

Selected micropollutants and their toxicity in groundwater

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1 Abstract

Clean drinking water is one of the main requisites for a healthy human population. However, global changes such as increase in industrialization, population and urbanization has led to over-exploitation of water resources and deteriorated water quality. The amount of potentially hazardous substances entering the ecosystem is increasing every day. With increase in usage of groundwater for drinking purposes, it is crucial to monitor groundwater quality. Micropollutants have emerged as a global concern due to their ubiquitous presence in the hydrological systems. Existing biological wastewater treatment plants are not specifically designed to remove micropollutants therefore discharges from WWTPs is considered as major point source of these chemicals in our environment. The occurrence and continuous input of organic micropollutants in receiving waters including groundwater is a growing environmental issue. Significance of micropollutants in determining the water quality is well understood in surface water in contrast to groundwater. Possible removal of micropollutants by the aquifer microbial communities has been receiving focus in the recent years. Aquifer microbial communities are of prime importance in governing groundwater quality due to their role in removal of contaminants in subsurface. Majority of the investigations that showed pollutant biodegradation in groundwater applied considerably high concentrations due to the experimental complexities. However, micropollutant concentrations in the environment range between ng/L to µg/L. Upon reflecting the significance of aquifer microbial communities and frequent occurrence of low concentrations of micropollutants in groundwater, a better insight about their influence on microbial communities is vital. In this study, fate and influence of 4 organic micropollutants (Sulfamethoxazole, Acesulfame, Benzotriazole and 4-Formyl-aminoantipyrene) in microbially active aquifer was monitored in mesocosm aquifer systems. The results show that low concentrations of micropollutants, in the range of several months (112 days), does not cause any significant changes to aquifer microbial activity under static and flow through conditions. Simulations performed considering transport processes suggest influence of dispersion and sorption on spreading of the micropollutants delaying their transport. However, it is necessary to monitor the experimental systems for a longer period and increase the complexity of the reactive transport model considering degradation kinetics facilitating improved and in-depth conclusions.

Keywords: aquifer, biodegradation, groundwater, micropollutants

Anotácia

Čistá pitná voda je jednou z hlavných potrieb pre zdravú ľudskú populáciu. Globálne zmeny, akými sú nárast industrializácie, obyvateľstva a urbanizácie viedli k nadmernému využívaniu vodných zdrojov a zhoršeniu kvality vody. Množstvo potenciálne nebezpečných látok vstupujúcich do ekosystému sa každým dňom zvyšuje. Pri náraste spotreby podzemnej vody na pitné účely je dôležité kvalitu týchto podzemných vôd monitorovať. Celosvetové znepokojenie vyvolali v posledných rokoch práve mikropolutanty z dôvodu ich prítomnosti vo všetkých hydrologických systémoch. Súčasné čistiare odpadových vôd nie sú špeciálne navrhnuté na odstraňovanie mikropolutantov, preto je vypúšťanie vôd z ČOV považované za hlavný bodový zdroj týchto chemických látok v našom prostredí. Výskyt a nepretržitý vstup organických mikropolutantov do recipientu vrátane podzemných vôd je rastúcim environmentálnym problémom. Na rozdiel od povrchových vôd, mikropolutanty v podzemných vodách nie sú natoľko prebádané, aby sme vedeli predvídať ich správanie. V posledných rokoch sa dostáva do popredia možné odstraňovanie mikropolutantov mikrobiálnymi komunitami vo zvodnenej vrstve. Mikrobiálne spoločenstvá zvodnených vrstiev majú prvoradý význam pri riadení kvality

podzemných vôd kvôli ich úlohe pri odstraňovaní kontaminantov v podzemí. Vo väčšine výskumov týkajúcich sa biologickej degradovateľnosti v podzemných vodách sa aplikovali vysoké koncentrácie skúmaných látok. Avšak, koncentrácie mikropolutantov v reálnom životnom prostredí sa pohybujú v rozmedzí od ng/l do µg/l. Po zohľadnení významu mikrobiálnych spoločenstiev v zvodnených vodách a častom výskyte nízkych koncentrácií mikropolutantov v podzemných vodách je nevyhnutné lepšie pochopenie ich vplyvu na mikrobiálne spoločenstvá. V tejto štúdií bol sledovaný osud a vplyv 4 organických mikropolutantov (Sulfametoxazol, Acesulfam, Benzotriazol a 4-Formyl-aminoantipyrín) v mikrobiálne aktívnych vodných tokoch v mesokozmových vodonosných systémoch. Výsledky ukazujú, že nízke koncentrácie mikropolutantov v rozmedzí niekoľkých mesiacov (112 dní) nevykazovali žiadne významné zmeny v mikrobiálnej aktivite vodonosnej vrstvy pri statických a prietokových podmienkach. Simulácie vykonané s ohľadom na transportné procesy naznačujú vplyv disperzie a sorpcie na šírenie mikropolutantov, ktoré odďaľujú ich transport. Tieto experimentálne systémy je však potrebné monitorovať na dlhšie obdobie a zvýšiť komplexnosť modelu reaktívneho transportu s ohľadom na kinetiku degradácie, ktorá uľahčuje vylepšené a hĺbkové závery.

Kľúčové slová: vodonosná vrstva, biodegradácia, podzemná voda, mikropolutanty

2 Introduction

Clean drinking water is one of the requisites for a healthy human population. However, the growing industrialization and extensive use of chemicals have increased the burden of unwanted pollutants in water in countries all over the world. Ground water contamination is nearly always the result of human activity. In areas where population density is high ground water is especially vulnerable. Micropollutants refer to residue from substances, use daily in modern society, including for example pharmaceuticals and personal care products (PPCPs), hormones, pesticides and industrial chemicals. Residue from these can be found in water bodies everywhere. Hazardous micropollutants usually end up in wastewater treatment plants (WWTPs) as a result of domestic uses of textiles, electronics, pharmaceuticals, and cosmetic and hygiene products. Other sources are industrial discharge, stormwater runoff from cities, and surface run-off from agricultural areas. Studies show that 70% of the pharmaceutical residue in the wastewater comes from household use (Herrmann, 2015). The amount of potentially hazardous substances entering the ecosystem is increasing every day. Existing biological wastewater treatment plants are not specifically designed to remove micropollutants therefore discharges from WWTPs is considered as major point source of these chemicals in our environment. The occurrence and continuous input of organic micropollutants in receiving waters including groundwater is a growing environmental issue. As a consequence to the growing population and high dependence of modern societies on chemicals especially pharmaceuticals, the amount of micropollutants in the environment is still expected to increase in the coming years. According to the UN World Water Development (2018) report, water quality is expected to further deteriorate in the coming years affecting human health and environment negatively on a global scale. Inadequacy of surface water to meet the water demand has promoted the users to opt for groundwater as primary water source for domestic and agriculture in many regions of the world (Li et al., 2017). However, anthropogenic activities along with the global changes and poor management of groundwater resources have led to deterioration of groundwater quality (Giri & Singh, 2015; Li et al., 2017). The increasing presence of organic micropollutants in different segments of the water cycle threatens future water resources.

Macro and micropollutants threaten groundwater quality while the fate and behavior of macropollutants are well demonstrated in comparison with micropollutants. Advancements in analytical methods have enabled to obtain a better insight about micropollutants in recent years (Richardson & Tarnes; 2017). Micropollutants are comprised of pharmaceuticals, life-style compounds, pesticides, industrial additives and by-products, and food additives (Sorenson et al., 2015) and commonly derived from municipal, agricultural and industrial sources. Micropollutants or emerging contaminants, present in low concentrations (ng/L to fewer µg/L) is a growing concern in aquatic systems and their presence in groundwater has drawn attention in recent years. Of all the studies investigating the fate of micropollutants in groundwater most of them applied the contaminants in much higher concentrations than their occurrence in the environment for experimental convenience.

In order to obtain a better insight about the behavior of micropollutants in aquifer, it is necessary to conduct studies with environmentally relevant concentrations (Sui et al., 2015; Lapworth et al., 2012).

Microbial communities in the subsurface influence groundwater quality, in the best case via degradation of pollutants, contaminants on the other hand may negatively impact the native microbes. Hence, it is important to understand the influence of micropollutants on the microbial communities and vice versa. There have been very few studies conducted to investigate the influence of contaminants on the microbial communities. Out of all the studies conducted, majority of the focus is on determining biodegradation kinetics. Any change in the microbial communities may change the microbial ecology of the site in a long-term and in consequence microbial ecosystem services. Therefore, interfering with the microbial catalysed contaminant removal processes which in turn can potentially change the groundwater quality.

The aim of this research is to understand micropollutant fate and transformation in natural systems and to study microbial biodegradation activity and its limitations. In this work we also investigate the impact of low concentrations of micropollutants on the natural microbial communities and their flow and transport in aquifers. Micropollutants for the study were chosen based on the observations at a riverbank infiltration site at river Main at Würzburg. Experiments in an artificial aquifer system were carried out to observe changes in the microbial activity caused by individual micropollutants and in mixture. The experiment in the artificial aquifer system also provided information about the flow and transport of micropollutants in low concentrations through a sandy alluvial aquifer.

3 Micropollutants in aquifer

In the last few decades, occurrence of micropollutants in aquatic systems has become a concern globally. Behaviour and fate of micropollutants in surface water and wastewater has been well understood in comparison with groundwater. In the last two decades, assessment of micropollutants in groundwater has grown due to the development of rapid and efficient screening techniques for micropollutants. In a review by Lapworth et al., 2012, sources and pathways for groundwater pollution by micropollutants were discussed. The review also considers the fate of micropollutants in subsurface. Point sources of micropollutants to aquifers includes municipal sewage treatment plants, waste disposal sites and industrial effluents (Lapworth et al., 2012; Heberer, 2002). Nonpoint source pollution occurs on a large geographical scale due to urban runoff, and leakage in the urban sewer system. Transport and fate of micropollutants in the subsurface depends upon the physicochemical properties of the contaminant, water flow and residence time, temperature and redox conditions (Lapworth et al., 2012). Hydraulic and geological properties, both influence the transport of contaminants in subsurface.

Processes that influence the transport of micropollutants to and in aquifers are adsorption and biodegradation. Sorption on to soil matrix and geological materials is an important process in attenuation of micropollutants in the subsurface. However, sorption capacity of contaminant varies depending on the properties and composition of the soil matrix and geological materials. For example, Chefetz et al., (2008) demonstrated the sorption-desorption behaviour of 3 pharmaceuticals in soil layers and significant influence of soil organic matter on the processes i.e. the compounds demonstrated increased mobility in soil layers with poor organic matter. Meffe and Bustamante (2014) reviewed the occurrence of 161 micropollutants in Italian surface and groundwater. According to their review, pesticides being persistent can travel long distances and infiltrate into aquifer without being attenuated. They are attributing the significantly less studies regarding the occurrences of micropollutants in groundwater to be due to the elaborate sampling.

4 Aquifer microbial communities

Groundwater quality and sediment properties are greatly influenced by the microorganisms in subsurface (Griebler & Avramov, 2015). Microbial activities catalyze reactions in subsurface like oxidation of organic matter to carbon dioxide, dissolution and precipitation of inorganic components. Aquifer microbial communities also degrade toxic compounds in the subsurface hence, playing a critical role in determining the quality of groundwater.

Since 1980s there have been a lot of studies carried out as an attempt to understand the aquifer microbial communities, their ecological significance and distribution. Slowly, with the evolution of sampling techniques and procedures of analysis a better picture about the microbial ecology in the aquifer has been obtained. A review about the Microbial diversity in groundwater ecosystems by Griebler and Leuders (2009), discusses the distribution of microorganisms in aquifers with most of the microorganisms being attached to sediments thereby aiding them to thrive in an environment poor with carbon and nutrients (Griebler and Leuders; 2009). Spatio-temporal variability and the components causing interference to the aquifer influence the microbial community in terms of composition and activity. Any disturbance caused to the microorganisms in the aquifers will also affect the microbially catalysed biogeochemical reactions thereby influencing the groundwater quality.

5 Studied micropollutants in groundwater

5.1 Sulfamethoxazole

Sulfamethoxazole (SMX) is a sulfonamide antibiotic inhibiting the folic acid synthesis required for bacterial growth. It is typically combined with trimethoprim and commonly known by trade names Bactrim and Gantanol. SMX is analogous to para-aminobenzoic acid (PABA) involved in the production of folic acid in bacteria. SMX competes with PABA inhibiting the production of a precursor of folic acid (US FDA, 2008). After absorption into the human body, approximately 30% of SMX is excreted in its original form along with two metabolites.

Increased production and consumption of SMX has led to its frequent occurrence in aquatic systems as ubiquitous contaminant. In a study conducted by the USGS throughout the nation in 2007 demonstrated that 23% of the samples had SMX at an average concentration of 1.11 µg/L (Barnes et al., 2008). In another study conducted by Underwood et al. (2011), the authors observed that sub-therapeutic concentrations of SMX alters the composition of enriched nitrate-reducing microcosms by inhibiting expression of the nitrification gene. Hence, SMX may co-occur with NO³⁻ contamination in the subsurface and alters the ecosystem in subsurface. SMX leads to the development of bacterial resistance in aquatic systems (Larcher and Yargeau, 2012) and because of its resistance to natural attenuation processes it has also been used as a tracer for subsurface contamination (Barber et al., 2008). SMX is photodegradable with a half-life of 19 days in the presence of sunlight. Observations of a study conducted by Avisar et al. (2009) indicates the ability of SMX to penetrate to deep subsurface (~1.3 Km) in saturated porous media.

SMX can also be biodegraded, anaerobic degradation rates for SMX are significantly lower than those measured under aerobic conditions in soil (Lin and Gan, 2011). At low concentrations (<1 µg/L) the degradation was observed to be slow with a half-life of 49 days, while at higher concentrations (>1 µg/L) it was 16 days (Baumgarten et al., 2011). On the other hand, there are studies reporting no removal of SMX in a pilot scale riverbank filtration system or in biological filters (Benotti et al., 2012; Zearley and Summers, 2012).

5.2 Acesulfame

Acesulfame (AC) is an artificial sweetener widely and commercially used as potassium salt known as acesulfame-K. AC is 180 times sweeter than sugar but also has a bitter aftertaste. The consumption of AC per capita in different European countries ranges from 4.9 mg/d to 17.6 mg/d. AC passes the human body unaffected thus quantitatively reaching the waste water and aquatic systems (Lange et al., 2012).

A study monitoring artificial sweeteners in a shallow urban aquifer for 6 years determined that 27.5% of the screened wells were contaminated with AC. Spatial distribution of the compound was found to be highly relevant to the pipeline leakages (Wolf et al., 2012).

Several studies have reported the presence of ACE in groundwater (Buerge et al., 2009; Scheurer et al., 2009). Half-life of AC in soil was determined to range between 17-19 days and its presence in

groundwater is mainly due to the infiltration of waste-water contaminated surface water (Buerge et al., 2011).

AC is present as an anion in the aquatic environment with $pK_a \leq 2$ thus, favouring a strong mobility of AC in sediments. AC is rather persistent to degradation hence it is used as a tracer to detect contamination of aquifers by wastewater. Storck et al., (2016) reported low sorption of AC to soils even with high content of organic carbon. They also demonstrated persistence of AC without degradation unless oligotrophic nutrient conditions were applied. Effects of acesulfame on microbial communities in subsurface are not well understood.

5.3 Benzotriazole

Benzotriazole (BT or 1H-Benzotriazole) is used as corrosion inhibitor in cooling systems and as additive to dishwashing detergents. Major source of BT in the environment is treated municipal wastewater. BT is also employed in anti-freeze and de-icing fluids. BTs are classified as toxic and cause long-term adverse effects to the aquatic environment. BT is readily soluble in water and has low adsorption tendency to organic matter in comparison to other micropollutants (Giger et al., 2006).

Aerobic biodegradation of BT follows a first order reaction with a half-life of 43 days. 72% of BT in water was observed to be degraded under aerobic conditions while, it reduced to 65% in an anaerobic environment. Addition of Fe (III) is predicted to enhance the anaerobic biodegradation of BT (Liu et al., 2012). Cancilla et al., (2003) discussed the contamination of groundwater and soil by BT (used in aircraft deicing and anti-icing fluids) of an air force base. At the Oslo airport, 20 top soil samples collected beside the runway were found to be contaminated with BT (Breedveld et al., 2002). In the same study, concentration of BT in groundwater was determined to range between 0.001 mg/m³ and 1100 mg/m³.

In a survey throughout Europe conducted by Loos et al. (2010), 53% of the groundwater samples analyzed were found to have BT. BT was also detected in four out of six groundwater pumping stations in a densely populated area in Zurich, Switzerland (Kahle et al., 2009). The range of concentrations of BTs as reported in literature are 10-1 to 104 ($\mu\text{g}/\text{kg d.w}$) in soils and sediments, and 1 to 10⁻⁶ mg/L in surface and groundwater.

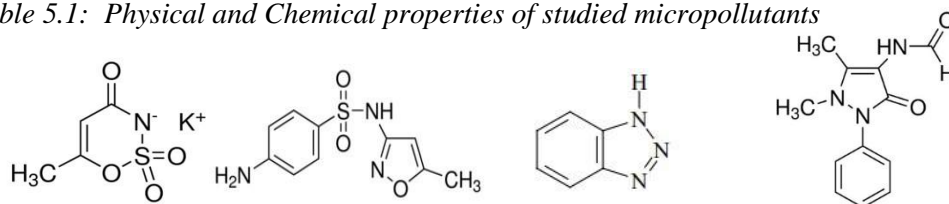
(Careghini et al., 2015) and Jia et al., (2006) studied the influence of BT on the microbial community in the unsaturated zone. They found that BT concentrations of 10 mg/L retarded the microbial degradation of toluene in the subsoil of 1-2m depth. Additionally, there was severe reduction in the growth yields.

5.4 4-formyl-aminoantipyrine

4-formyl-aminoantipyrine (FAA) is a metabolite of metamizole which is a non-steroidal anti-inflammatory drug. Hydrolytic cleavage of metamizole results in the primary metabolite 4-methyl-aminophenazone which is further metabolized in the human body to aminoantipyrine, 4-acetylaminoantipyrine and FAA. Among the four metabolites of metamizole, FAA remains persistent during the sewage treatment.

The presence of FAA in groundwater has been reported by a few studies, however, the fate and behaviour has not been well understood. Candela et al., 2013 reported 96ng/L of FAA in the monitoring wells samples near an artificial aquifer recharge site in Barcelona, Spain. During a 7-year study, the detection frequency of FAA in groundwater was determined to be 25%, the authors also described distribution co-efficient (K_d) as 0.86 L/Kg.

Table 5.1: Physical and Chemical properties of studied micropollutants



Property	Acesulfame-K (AC)	Sulfamethoxazole (SMX)	Benzotriazole (BT)	4-Formylaminoantipyrine (FAA)
Use	Artificial sweetener, non-nutritive sweetener	Antibacterial drug. Mainly used in treatment of acute urinary tract infections	Corrosion inhibitor, deicing and defogging agent, used in cooling systems and dishwashing detergents for metal protection.	Metabolite of metamizole (non-steroidal anti-inflammatory drug) used as analgesic and antipyretic drug.
Alternative names	Acesulfame-potassium Acetosulfame-	Sulfamethoxazol 3-(para-Aminophenylsulfonamido)-5-methylisoxazole 5-methyl-3-sulfanilamidoisoxazole	1,2-Aminozophenylene Azimidobenzene 1H-Benzotriazole Benzisotriazole	4-Formamidoantipyrine
CAS-No.	55589-62-3	723-46-6	95-14-7	1672-58-8
Molecular formula	C ₄ H ₄ KNO ₄ S	C ₁₀ H ₁₁ N ₃ O ₃ S	C ₆ H ₅ N ₃	C ₁₂ H ₁₃ N ₃ O ₂
Molecular weight (g/mol)	201.237	253.28	119.13	231.251
Water solubility	Readily soluble	610 mg/L at 37°C	1-5g/L at 23.7°C	-
Half-life	17-19 days in loamy arable soil (Buerge et al., 2011)	9-11.4 days in non-sterile soil (Lin and Gan 2011)	29±2 days in non-sterile columns with anaerobic sediment (Alotaibi et al., 2015)	≈420 days sediment (Dimkic et al., 2017)
Concentration in groundwater	4.7µg/L (Buerge et al., 2009)	1.1 µg/L (Barnes et al., 2008)	0.016–0.0777µg/L (Kahle et al., 2009)	96ng/L (Candela et al., 2016)
Sorption Coefficient (Kd)	-	0.77 L/Kg in sandy loam (Srinivasan et al., 2010)	0.26 L/Kg in anaerobic sediment	0.86 L/Kg (Dimkic et al., 2017)
Degradation kinetics	-	0.0766 ± 0.0115 d ⁻¹ (Lin and Gan 2011)	-	-

From the literature, it is evident that there is less literature available focusing on the study of the above discussed micropollutants in groundwater in comparison with surface water. Also, most of the studies carried out to study the transport of micropollutants were found to have used higher concentrations in the mg/L range. However, environmental concentrations are typically in µg/L or ng/L concentrations (Sui et al; 2015). Additionally, there have been very few studies focusing on the effect of the environmental concentrations of micropollutants on the natural microbial community.

6 Materials and Methods

Chemicals Benzotriazole, Acesulfame, Sulfamethoxazole, and 4-formylaminoantipyrine were purchased from Sigma-Aldrich (Germany).

Indoor Aquifer Experiment

The experiment was conducted in an indoor artificial aquifer system with 4.8m length, 0.8m width and 0.7m long filled with homogenous natural sediment of grain size between 0.63mm to 2mm. The indoor aquifer was saturated with a water level difference of 9cm along 4.8 m flow length. The indoor aquifer was organized in 5 transects (A, B, D, C, E) in a longitudinal direction, each with 10 sampling

ports and oxygen sensors oriented in the vertical direction. For the current experiment, 3 of the longitudinal sampling points (B, D and E) and 3 depths each (25cm, 45cm, 60cm) were chosen for sampling. Samples were collected through a stainless-steel capillary connected to a fluran tubing and peristaltic pump. The aquifer was constantly maintained before and during the experiment. Water flux, oxygen concentration and temperature at the inlet and outlet were monitored every week.

The container with the artificial aquifer is of 5m length when 10cm on both the ends acts as inlet and outlet chambers. At the inlet, the aquifer can be supplied in 4 sections however; this separation is restricted to this chamber and does not extend throughout the length of the aquifer. Design of the artificial aquifer experiment is as demonstrated in figure 6.2.

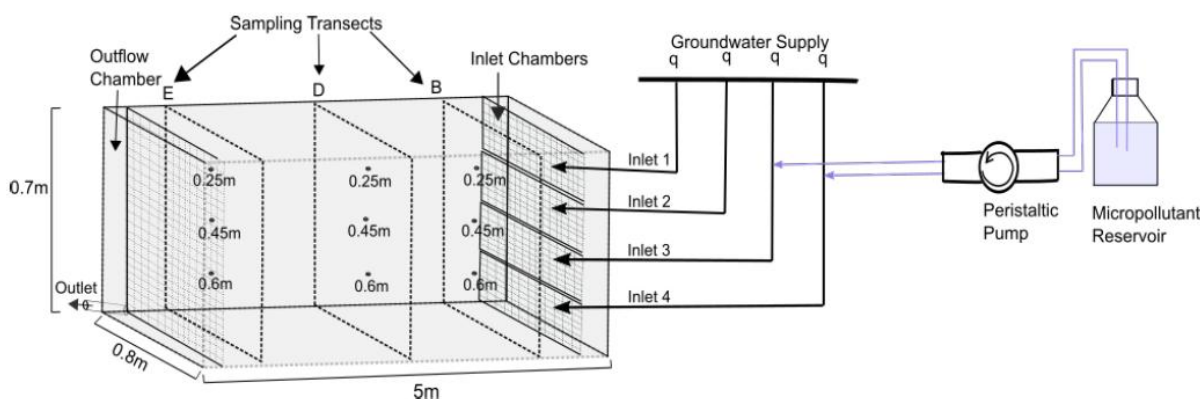


Figure 6.1: Experimental Set-up of Indoor Aquifer (Adapted from Herzyk et al., 2017)

Micropollutant Injection and Sampling in Artificial Aquifer

Concentration of micropollutants were determined based on observations of the riverbank infiltration study along river Main in Würzburg. A 5 L reservoir of micropollutant mixture was prepared in sterile water, tightly sealed and covered to avoid sunlight. The reservoir was connected to the peristaltic pump with two stainless steel capillaries and two fluran tubings in line. After the peristaltic pump, the mixture of the micropollutants was continuously injected to the two lower inlet chambers at a flow rate of $0.12 \text{ L}\cdot\text{day}^{-1}$.

Table 6.1: Final concentration of micropollutants in the groundwater supplied to the two lower inlet sections artificial aquifer system (see Figure 6.1)

Compound	Concentration ng/L
Acesulfame	1000
Sulfamethoxazole	50
Benzotriazole	1000
4-Formyl-aminoantipyrine	500

In order to examine the changes in the artificial aquifer, both sediment and water samples were collected. Samples were collected at the following positions:

- Sampling Point 1 - Micropollutant mixture source before the peristaltic pump
- Sampling Point 2 - After the peristaltic pump-In order to observe if there was sorption to the plastic tubing of the pump
- Sampling Point 3 - All the four inlet chambers
- Sampling Point 4 - At transects B, D and E of the aquifer system at depths 25cm, 45cm and 60cm

During each sampling, 100 ml water samples were collected in a sterile Schott bottles for measuring total ATP concentration, external ATP concentration and total organic carbon.

7 Analysis performed

Estimation of viable microbial activity by ATP analysis

Microbial activity can be estimated via adenosine tri-phosphate (ATP) measurements in water and microbial biomass (Eydal and Pederson; 2007). Furthermore, ATP measurements can also be performed with sediment samples.

Considering the sensitivity of the assay, all samples collected were stored at 4°C and processed within 48 hours of sampling. Total ATP concentrations in the water samples were measured by warming 1ml of samples and BacTiter-Glo™ reagent for 2 minutes. Followed by addition of 50 µL BacTiter-Glo™ reagent to the warmed samples, incubated for 1 minute and measured for luminescence.

For sediment samples, 200mg of sediment samples were suspended in 100µl of phosphate buffer (pH 7) and warmed to 30°C for 3 minutes. Subsequently, 200µl BacTiter-Glo™ Assay reagent was added to the sediment-buffer mixture and incubated at 30°C for 1.5 minutes. Samples were centrifuged at 12000 x g for 30 seconds and 200µl was transferred into a sterile tube to measure luminescence under conditions mention in Herzyk et al. 2017.

Dissolved Organic Carbon (DOC) Analysis

Water samples collected from the artificial aquifer were analysed for the dissolved organic carbon. 7ml of water samples were filtered through 0.45µm syringe filters and collected into clean dry glass tubes. To the filtered water samples a drop of concentrated HCl was added before placing them into the TOC analyzer (TOC-VCPH; SHIMADZU). All the samples were processed in analytical triplicates. Before and after the water samples DOC standard solutions and MQ water were measured for DOC.

8 Results and discussion

During the artificial aquifer experiment pH, electrical conductivity, major cations and major anions are determined in order to evaluate the changes. Also, oxygen concentrations and temperature are monitored every week using oxygen sensors.

Estimation of microbial activity in aquifer sediments

In the indoor aquifer, microbial activity was estimated by observing the changes in the ATP concentrations (mol/L). A mixture of 4 compounds at low concentrations is introduced into two lower inlet chambers of the indoor aquifer. There is a continuous input of the micropollutants along with groundwater flowing through the system.

Standard curve applied for determining total ATP concentrations is used for the estimations in the artificial aquifer samples. The variation in the ATP concentration along the artificial aquifer (at transects B, D and E) at three depths i.e. 25cm, 45cm and 60 cm from the top. The depths chosen were to determine the difference in the microbial biomass in the contaminated and uncontaminated regions within the aquifer system.

At transect B, closest to the inlets variations in ATP concentrations are as observed in the figure 8.1. The depth profiles of ATP concentrations at transect B over time indicates that there are no pronounced variations in the ATP concentration. The ATP concentrations measured on day 7, 28 and 56 fall in the same range as the pristine samples collected prior to the injection of contaminants to the aquifer system. The faint difference relatively obvious in the graph is the slight increase in ATP concentrations with depth on day 28 and contradicting decrease in ATP concentrations with depth in

day 56 samples. However, these minute variations observed can be a result of analytical error, trend in the ATP concentrations were measured over a shorter period of time in order to determine the importance of the variability in the data. Also, transect B being closest to the inlet, it was expected that the influence on microbial biomass will be first noticed in these observation sites.

Depth profiles of ATP concentrations at transect D and E in the aquifer system is depicted in figures 8.2 and 8.3. Similar to the observations in transect B, no significant variations were observed in the ATP concentrations. Although close observation of the total ATP concentrations across the aquifer indicates a slight increase from transect B to E along the entire depth. As the variations in not only observed at depths 45cm and 60cm where the micropollutants are injected, ATP concentration at 25cm also increases indicating the changes observed cannot be concluded as the effect of micropollutants.

Critical analysis of the data indicated that although there are minute changes in the ATP concentrations it is necessary to understand if these indicate any modifications in the microbial biomass. In order to determine the contribution of effect of micropollutants on the microbial biomass, the amount of cells contributed by each micropollutant at the concentrations studied is estimated.

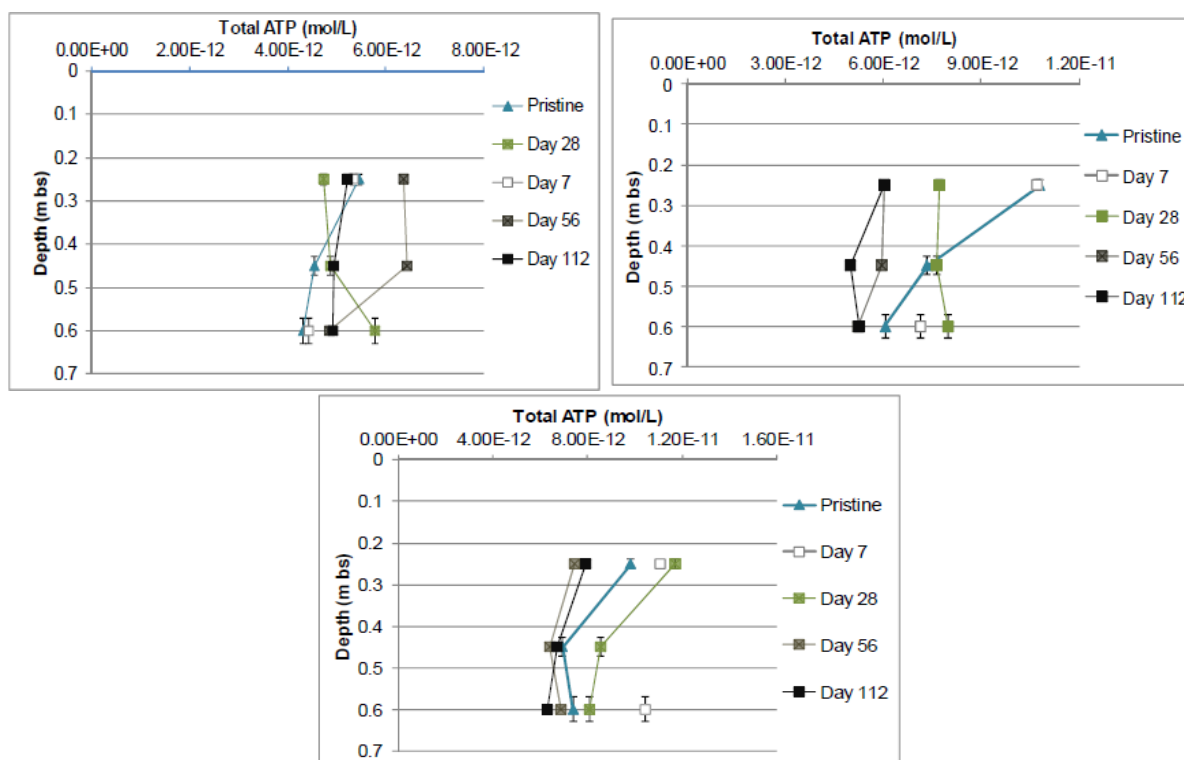


Figure 8.1 – 8.3: Depth profile of the total ATP concentrations in water (mol/L) at transects B, D, E over 112 days.

Estimation of microbial activity on the aquifer sediments

Wet sediment samples from the artificial aquifer were collected prior to the start of the experiment and on day 7, day 28. ATP concentration (mol/g of wet sediment) is determined using a standard curve. Variations in the ATP concentration at all these transects are as depicted in the figure 8.4 – 8.6. The ATP concentration in sediment samples from all the three transects is in the range of 10⁻⁸ mol/g of wet sediment. Samples estimated from the pristine aquifer system (prior to the experiment) and day 28 indicates there is no significant variation caused by the micropollutants on the living biomass. The sediment samples collected on day 7 indicates a faint increase in the ATP concentration. However, the

changes observed can be due to the analytical variations. Also, ATP concentrations do not demonstrate noticeable changes along the depth.

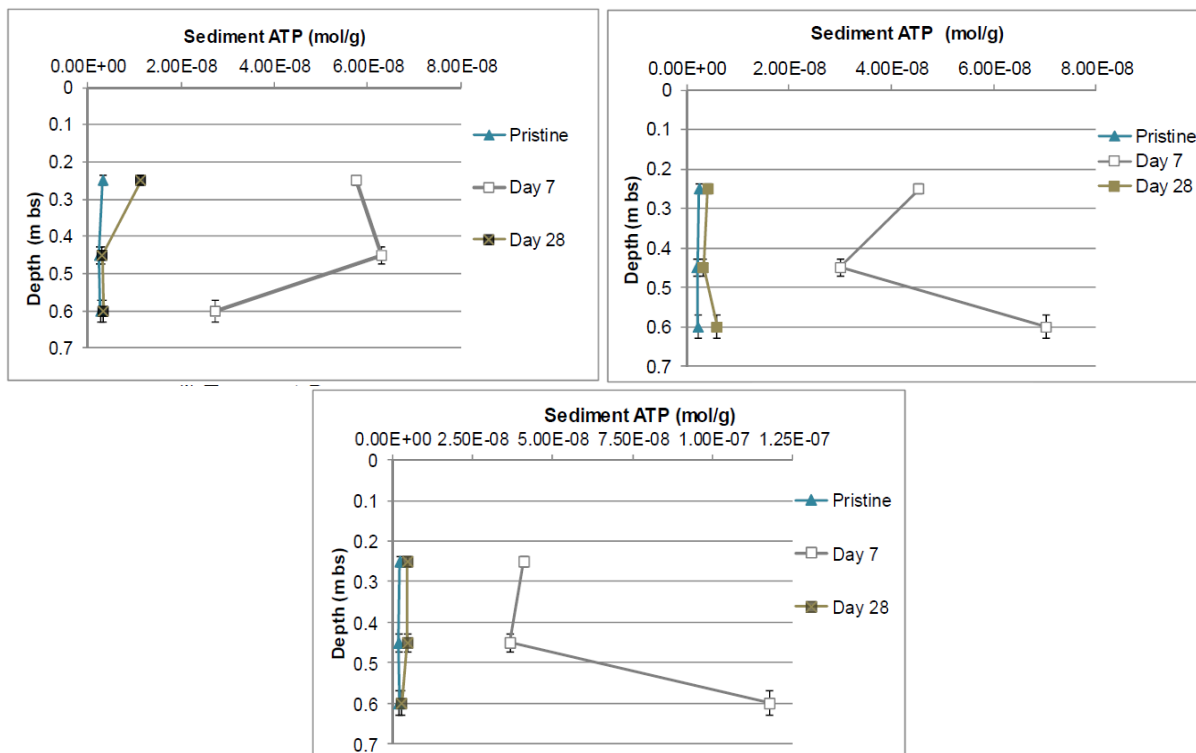
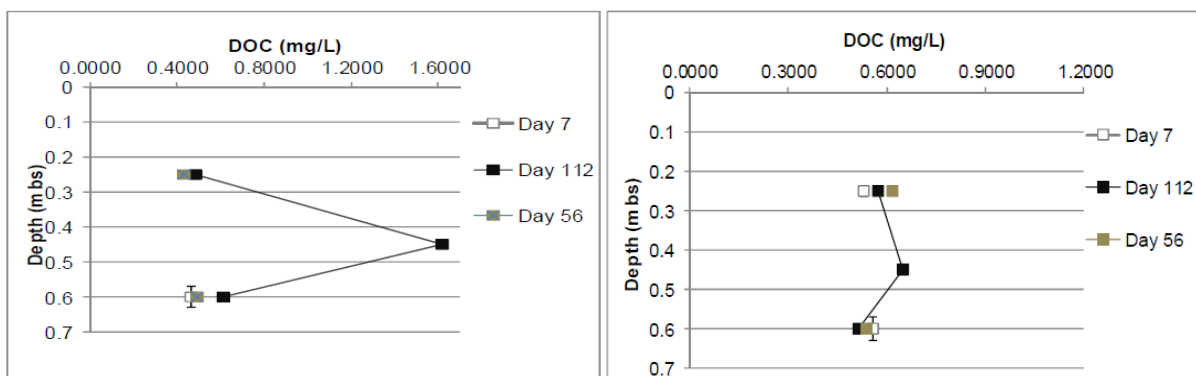


Figure 8.4 – 8.6: Depth profile of the total ATP concentrations in wet sediment (mol/g) at transects B, D, E over 112 days.

DOC Analysis

DOC is a measure indicating the total amount of dissolved organic carbon in the water. In this study, DOC measurements revealed the amount of DOC in day 28 water samples is observed to be low at all transects while samples collected on day 56 did not follow an extremely decreasing trend. The variations observed during day 28 samples are due to the analytical deviation related to the instrument. Hence, the any variation in day 28 samples may not be considered significant until the trend continues in further sampling periods. Along the three transects chosen for observations, DOC is observed to decrease from 0.6 mg/L in the pristine samples to 0.4 mg/L in day 56 samples. From the observations, increase in DOC at transects D and E over time is evident. Dissolved organic carbon did not vary significantly with depth. The range in the DOC concentration in the samples analysed is 0.4-1.0 mg/L, which is somewhat similar to earlier observations (Herzyk et al., 2017) and literature observations ranging from 0.4 to 0.8 mg/L (Zhang et al. 2015).



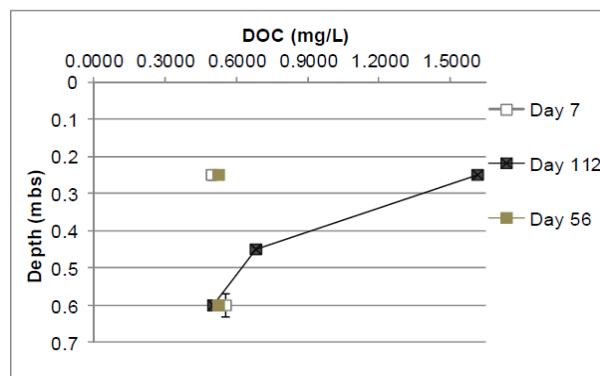


Figure 8.7–8.9: TOC (mg/L) monitored along the artificial aquifer system at transects B, D and E

Aquifer experiments were successfully set-up to evaluate the impact of micropollutants on the microbial biomass. Samplings were performed in the experiments and analysed the microbial activity. There are three possible changes in the microbial biomass that can be caused upon addition of micropollutant. (1) Increase in the biomass, (2) inhibit growth of the biomass (3) cause no effect.

There is no noticeable change in the microbial biomass in both the experiments conducted. As previous discussed, low concentrations of micropollutants used in the experiments might not contribute to a significant increase in the microbial biomass. Inhibition of microbial biomass is caused by interference of micropollutants with metabolic processes. Inhibition of microbes leading to a decrease in microbial activity and/or biomass occurs when the micropollutant is toxic. From the observations, there is no inhibition of microbes by the micropollutants observed until day 112. However, further samplings will aid to analyze the long-term and thus chronic impact of micropollutants at low concentrations over long period of time. Also, analyses of micropollutant concentrations will facilitate the determination of sorption and degradation parameters.

According to the literature, all the 4 organic micropollutants have demonstrated weak sorption and no or weak degradation in aquifers under natural conditions (Larcher and Yargeau 2012; Avisar et al. 2009; Baumgarten et al. 2011; Storck et al. 2016; Jia et al. 2006). Hence, upon considering the observations in the literature low concentrations of micropollutants can get transported in aquifers without being transformed or delayed. High dispersion and weak sorption might lead to the spread of contaminants to non-contaminated regions thus also widening the zone of natural microbial communities affected by micropollutants.

9 Conclusion

This study was set out to evaluate the influence of low concentrations of micropollutants on aquifer microbial activity due to their significance in biogeochemical processes and groundwater quality. The choice of micropollutants and the concentrations to be studied in the experiments was made based on the mean concentrations of the same micropollutants observed in a field study conducted at the riverbank infiltration site at Würzburg, Germany.

In indoor aquifer experiments conducted with the 4 selected organic micropollutants (SMX, AC, BT and FAA), no significant variation in the microbial activity was observed during the 112 days. The mixture of these low concentration micropollutants was continuously injected. The measurement of ATP concentrations along the length of aquifer at 3 different depths also demonstrated that injection of micropollutants at low concentrations may not influence the microbial communities. The aqueous phase contributes to only 1% of the total microbial biomass in aquifers, while 99% of the microbes are attached to the sediment. Hence, noticeable variation in the microbial biomass and activity in sediment samples is expected to take longer time.

Microbial biomass requires an energy source to be metabolically active. In the indoor aquifer being a flow-through system, there is continuous input of organic carbon. In this system, in case micropollutants act as carbon source upon entering the flume it is expected to be controlled by the microbial activity near the inflow leading to accumulation of the biomass near the inlet over a long term. However, even the faint increase in the ATP concentrations observed in this study were higher in transect closer to the outlet.

Duration of the experiment on the microbial activity: The impact of micropollutants on the microbial activity remains unaffected by the time span of the experiment until 112 days. However, from estimations of carbon yield by each micropollutant in the flow through experiment, it is evident that increase in microbial activity due to the micropollutant will require a longer incubation time.

Investigations suggested in order further study the impact of environmental concentrations of micropollutants on the natural microbial communities in the aquifer are as are comprehensively listed below:

1. Monitoring the indoor aquifer experiments for a longer period of time will provide insight about the impact of environmental concentrations of micropollutant on the microbial activity.
2. Determination of micropollutant concentrations in the aqueous will aid in determination of sorption and degradation kinetics under the experimental conditions.
3. Further molecular analysis of the samples collected will provide information about the changes in the composition of the microbial ecosystem in the aquifer system.
4. Monitoring the flow and transport of micropollutants under varying hydraulic parameters in the artificial aquifer system will help in understanding their significance on the interaction between low concentration micropollutants and natural microbial communities.

From the observations it can be explained that environmental concentrations of micropollutants does not inhibit the microbial communities in aquifers over a period of 112 days. Also, there is no significant increase in the microbial activity suggesting that micropollutants are not degraded. Upon considering the weak sorption of all the 4 micropollutants as observed in the literature, it can be concluded that the micropollutants remain in the water phase unchanged. This claim is supported by the observations of micropollutant analysis. In summary, environmental concentrations of micropollutants may remain unchanged in groundwater for 112 days and hence has to be monitored and remediated prior to use in order to avoid negative effects on environmental and human health. However, further long term investigations are necessary to understand the chronic effects of micropollutants in aquifers.

7 References

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