty et al. (2009) used the organic compound resazurin to quantify the metabolically active transient storage in a stream. Caffeine, as an often discussed micropollutant (Buerge et al., 2003, 2006; Swartz et al., 2006), possesses promising sorption and degradation properties to determine the attenuation potential of a karst aquifer and therefore indicator properties for reactive transport at large. Caffeine is readily degradable in wastewater treatment plants. In lakes and porous aquifers the degradation was observed to be much lower (Buerge et al., 2003; Swartz et al., 2006). The German Federal Environment Agency classified caffeine as lowly water-hazardous (lowest hazard class). Within the context of controlled and specially designed experiments, the mass loss of caffeine has a potential to indicate the attenuation capacity of a karst aquifer along the tracer flow path.

Mass loss of a tracer resulting from degradation and the respective quantification can be uniquely identified from the appearance of metabolic products or by the simultaneous injection of an inert reference tracer (i.e. multitracer test; Geyer et al., 2007). Since primary or secondary metabolites are unlikely to be produced by oligocarbotroph microorganisms (Wainwright et al., 1993), and laboratory experimental observations indicate that degradation products cannot be expected from the degradation of caffeine (Kurtzman and Schwimmer, 1971; Mazzafera et al., 1996), an inert reference tracer, e.g. uranine, has to be used to determine the mass loss of caffeine in the investigated karst aquifer and therefore demonstrate the natural attenuation capacity of that system.

This study presents results from a dualtracer experiment, employing caffeine as an indicator for the natural attenuation capacity of a karst aquifer. Apart from caffeine, uranine was injected simultaneously as inert reference tracer for model calibration. Transport parameters were estimated with the numerical modelling approach CXTFIT (Toride et al., 1995).

2. Materials and methods

2.1. Dualtracer test

The selected field site for the dualtracer experiment is located in the catchment area of the Gallusquelle spring in southwest Germany (Fig. 1). The spring drains a catchment area of approximately 45 km^2. Annual discharge averages to 500 L s^{-1}, ranging from less than 100 to 2500 L s^{-1}. A small fraction of the outflow is expected to occur below the gauging station. Estimations of this discharge component range up to 200 L s^{-1}. The general flow direction in the catchment is NW–SE. Hirsch (1986) stated groundwater to be oligotrophic, based on low

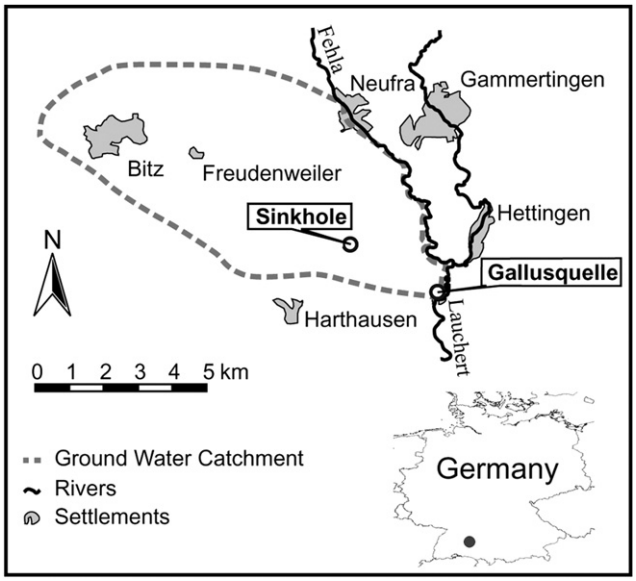

Fig. 1 – Catchment area of the Gallusquelle spring. The sinkhole for the injection of the tracers is located at a distance of 3000 m from the spring (from Birk et al., 2005).

concentrations of organic carbon (1–10 mg/L). These conditions also apply for the investigated aquifer (1–3 mg/L; Heinz et al., 2009), which is therefore classified to be oligotrophic. However, the accidental, irregular and event-based inflow of wastewater related micro-contaminants was demonstrated in previous studies (Heinz et al., 2009; Hillebrand et al., 2012; Nödler et al., 2012).

A tracer experiment was performed on June 27th 2011. A sinkhole 3 km northwest of the spring was selected as tracer injection location (Fig. 1). The characteristics of the sinkhole injection site were previously investigated by two artificial tracer tests (Birk et al., 2005; Geyer et al., 2007), which demonstrated the point-to-point connection between injection point and the Gallusquelle spring. The thickness of the unsaturated zone in the area of the sinkhole is approximately 100 m (Geyer et al., 2007). In order to minimize the influence of the unsaturated zone on the tracer injection and facilitate an introduction of the tracers into the conduit system, the sinkhole was flushed with tap water before and after the injection. Before the injection of the tracers 105 m^3 of water was used (~4 h with a flow rate of 6.9 L s^{-1}) to temporarily obtain near saturated conditions along the flow path in the vadose zone. Shortly before injection the tracers (30 g of caffeine and 500 g of uranine) were dissolved in 1 m^3 of tap water. The tracer injection was followed by 81 m^3 of water to flush the sinkhole over a period of ca. 3.5 h with a flow rate of 6.5 L s^{-1} to force the injected tracer cocktail through the unsaturated into the saturated zone.

Uranine was simultaneously injected with caffeine since (i) uranine can easily be monitored online by a fluorometer providing an indication for the times when samples for the analysis of the caffeine concentrations need to be taken and (ii) uranine serves as a conservative reference tracer, i.e. it is neither retarded nor degraded (Geyer et al., 2007), to quantify the potential mass loss of caffeine.

2.2. Sampling

The uranine concentration was monitored over a period of 16 days with the field spectrofluorometer GGUN-FL30 (excitation: 470 nm, detection: Wratten orange filter). The measuring interval was initially set to 10 min, and decreased to 1 min during tracer breakthrough. The detection limit for uranine in the investigated spring water is stated to be 0.02 $\mu g\ L^{-1}$ (Geyer et al., 2007). As quantification limit, a threefold detection limit of 0.06 $\mu g\ L^{-1}$ was assumed in this study. Concentrations below this value were set to zero. For the calibration of the device three calibration levels were prepared by subsequently diluting a uranine stock solution (1 $mg\ L^{-1}$) with water from the Gallusquelle spring. The calibration levels were 1, 10 and 100 $\mu g\ L^{-1}$. As no natural recharge occurred for the duration of the tracer test (no changes in turbidity) and the calibration was performed with spring water, interferences with fluorescent humic substances can be excluded to affect the quantification of uranine.

Water samples to be analyzed for caffeine and selected metabolites were taken over a period of 7 days. In total 93 spring water samples were taken. The sampling interval for caffeine varied between several hours and 10 min for the time of the increasing limb of the tracer breakthrough curve (TBC), achieving a high temporal resolution of the caffeine TBC. The water samples were preconcentrated within a few hours (<8 h) after sampling by solid phase extraction (SPE) as described in Section 2.3.2. The volume of the spring water samples varied between 500, 250 and 200 mL depending on the expected caffeine concentration, estimated from the measured uranine concentrations.

The electrical conductivity and the turbidity of the spring water were monitored every 20 min by a pre-installed multiparameter probe system. The discharge of the Gallusquelle spring was acquired from a spring gauging station.

2.3. Laboratory analysis

2.3.1. Chemicals

Methanol (LC/MS grade) and caffeine were purchased from Fisher Scientific (Schwerte, Germany), ethyl acetate and ammonium acetate (all analytical grade) were purchased from VWR (Darmstadt, Germany). Paraxanthine, paraxanthine-D_6, theobromine, theophylline, 1-methylxanthine and 3-methylxanthine were obtained from Sigma Aldrich (Steinheim, Germany). Uranine was purchased from Acros Organics (Geel, Belgium).

2.3.2. Analysis of caffeine and its metabolites

An analytical method based on SPE and high-performance liquid chromatographic separation with tandem mass spectrometric detection (HPLC/MS–MS) was used for the analysis of caffeine and its metabolites paraxanthine, theobromine, theophylline, 1-methylxanthine and 3-methylxanthine. Details were published previously (Nödler et al., 2010). In brief, 500 mL of sample volume was buffered at neutral pH (phosphate buffer) and extracted by SPE (500 mg OASIS HLB; Waters, Eschborn, Germany). Samples of smaller volume than 500 mL were filled up with ultrapure water. Prior to extraction, 400 ng of paraxanthine-D_6 was added as internal standard for the quantification of the analytes.

After extraction the sorbent was rinsed with ultrapure water and dried by drawing air through the cartridges under vacuum. The cartridges were wrapped in aluminum foil and kept frozen (−18 °C) until analysis. The analytes were eluted with methanol and ethyl acetate, successively. The solvents were evaporated and the dry residue was re-dissolved in 1 mL of an aqueous 5 mM ammonium acetate solution, containing 4% methanol. The method quantification limits (MQL) of the analyzed substances were: 4.3 ng L^{-1} (caffeine), 3.2 ng L^{-1} (paraxanthine), 5.1 ng L^{-1} (theobromine), 3.4 ng L^{-1} (theophylline), 21 ng L^{-1} (1-methylxanthine) and 28 ng L^{-1} (3-methylxanthine).

Recovery rates for caffeine were determined by the extraction of 500 mL of the original spring water spiked at levels of 100 and 1000 ng L^{-1}. The results were 109% (± 0.6%) and 100% (± 6.6%), respectively. The influence of uranine on the quantification of caffeine was investigated by analyzing 500 mL of spring water spiked with 1000 ng L^{-1} caffeine and 30,000 ng L^{-1} uranine. No significant influence on the recovery rate of caffeine was observed. All experiments on recovery rates were conducted in duplicates.

2.4. Modelling

Birk et al. (2005) demonstrated that a simple advection–dispersion model (ADM) fails to reproduce the tailing of TBCs in the investigated karst aquifer. In order to achieve a better model fit and reliably interpret the TBCs of uranine and caffeine, the suggested non-equilibrium ADM was applied for TBC interpretation: CXTFIT 2.0 (Toride et al., 1995) was used as part of STANMOD (Simunek et al., 1999). The CXTFIT 2.0 code implements a uniaxial, two-region non-equilibrium transport model. Field and Pinsky (2000) introduced the application of two-region non-equilibrium transport models to analyze large-scale artificial tracer tests in karst aquifers. The approach considers the fluid in a karst conduit as divided into a mobile and immobile (stagnant relative to the direction of flow) fluid region, described previously (Field and Pinsky, 2000; Hauns et al., 2001; Geyer et al., 2007). Thus immobile fluid regions are characterized by higher residence times, as the water is not displaced by plug flow. Possible immobile fluid regions are vortices and eddies resulting from irregular cross-sections of the conduits. As input function for the model a pulse input was used, i.e. the input duration was assumed to be negligible in comparison to the total duration of the tracer test.

Solute transport processes considered in this study include advection, dispersion, mass transfer between the two fluid regions (mobile and immobile), reversible sorption and tracer attenuation. The analytical equations for the one-dimensional, two-region non-equilibrium model are given as follows (modified from van Genuchten and Wagenet, 1989):

$$\beta R\frac{\partial c_m}{\partial t} = D\frac{\partial^2 c_m}{\partial x^2} - v\frac{\partial c_m}{\partial x} - \alpha(c_m - c_{im}) - \beta R\mu_1 c_m \quad (1)$$

$$(1-\beta)R\frac{\partial c_{im}}{\partial t} = \alpha(c_m - c_{im}) - (1-\beta)R\mu_2 c_{im} \quad (2)$$

with the retardation coefficient, defined as:

$$R = 1 + \frac{A}{V} K_a \tag{3}$$

for non-porous matrix blocks. β, the solute partitioning coefficient between mobile and immobile fluid regions is given as:

$$\beta = \frac{\theta_m + f(R-1)}{R} \tag{4}$$

t is time, x is the space coordinate, D is the dispersion coefficient, v is the average flow velocity, α is a first-order mass transfer coefficient between mobile and immobile fluid regions. c_m and c_{im} are the solute concentrations in, μ_1 and μ_2 are first-order attenuation rates within the mobile and immobile fluid region respectively. In this study a uniform attenuation rate in the mobile and immobile region was considered ($\mu_1 = \mu_2 = \mu$). θ_m is the volumetric fraction of the mobile fluid region, while $\theta_m + \theta_{im} = \theta = 1$ for a fully saturated conduit, θ_{im} being the volumetric fraction of the immobile fluid region. A/V represents the surface to volume ratio of a karst conduit, K_a is the linear distribution coefficient defined as the ratio of tracer mass per unit surface area of the solid phase to the unit concentration of the tracer within the conduit. The parameter f refers to the fraction of reversible adsorption sites that equilibrates with the mobile liquid phase. The retardation coefficient R captures the retardation of unpolar sorption as well as from reversible polar interactions as shown by Geyer et al. (2007). Rearranging Eq. (4) and inserting physically reasonable values for f (between 0 and 1) allows to constrain β (Geyer et al., 2007):

$$\frac{\theta_m}{R} \le \beta \le 1 - \frac{\theta_{im}}{R} \tag{5}$$

To reliably interpret TBCs of reactive tracers a step-wise calibration strategy can be applied (Geyer et al., 2007). Fitting the TBC of a conservative tracer yields estimates for the parameters v, D, α and θ_m. The application of uranine as conservative tracer in karst hydrology has been shown in several large-scale field studies (Birk et al., 2005; Geyer et al., 2007). Conservative transport parameters can be assumed to be equal for conservative and reactive solute tracers (Geyer et al., 2007). Consequently, the calibration of the reactive transport model is reduced to the transport parameters R, β and the attenuation coefficient μ if a conservative reference tracer is applied simultaneously.

As transport distance, the linear distance of 3000 m between the injection-point and the Gallusquelle spring was used. The initial values for v and D for the calibration of the model were derived from the method of moments, using the software QTRACER (U.S. EPA, 2002). Estimates for α are not generally possible and the initial value for θ_m was obtained from the ratio of the mean tracer velocity and the peak tracer velocity (modified from Goltz and Roberts, 1988; Field and Pinsky, 2000).

3. Results and discussion

Precipitation events and associated infiltration can have an impact on the spring discharge and the flow regime in the aquifer, because they impose a temporally variable discharge rate. Therefore, the interpretation of TBCs becomes considerably more complex, since the mass flux of uranine and caffeine are calculated based on the spring discharge. To avoid these complications, the tracer test was performed during a dry spring recession period. During the whole investigation period spring discharge was relatively constant at ca. 175–200 L s^{-1}. Turbidity and electrical conductivity measurements were stable at 0.12 FNU and 650 µS cm^{-1}, demonstrating the absence of disrupting recharge events.

Background effects with respect to caffeine in the spring water originating from wastewater infiltration can be excluded for the tracer test. The caffeine concentrations are comparatively small (Hillebrand et al., 2012) and the wastewater infiltration does not occur evenly distributed over time, but simultaneously to precipitation events (Musolff et al., 2010), which were absent for the duration of the tracer test.

The mass recovery of injected uranine was found to be 49% (246 g). The mass loss of uranine was likely to be caused by groundwater discharge below the gauging station. Geyer et al. (2007) stated for the same catchment area that the proportion of mass loss increases with lower discharge. The observations of this study emphasize this finding.

The recovered mass of injected caffeine was only 27%, indicating an additional mass loss in comparison to uranine, i.e. caffeine shows reactive transport behaviour in the investigated aquifer system. Furthermore, caffeine exhibited a longer tailing (Fig. 2). While the recovery of the total uranine mass was achieved after 127.5 h, caffeine concentrations took 164 h before dropping below the limit of quantification after the tracer peak. This may be attributed to the lower limit of quantification for caffeine. However, the lower recovered mass and the smaller peak indicate a significant mass loss relative to the conservative tracer uranine. Irreversible sorption is unlikely to occur since caffeine is highly soluble (Gardinali and Zhao, 2002) and has a negative (−0.07; Maeng et al., 2011) log K_{ow} (octanol–water partitioning coefficient). Several authors emphasize the degradability of caffeine and thus being the main process in its attenuation especially in treatment plants, but also in the environment. Buerge et al. (2003, 2006) calculated biodegradation rates of caffeine to be in the order of 0.003–0.006 d^{-1} in a lake. Swartz et al. (2006) observed caffeine degradation in a porous aquifer at a rate of 0.07 to 0.014 d^{-1}.

The metabolites of caffeine considered in the analytical method could only be found sporadically and at insignificant levels. The metabolites were either not detectable due to the high dilution, not produced during degradation, or metabolized further at a higher rate than caffeine. The latter is consistent with findings from laboratory experiments (Kurtzman and Schwimmer, 1971; Mazzafera et al., 1996). Moreover, Wainwright et al. (1993) stated the production of primary or secondary metabolites to be unlikely for oligocarbotrophs. Due to the high discharge, a decrease of oxygen concentrations or changes of the redox potential in the spring water cannot be resolved.

In the model a general attenuation rate is determined, which is comprised of all possible mechanisms for the additional mass loss (e.g. degradation, irreversible sorption) of caffeine relative to the conservative reference tracer uranine.

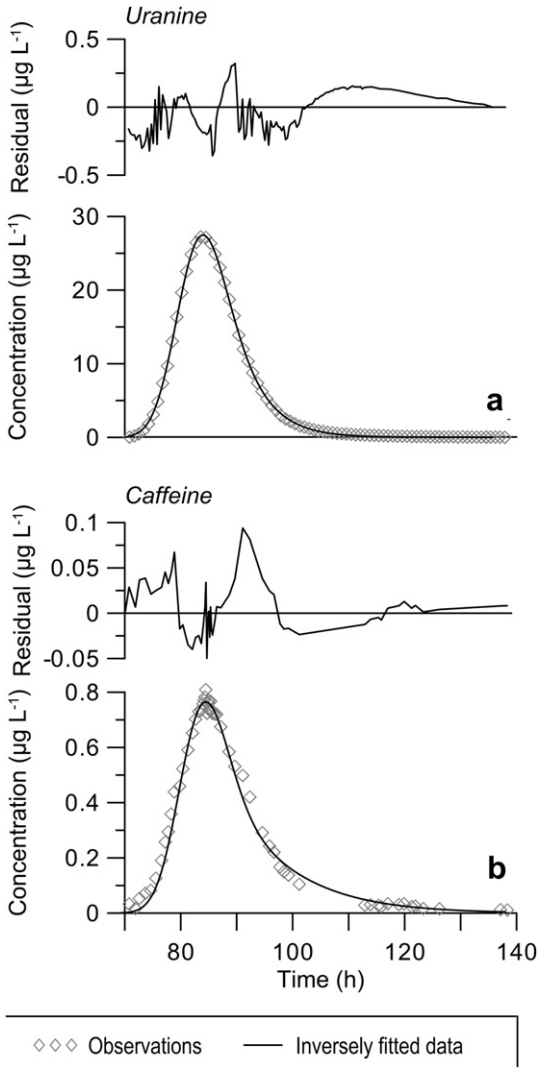

Fig. 2 – Tracer breakthrough curves of uranine (a) and caffeine (b) with their respective fitted models and residuals. For the graphical illustration of the uranine breakthrough only every 50th observation point is displayed.

Table 1 – Parameter estimates for a uniaxial, two-region non-equilibrium model to observed tracer breakthrough curves.

Tracer	Uranine	Caffeine
v (m h^{-1})	*34.9*	*34.9*
D (m^2 h^{-1})	*135.2*	*135.2*
α (h^{-1})	*8.91E-3*	*8.91E-3*
β (–)	0.9683	0.9340
R (–)	1	1.046
μ (h^{-1}); $T_{1/2}$ (h)	0	0.0067; 104
r^2	0.9997	0.9924
RMSE (µg L^{-1})	0.487	0.027
m_m (g)	246	14.8
m (g)	500	30

v = average flow velocity; D = dispersion coefficient; α = mass transfer coefficient; β = partitioning coefficient between mobile and immobile fluid regions; R = retardation coefficient; r^2 = coefficient of determination; μ = first-order attenuation rate; $T_{1/2}$ = half-life; RMSE = root mean square error; m_m = tracer injection mass used in the model; m = tracer mass injected into the sinkhole.
Note: Values in italics represent fitted values, while underlined values are prescribed values.

3.1. *Modelling results*

Calibrating the model (using the TBC of uranine) resulted in a very good agreement between observed and fitted concentrations (Fig. 2). The flow velocity v, dispersion coefficient D, volumetric fraction of the mobile fluid region θ_m as well as the mass transfer coefficient α (Table 1) are in good agreement with the results of a previously conducted study by Geyer et al. (2007).

In the model for the caffeine TBC the attenuation rate μ as well as solute-specific values for the retardation coefficient R and the partitioning coefficient β are considered additionally. Fig. 2 and Table 1 show the estimated parameters for uranine and caffeine.

The mass loss of caffeine was modelled by an attenuation rate of 0.0067 h^{-1} (i.e. a half-life of 104 h). This rate is surprisingly high in comparison to degradation values from the literature observed in a lake and a porous aquifer environment (Buerge et al., 2003, 2006; Swartz et al., 2006). The estimations of these authors for the half-life of caffeine range from weeks to months. In general it is assumed that bacteria are associated with sediment and rock surfaces (Holm et al., 1992). For karst aquifers the attenuation rate of caffeine was expected to be lower than the attenuation rate within the porous aquifer, as the contact area of water to the solid matrix, implying a reduced bacteria count for karst aquifers and less reactive interfaces. The relatively high attenuation rate may be related to the influence of wastewater leakage and the redox condition in the subsurface, as proposed by Bradley et al. (2007).

The literature values on *in-situ* degradation of caffeine mentioned above refer to sub-oxic to anoxic conditions (Swartz et al., 2006) and to conditions with low oxygen (Buerge et al., 2003, 2006). In the investigated aquifer oxic conditions prevail (data not shown). The increased degradability of caffeine under oxic conditions has been emphasized by Bradley et al. (2007). Furthermore, the investigated aquifer is known to be affected by wastewater leakage (Hillebrand et al., 2012; Nödler et al., 2012) and the periodical occurrence of overflow events of a wastewater retention basin (Heinz et al., 2009). With the percolating wastewater caffeine is introduced into the aquifer. The regular exposition of the aquifer bacteria to wastewater and therefore to caffeine may result in an adaption of the bacteria to caffeine or rather wastewater related micropollutants in general. This scenario and to a larger extent the sufficiently provided oxygen in the aquifer may explain the effective attenuation of caffeine observed during the tracer experiment. Moreover it is possible that the flow through the unsaturated zone affected the determined attenuation rates.

It has to be emphasized here that the attenuation rate determined for caffeine is an integrated value. No statements with respect to the temporal and spatial distribution can be

made. The attenuation along the flow path may have occurred uniformly or at different rates.

A slight shift of the caffeine TBC peak was taken into account by a retardation coefficient of 1.046. This value refers to the best fit of the model and may be affected by the scattering of the measured caffeine concentrations at the peak maximum. If the retardation is due to unpolar or polar interactions with organic carbon or the aquifer material cannot be determined.

From the observed mass loss of caffeine relative to the conservative reference tracer uranine, an attenuation capacity of the aquifer along the flow path of the tracers can be deduced. The high attenuation rate highlights the potential of caffeine as groundwater tracer to indicate the natural attenuation potential even in rapid flowing systems. Due to its low limit of quantification, very little amounts of caffeine can be used while still producing a pronounced TBC. Together with the fluorometrically detectable uranine an inexpensive and environmentally benign method for the indication of the *in-situ* attenuation potential along the tracer flow path is presented. In contrast to laboratory experiments, this method determines the natural attenuation potential and the risk posed by micro-contaminants in aquifers *in-situ*. The complexity of the system is captured and considered by lumped parameters, i.e. spatially averaged values across the length of the whole flow path.

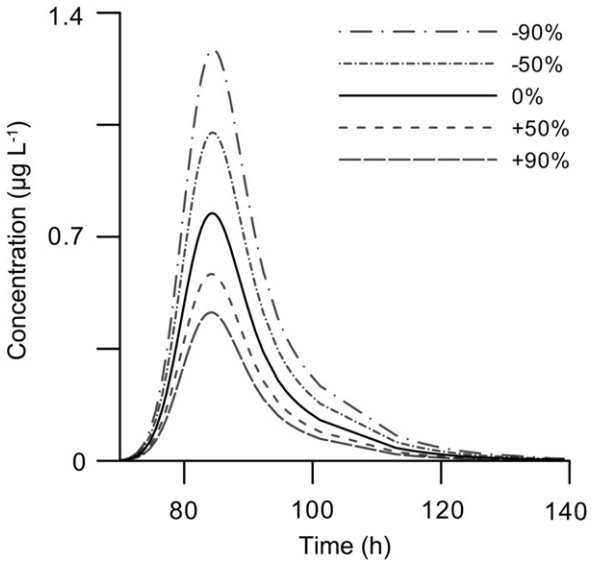

Fig. 4 – Effect of varying the attenuation rate μ in the model for caffeine. The higher the attenuation rate, the lower the peak and vice versa. The shape of the tracer breakthrough curve is not affected by the variation of the attenuation rate.

3.2. *Sensitivity analysis*

By varying single parameters and comparing the root mean square errors (RMSE), the sensitivity of the modelled concentrations to each parameter was assessed. Geyer et al. (2007) discussed the sensitivity of the model concentrations with respect to the parameters v, D, θ_m, α and β. The sensitivity of the model to the parameters μ and R were evaluated for caffeine (Fig. 3). The parameter R is investigated, varying $(R-1)$ instead of R, since the difference to 1 quantifies the shift of the TBC. The effect of varying the attenuation rate μ rate is illustrated in Fig. 4. The higher the attenuation rate, the smaller is the peak and vice versa. Except for a shorter tailing for high attenuation rates, the shape of the TBC is not affected by changes in the attenuation rate.

3.3. *Implication*

The determined attenuation rates from the large-scale artificial dualtracer test could be used to improve the estimation of wastewater volumes infiltrating the aquifer within the catchment area (Hillebrand et al., 2012). In that study wastewater volumes appearing in the spring water were quantified, employing caffeine concentrations. An attenuation of caffeine between source and spring was neglected as intrinsic attenuation data were missing. The mean rate of infiltrating wastewater was determined to be 2.2 ± 0.5 m^3 d^{-1}. Taking the here presented results into account, the impact of caffeine

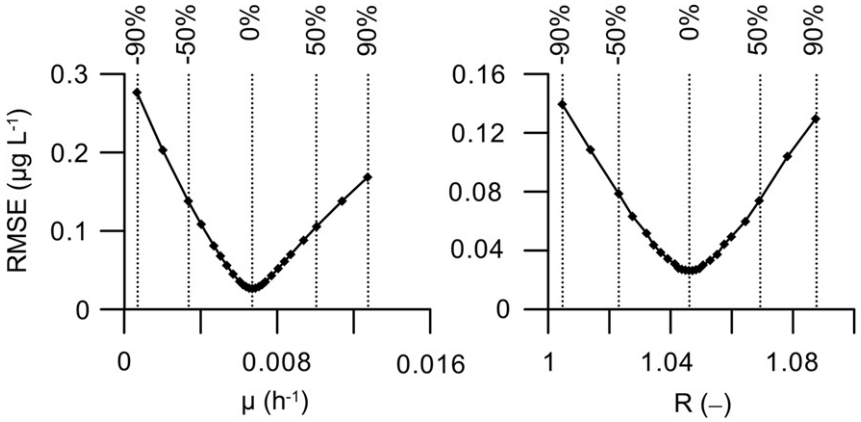

Fig. 3 – Results of the sensitivity analysis for the parameters μ and R. The values were obtained from variations of parameters in the model for caffeine transport. Please note the different scales of the ordinates. μ = attenuation rate, R = retardation coefficient. The percentages above the graphs indicate the magnitude of variation of each parameter.

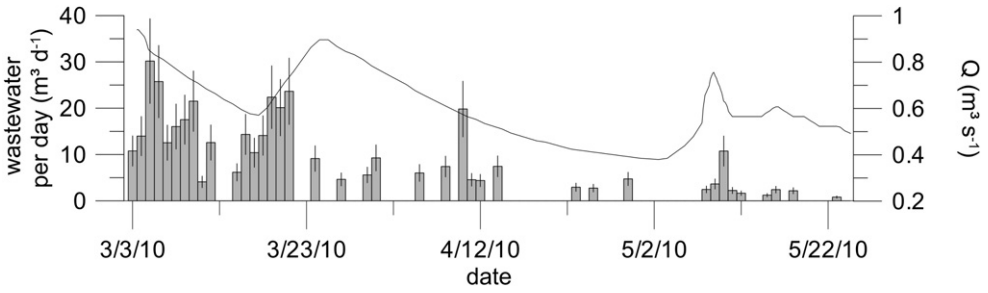

Fig. 5 – Calculated volumes of wastewater at the investigated spring under consideration of the determined first-order attenuation term. Adapted from Hillebrand et al. (2012).

attenuation should be included in the wastewater impact estimation. Extending the formula stated by Hillebrand et al. (2012) with a first-order attenuation term leads to:

$$WW = \frac{c \cdot e^{\mu \cdot t} \cdot WC \cdot Q}{I} \quad (6)$$

where WW is the volume of wastewater discharging at the spring per day; c the caffeine concentration at the spring; μ the first-order attenuation rate (0.0067 h^{-1}); the mean residence time of wastewater in the subsurface t (115 ± 20 h); the daily water consumption per capita in the spring catchment WC (134 L d^{-1} $person^{-1}$); spring discharge Q and the load of caffeine in untreated wastewater I (15.8 ± 3.8 mg d^{-1} $person^{-1}$; Buerge et al., 2003). A mean wastewater infiltration rate of 4.7 ± 1.4 m^3 d^{-1} could be calculated. The temporal distribution is shown in Fig. 5.

The sensitivity of the wastewater estimation method is affected by the effective attenuation of caffeine as well. Considering the method quantification limit of caffeine (4.3 ng L^{-1}) and a mean spring discharge of 0.5 m^3 s^{-1} the minimum volume of wastewater, which can be quantified is 3.4 ± 1.0 m^3 d^{-1}.

4. Conclusion

- A methodology to identify the attenuation potential of a karst aquifer is presented employing a dualtracer test with uranine and the reactive indicator caffeine.
- Surprisingly high attenuation rates for caffeine indicate a higher attenuation potential of the investigated karst aquifer than expected.
- To identify reactive transport and potential attenuation, the use of a conservative reference tracer (e.g. uranine) is a prerequisite.
- The application of uranine and caffeine during a dualtracer experiment is an inexpensive and environmentally benign approach for the assessment of the *in-situ* attenuation potential even in rapidly flowing groundwater systems.

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