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D311/315 Identification of biodegradation processes (D311) and assessment of water quality during soil infiltration, aquifer storage and recharge (D315)

D317/503 Water quality analysis in waste water and recharged groundwater (D317) and Assessment of groundwater risk by treated waste water (D503)

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Abbreviations

AOI	Adsorbable organic iodine
BDOC	Biodegradable dissolved organic carbon
BGR	Bundesanstalt für Geowissenschaften und Rohstoffe (Federal Institute for Geosciences and Natural Resources - Hannover)
COD	Chemical oxygen demand
DOC	Dissolved organic carbon
E1	Estrone
E2	17 β -Estradiol
E3	Estriol
EE2	17 α -Ethinylestradiol
EP	Emerging Pollutants
GIZ	Deutsche Gesellschaft für Internationale Zusammenarbeit GmbH (German Society for International Cooperation)
JUA	Jordan University Amman
JVA	Jordan Valley Authority
KIT	Karlsruhe Institute of Technology
MAR	Managed Aquifer Recharge
MBR	Membrane Bioreactor
MEK	Mekorot Water Co.
MWI	Ministry of Water and Irrigation
PHG	Palestinian Hydrology Group
QUDS	Al-Quds University Jerusalem
SAT	Soil aquifer treatment
STP	Sewage treatment plant

TEA	Terminal electron acceptor	
TZW	Technologiezentrum Wasser Technology Center - Karlsruhe)	(Water
WWTP	Waste water treatment plant	

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1. Preface

Within work package 3.2 treatment requirements for waste water used in artificial recharge schemes are to be identified, focusing on emerging pollutants and microbiological parameters. In order to assess the level of treatment, knowledge of the natural attenuation processes during soil infiltration and storage in the underground are of utmost importance. By identifying the capacity of the soil passage for microbial degradation of trace organics and the removal of hygienically relevant microorganisms, favorable conditions for engineered and natural elimination processes can be recommended. It is the overall aim to maximize the use of naturally occurring degradation processes and thereby minimize the use of energy and cost intensive waste water treatment prior to its recharge. Subsequently trace pollutants still contained in the water, which is stored in the underground, will undergo further degradation. It is therefore crucial with regard to the recovery of the recharged groundwater to know the time frame in which these processes take place.

In order to gain data on the occurrence of these micropollutants in the model region Lower Jordan Valley extensive sampling campaigns have been conducted between 2007 and 2012 with the results presented in chapter 6. The improvement of water quality during infiltration and aquifer storage has been assessed so far in the laboratory to be able to identify the processes under more defined conditions. The results from these experiments are presented in chapter 4 and 5 and the perceptions gained from these studies are presented in chapter 7. They shall help to support MAR applications at selected sites within the SMART project region.

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2. Theoretical Background

2.1. Occurrence of emerging pollutants in the environment

The release of emerging pollutants (EP) such as pharmaceutical compounds and their metabolites into the aquatic environment has become an issue of increasing concern over recent years. The first record of a pharmaceutical in the aquatic environment was denoted in 1976 in the U.S., where the lipid lowering agent clofibrac acid was found in treated waste water in the range of 0.8–2 µg/L (Fent et al. 2006 and reference therein). Due to the continuous development of analytical techniques, enabling measurements within trace quantity ranges (ng/L–µg/L), knowledge about the environmental occurrence of pharmaceuticals has increased to a large extent. The list of pharmaceutical drugs includes for example antidepressants and antiepileptics (carbamazepine, diazepam), analgesics (e.g. diclofenac, ibuprofen), beta-blockers (e.g. atenolol, propranolol), lipid lowering agents (e.g. bezafibrate, gemfibrozil) and estrogens (e.g. 17 α -ethinylestradiol, 17 β -estradiol). Additionally xenobiotics with endocrine disrupting effects such as bisphenol A used in plasticizers or trialkyl phosphates with a carcinogenic potential used as flame retardants belong to the group of emerging pollutants. They are released to the environment during their manufacturing, usage and disposal.

Hormones and pharmaceuticals can enter waste water via feces and urine or by improper disposal of medicinal products flushed down the drain or toilet. Pharmaceuticals not readily degraded in the sewage treatment plant (STP) are being discharged in treated effluents resulting in the contamination of rivers, lakes and rarely groundwater and drinking water. Where sewage sludge is applied to agricultural fields, contamination of soil, runoff into surface water but also drainage to the groundwater may occur. Waste water reuse can lead to the accumulation of emerging pollutants. In drinking water in Germany 270 ng/L of the blood lipid regulator clofibrac acid have been detected, and recharged groundwater, contaminated by sewage was considered as the source of contamination (Heberer et al. 1998).

With urine being the major path of entry (Winker et al. 2008) more than 80 pharmaceuticals and some of their metabolites have been detected in influents and effluents of European and US waste water treatment plants (WWTPs). In minor concentrations some of these compounds also have been found in surface water and groundwater. Concentrations in the range of ng/L up to µg/L were reported in surface waters, e.g. diclofenac 1.4 – 54 ng/L, ibuprofen 0.3 – 1215 ng/L, gemfibrozil 2.3 – 1105 ng/L, carbamazepine 56.3 – 900 ng/L, or naproxen 9.1 – 790 ng/L (Hoppe-Jones et al. 2010, Rabiet et al. 2006). Up to 590 ng/L of diclofenac and 900 ng/L of carbamazepine were found for instance in groundwater samples in Germany (Sacher et al. 2001). Metabolism of pharmaceuticals first occurs in the human body, from whereon the metabolites and unaltered parent compounds will be subjected to further transformation in sewage treatment plants. Literature shows that many of these substances are persistent and are discharged into receiving surface waters (Clara et al. 2005, Joss et al. 2004, Kolpin et al. 2002). It is also known that some metabolic conjugates can be converted back to their parent forms (Ternes 1998). Due to the high persistency some of these compounds display, accumulation in the environment through a constant input is supposed. On the other

hand, pharmaceuticals with a high transformation rate might still accumulate via high consumption rates and a continuous replacement in the environment (Daughton & Ternes 1999).

Over time this chronic exposure bears the risk to cause subtle behavioral or genetic modifications in ecosystems, which are not distinguishable from natural processes of adaptation or ecological succession (Daughton & Ternes 1999). Moreover additive effects, caused by the presence of numerous micropollutants with similar actions have to be considered. Pharmaceutical drugs are used in a broad and diverse range of therapies, but knowledge of the biochemical pathways is still limited, favoring the development of unpredicted side effects despite low concentrations. Also non-target organisms might be affected by a continuous exposure of these substances, as is especially the case with aquatic organisms. Hazardous effects at ng/L concentrations in aquatic organisms like turtles, trouts and minnows, which may be sexually inhibited (Ankley et al. 2001, Khanal et al. 2006) and renal and hepatic alterations in the rainbow trout, were reported (Hotchkiss et al. 2008, Ruyter et al. 1997, Triebskorn et al. 2004). Bioaccumulation of gemfibrozil occurred in blood plasma of goldfish after exposure over 14 days at 113 times higher levels than in water (Mimeault et al. 2005) and accumulation of diclofenac in the prey of vultures resulting in a severe decrease of the population was reported by Oaks et al. (2004) and is the most prominent case of detected effects of a pharmaceutical substance on non-target organisms.

In the European Union (EU) about 3000 different substances are used in human medicine (Fent et al. 2006). The world-wide consumption of 100,000 metric tons per year corresponds to a per capita consumption of 15 g/a (Kümmerer 2004). In Germany 25.000 tons are being prescribed annually, irrespective of over-the-counter sales, which, if considered, would probably increase the amount significantly for some pharmaceuticals (UBA/ISOE, 2010). The broad spectrum of substances makes the evaluation of the impact in the water cycle difficult. Additionally the pattern of consumption varies from country to country and over the time, since new products are introduced to the market and others become less popular. In England, Germany and Austria, the amounts for the most frequently used drugs are in the hundreds of tons per year (Jones et al. 2002, Huschek et al. 2004, Khan and Ongerth, 2004). Some drugs are regularly documented within the most frequently applied range, such as the class of non-steroidal anti-inflammatory drugs (NSAID) ibuprofen and diclofenac, as well as the antiepileptic carbamazepine (Fent et al. 2006).

Environmental concern arises if environmental persistence and a harmful biological activity are exhibited by a certain pharmaceutical. As exemplified by the synthetic steroid hormone 17 α -ethinylestradiol (EE2) in contraceptive pills, the annual production lies in a couple of hundreds kilograms per year in the EU, yet it is extremely potent, quite persistent in the environment and shows estrogenic activity in fish at 1–4 ng/L or lower. Hence Fent et al. (2006) concluded that pharmaceuticals having environmental relevance share the following properties: often, but not always, high production volume combined with environmental persistence and biological activity.

2.2. Fate of emerging pollutants during waste water treatment, aquifer recharge and storage

Studies on the elimination of emerging pollutants during waste water treatment are mainly based on measurements of influent and effluent concentrations in waste water treatment plants (WWTPs). They vary to a great deal according to the construction and treatment technology, hydraulic retention time, sewage age, season and overall performance of the WWTP (Roberts & Thomas 2006). Some studies (Ternes 1998, Stumpf et al. 1999, Carballa et al. 2004) show that elimination efficiencies of the same compound can span a large range (0–99%). Emerging pollutants like pharmaceuticals form a heterogeneous group consisting of compounds with diverse chemical properties and thus variation in elimination rates between these different substances is to be expected.

Waste water treatment plants are by design highly efficient in the elimination of easily degradable organic compounds but for low concentrated complex organic substances their efficiency is usually low. Mechanisms of removal of these substances are sorption, biodegradation or abiotic degradation.

Abiotic transformation can take place via photolysis or hydrolysis with the latter process being negligible due to the resistant design of the considered emerging pollutants to this process. Photolysis has been identified as a primary pathway in the transformation of some micropollutants in surface waters (Nikolaou et al. 2007). Degradation of drugs due to direct and indirect photolysis has been also demonstrated by e.g. Buser et al. 1998, Andreozzi et al. 2003 or Doll and Frimmel 2003.

In general one important parameter controlling the fate of micropollutants in waste water treatment is their water solubility. The octanol-water partition coefficient ($\log K_{ow}$) is a measure for the hydrophobicity of a substance with a high value indicating a high affinity of a substance to organic matter and subsequently lower water solubility. Sorption to sludge is thought to be an important mechanism in the removal of the hydrophobic 17α -ethinylestradiol ($\log K_{ow}$ 4.2) from waste water. As degradation in sludge is not significant, EE2 was found in concentrations of 17 ng/g (Ternes et al. 2002). The acid dissociation constant (pKa) further influences the removal efficiency of a compound. Acidic pharmaceuticals such as ibuprofen, naproxen and diclofenac with pKa values of 4.91, 4.2 and 4.16, as well as clofibric acid (pKa 3.0), bezafibrate (pKa 3.6) and gemfibrozil (pKa 4.8) occur as ions at neutral pH with low adsorption affinity to the sludge. At neutral pH these pharmaceuticals are negatively charged and occur mainly in the dissolved phase in the waste water. Basic pharmaceuticals and dipolar ions however, can adsorb to sludge to a significant extent at neutral pH.

Tertiary treatment steps as activated carbon, advanced oxidation, ozonation and membrane filtration have been reported to efficiently improve the removal of some of these compounds. Ozonation for instance showed substantial removal for most pharmaceuticals measured in a full-scale pilot plant (Hollender et al. 2009, Reungoat et al. 2010). Application of powdered activated carbon also resulted in a good removal of a broad spectrum of micropollutants (Nowotny et al. 2007, Snyder et al. 2007). However, sufficient elimination of iodinated X-ray contrast media cannot be accomplished by this method. Overall these advanced treatment technologies are cost-intensive considering also the huge volumes that have to be treated. The

further understanding and utilization of natural degradation processes is a promising economic alternative.

2.2.1. Biological degradation

For dissolved emerging pollutants biodegradation is thought to be the most important elimination process in waste water treatment. Biological degradation is mainly ascribed to aerobic and anaerobic activated sludge treatment as well as anaerobic sludge digestion. In activated sludge treatment biological degradation of micropollutants is enhanced by increased hydraulic retention time and sludge age. Diclofenac has been shown to be substantially biodegradable only after at least 8 days of sludge retention time (Kreuzinger et al. 2004). Very persistent micropollutants like the antiepileptic carbamazepine however, show no dependency on sludge age or hydraulic retention time (Metcalf et al. 2003 a, b).

Other important factors for an efficient biotic removal are the concentration of the micropollutants in waste water, the structure of the molecules and the presence of organisms capable of degrading those compounds. Molecules with long, highly branched side chains are generally less biodegradable than unbranched compounds with shorter side chains. Unsaturated aliphatic compounds are generally more accessible to degradation than saturated analogues or aromatic compounds with compact aromatic ring structures and sulfates or halogen groups (Jones et al., 2005). The concentration of drugs in the influent of a WWTP in general is thought to be below saturation levels for the respective organisms to induce enzyme production and further on growth. Still microorganisms capable of degrading micropollutants are possibly specialists living in environments with low carbon fluxes (Egli et al. 2010). Due to the high organic load this kind of specialist group is not to be expected in waste water treatment but rather plays a role in surface water and ground water.

Microbial degradation of organic compounds is either conducted productively or co-metabolically. Productive degradation is characterized by use of the substance as a substrate, namely a carbon and/or energy source and is linked to enzyme production and biomass growth. A growth substrate after is an electron donor while oxygen serves as the electron acceptor under aerobic conditions. Anaerobic conditions provide other electron acceptors like nitrate, manganese (Mn(IV)), iron (Fe(III)) and sulfate. The electron transfer provides energy for cell growth and maintenance metabolism and a complete mineralization of the substrate liberates CO₂, H₂O, nitrate, phosphate and sulfate as inorganic end products.

Due to the lack of specificity of some enzymes co-metabolism of non-growth supporting compounds can occur as a non-directional side effect. Co-metabolic substrates do not support cell proliferation, since the parent substrate does not support microbial growth (Fuchs & Schlegel 2006). When further oxidized however, the resulting transformation products might represent a favorable carbon and energy source (Arp et al. 2001). Thereby oxidation can occur for instance by radicals which have been build up out of the monooxygenase activity, an enzyme which often initiates the oxidative degradation of a molecule (Fuchs & Schlegel 2006). Another reason for the existence of non-specific enzymes conducting co-metabolism might be the possibility for microorganisms to use these less beneficial substrates as supplementary carbon sources under oligotrophic conditions (Arp et al. 2001). Co-metabolic

processes contribute to the degradation of industrial chemicals including aromatic compounds, chlorinated organics and pesticides and the bacteria able to carry out a co-metabolic activity belong to different groups (e.g. *Nocardia*, *Pseudomonas*, *Flavobacterium*, *Rhodococcus*, *Nitrosomonas*, methanotrophs, etc). Also micro fungi and yeast present in soil and waste water may contribute to co-metabolic degradation of organic pollutants (Fritsche 1999).

2.2.2. Removal during managed aquifer recharge (MAR)

Concentrations of emerging pollutants in WWTP effluents are generally in the ng/L to µg/L range. Through dilution effects and further abiotic and biological degradation processes concentrations in rivers, lakes and seawaters are rather in the ng/L range (Buser et al. 1998, Kolpin et al. 2002, Ashton et al. 2004, Thomas and Hilton 2004). The rather persistent antiepileptic carbamazepine, and clofibric acid, a metabolite of the lipid lowering agents clofibrate, etofibrate and etofyllin clofibrate, have been detected in STP effluents, freshwater (rivers and lakes) and even in seawater (Buser et al. 1998, Weigel et al. 2002). If not transformed through biodegradation or photolysis micropollutants in surface water will enter the soil passage unchanged. Another pathway for e.g. pharmaceuticals to enter the soil layer is through adsorption onto sludge which is utilized as agricultural fertilizer. Further on waste water reuse in irrigation and artificial recharge of groundwater are important measures to meet the increasing water demand in areas of extreme water scarcity but represent relevant sources of diffuse input as well.

Managed Aquifer Recharge (MAR) has many applications with different options, soil aquifer treatment (SAT) being one of them. This specific method in MAR does not only serve the valuable supply of recharge water to the aquifers to overcome for instance water shortages in dry seasons. It also uses the natural microbial degradation processes occurring in the vadose zone during infiltration for the removal of different kinds of chemical and biological pollutants. Potentially harmful organic pollutants, particulate matter and the chemical and biological oxygen demand can be reduced by filtration and degradation processes within the unsaturated zone (Icekson-Tal et al. 2009). Furthermore, aquifers can be seen as biochemical reactors with temperature, nutrient status and redox potential influencing the biodegradation of organic chemicals (Dillon et al. 2010). Many studies identified the availability of oxygen as a premise in the elimination of emerging contaminants such as phenazone (Massmann et al. 2006). On the other hand Drewes et al. (2001) showed partial elimination of organic iodine as a sum parameter for iodinated X-ray contrast media under anoxic and anaerobic conditions but a high persistency under aerobic conditions. Furthermore Schmidt et al. (2007) identified anaerobic redox conditions to be beneficial for the removal of recalcitrant substances like clofibric acid (lipid lowering agent), carbamazepine (antiepileptic) and diatrizoic acid (X-ray contrast agent). Drewes et al. (2002) found that SAT can efficiently remove anti-inflammatories and lipid lowering agents within retention times of 6 to 18 months, while the antiepileptic carbamazepine persisted even after a travel time of over 6 years.

Consequently substances which are highly recalcitrant have to be identified and eliminated prior to aquifer recharge or if interactions in the aquifer itself can be excluded, appropriate

advanced treatment after recovery has to be applied. Treating water to drinking water standards before recharge is not recommended since high chloroform levels due to chlorination, elevated arsenic concentrations as a result of reactions between injection water and aquifer minerals, or dissolution of minerals by highly purified desalinated water might be just some of the consequences. Another aspect to be considered is the low level of nutrients contained in drinking water which would impede possible co-metabolic processes (Dillon et al. 2010). Rather adequate pre-treatment measures have to be defined to which the studies presented in this report contribute.

2.3. Fate of pathogenic microorganisms and indicator organisms during soil passage

Relevant waterborne pathogens which are transmitted mainly fecal-orally belong to bacteria (e.g. *Salmonella* spp., *Campylobacter jejuni*, *Vibrio cholerae*), viruses (e.g. Enterovirus, Adenovirus, Rotavirus, Norovirus) and protozoa (e.g. *Cryptosporidium parvum*, *Giardia lamblia*). Usually the hygienic property of ground- and drinking water is monitored via indicator organisms like *Escherichia coli*, Enterococci or *Clostridium perfringens*, with their presence pointing towards fecal contamination of the tested water.

Viruses are 0.02 – 0.2 μm in size and different from living organisms as they do not have their own metabolism and are not able to autonomously replicate themselves. They need a host cell that they infect to adapt its metabolism to the production of viruses. If no host cells are present, some viruses can still persist for a long time. They are a potential risk for the health of humans and animals. Enterobacteria like coliforms are not always suitable for water quality assessment in terms of viruses due to their higher sensibility towards different kinds of water treatment (coagulation, sedimentation, filtration, disinfection). Furthermore they show a different behavior under unfavorable environmental conditions compared to viruses or protozoa. As possible indicators for viruses bacteriophages are discussed. These are viruses that replicate at the expense of bacteria like coliform bacteria and are not harmful to humans. Bacteriophages such as the coliphage MS2 occur in all kinds of water resources that are under anthropogenic influence. They have been reported to exhibit higher persistency during soil passage (Schijven et al. 2003) as well as in ground- and waste water (Nasser et al. 1993) than human pathogenic viruses. Compared to viruses bacteriophages have the advantage, that their analysis is less elaborate and can be carried out by routine laboratories. Bacteriophages have been used as tracers in the investigation of migration in soils and karstic aquifers (Auckenthaler et al. 2002, Flynn and Sinreich 2010, Gitis et al. 2011). An advantage over chemical tracers is that they diffuse less into smaller pores, which would lead to retardation effects decreasing the migration velocity.

The most common way of infection with waterborne pathogens is via oral intake of contaminated water or food. During waste water treatment sludge is being produced that is brought onto the field as manure. From thereon pathogenic organisms contained in the sludge can reach the soil and finally the groundwater during infiltration. Groundwater used as a resource for drinking water production can still contain some of the infectious organisms if no

appropriate treatment is conducted. However a lot of the pathogenic load can be retained by the infiltrating top soil layers where pathogens are inactivated over time.

The elimination of particles and organisms during infiltration into the underground is triggered by physical and chemical processes as well as biological interactions. Generally the retardation and transport of microorganisms in the unsaturated and saturated zone is based on the same processes as it is the case with other suspended compounds or particles. Most important mechanisms are the processes of filtration and sorption through surface interactions. Their efficiency is influenced by different factors and the aquifer characteristics. Pathogenic microorganisms are mainly retained within the first 10 cm of the top soil layer (Dürkopp 2000).

Microorganisms essentially contribute to the purification of the infiltrating water, the recovery of the infiltration zone and adaptation efficiency of the soil system towards changing chemical and hydraulic conditions. With increasing depth allochthonous microorganisms that are introduced as non-residential organisms to the soil passage via surface and seepage water, are confronted with environmental conditions, which they are not adapted to. Together with antagonistic effects of the autochthonous (native) soil and groundwater population the unfavorable conditions will lead to the inactivation and elimination of the introduced organisms in the aquifer over time. Especially these biological processes make river bank filtration and artificial groundwater recharge effective processes for a sustainable water management.

Antagonistic effects are the production of antibiotic compounds und lytic exoenzymes through bacteria, funghi and algae, as well as the grazing of protists. Further factors of influence are ionic strength, pH of the soil water or the infiltrating media, the amount of organic compounds as well as nutrient status and temperature. Viruses and bacteria are known to persist longer at lower temperatures. A rise in temperature can cause changes in the viral surface structure (capsid) and the genome. Additionally dehydration of the pathogenic organisms will lead to inactivation. Soil moisture on the other hand favors the soaking of organic and inorganic soil compounds, by which the surface for microbial adsorption is enlarged.

Flow velocity, defined by porosity of the soil and aquifer, influences the retardation of organisms in the infiltrating water as well. High flow velocities allow for a continuous transportation, overcoming the top soil layers with its microbial activity. Filtration efficiency of soils is related to the variance of the soil structure, like the amount of sand and clay and subsequently the grain size distribution as well as the organic content. In addition singular events like storm waters or floods can influence the hydraulic conditions in the underground. Macropores ($> 3 \text{ mm}$) can support the migration of microorganisms in combination with storm water events. Generally microorganisms are transported further and faster at higher flow velocities because the contact time and distance to the soil surfaces alleviate adsorption. Of special importance is the case of karstic aquifers. Here, water contaminated with viruses moves very fast ($>100\text{m/h}$) through the fractures and fissures and thus retardation and inactivation through adsorption and the soil biocoenosis, cannot act as detention factors (Dürkop 2000).

In summary, most waterborne pathogens belong to bacteria or viruses with the latter one being less sensitive towards different kinds of water treatment. As surrogates for viruses, bacteriophages are being currently under investigation. Their advantage is the less elaborate analysis compared to human pathogenic viruses. Through filtration and sorption pathogens can be prevented from entering the groundwater level through e.g. usage of manure in agriculture. Abiotic factors such as temperature, soil moisture or the amount of organic matter influence the inactivation and retardation of microorganisms. Next to these parameters the autochthonous microorganisms are thought to play an important role in the elimination of pathogens either by direct grazing on viruses or indirectly by the formation of antibiotic compounds and exoenzymes with antiviral effect.

3. Material and Methods

3.1. Chemical analysis

Standard parameters pH and oxygen were measured with a Multi 340i device using a Sentix 41 and a Cellox 325 (WTW, Weilheim, Germany), respectively. The redox potential in the batch assays was measured with a Sentix ORP redox electrode (WTW).

Dissolved organic carbon (DOC) was analyzed using a Dohrmann Carbon Analyzer with UV radiation and potassium peroxydisulfate oxidation and measurement through infrared-spectroscopy. Removal of particulate matter was conducted via filtration through a 0.45 µm-polyether-sulfonate filter (PALL Corporation, Dreieich, Germany). Carboxylic acid was removed through addition of one drop of ortho-phosphoric acid (85%) and subsequent stripping of the evolving carbon dioxide with nitrogen.

Analysis of anions was carried out via ion chromatography (761 compact IC, Metrohm, Filderstadt, Germany), using a metrosep A sup 5 column (L 150 mm, D4 mm) for separation. The eluent consisted of a buffer solution of 3.2 mM Na₂CO₃, 1.0 mM NaHCO₃ and acetone (2%). A solution of H₂SO₄ (0.1%) served as regenerant. Prior to analysis samples were filtered through a 0.2 µm-cellulose-acetate filter (Schleicher and Schuell, Dassel, Germany). Limits of detection were 0.5 mg/L, 1 mg/L and 2mg/L for nitrite, nitrate and sulfate, respectively.

For detection of ammonium, COD, Fe(II), total iron, Mn(IV) and Mn(II) NANOCOLOR[®] tube and standard tests were used (Macherey & Nagel, Düren, Germany). For measurement of ammonium in column samples and Mn(II) in the batch samples filtering through a 0.2 µm-cellulose-acetate filter (Schleicher and Shuell) was necessary prior to analysis. To avoid oxidation of the reduced iron and manganese forms under aerobic conditions 200 µL of half diluted hydrochloride acid were added to each 10 mL sample. For the NANOCOLOR[®] tests a 3 % error is given by the company and together with an estimated error of 2 % due to handling and matrix effects of the samples a total error of 5 % is assumed for these measurements.

Analysis of pharmaceutical residues was done by HPLC ESI MS-MS following a previously described procedure (Sacher et al., 2008). Also the analysis of X-ray contrast agents has been described previously (Sacher et al., 2005). Analysis of the trialkyl phosphates was done by a GC-MS method using a GC-MSD Agilent 5973 (Agilent Technologies, Santa Clara, California) after solid-phase extraction on plastic cartridges filled with 200 mg of Bakerbond SDB 1 material (Mallinckrodt Baker, Deventer, The Netherlands). For GC separation, a DB-5 column (60m) (Agilent Technologies, Santa Clara, California) was used. Injection temperature was 250°C. The injection volume was 2 µL and total flow was 7.4 mL/min with helium as carrier gas. The temperature program started at 60°C (held for 2 min), set at 20°C/min to 130°C (held for 5 min), set at 1°C/min to 205°C, set at 5°C/min to 300°C (held for 10 min). Detection with the MSD device was done in EI mode (SIM). Calibration was done between 20 and 500 ng/l. Detection of endocrine disruptors (steroid hormones,

bisphenol A) was carried out by a GC-MS method using an Agilent 7890 A with a MSD 5975 C (Agilent Technologies, Santa Clara, California) after solid-phase extraction on plastic cartridges filled with 100 mg of STRATA X material (Phenomenex, Aschaffenburg, Germany). For GC separation, a Rtx®-5Sil MS (Restek Corporation, Bellefonte, Pennsylvania) was used. Injection temperature was 280°C. The injection volume was 1 µL and total flow was 1.2 mL/min with helium as carrier gas. The temperature program started at 120°C (held for 1 min), set at 15°C/min to 180°C, set at 5°C/min to 290°C (held for 10 min). Detection with the MSD device was done in EI mode (SIM). Calibration was done between 1 ng/L and 500 ng/L. Regardless of the analytical methods applied, all water samples were put onto SPE cartridges after the main particles had settled, considering only dissolved compounds for the analysis. Analytical errors of measurement were between 25% to 36% for pharmaceutical residues and 50% for endocrine disruptors.

Limits of detections for the column studies were in the range between 0.001 µg/L and 0.5 µg/L for hormones and 0.05 µg/L – 13 µg/L for 4-*tert*-octylphenol, 4-*iso*-nonylphenol and bisphenol A. The pharmaceutical substances had detection limits between 0.05 µg/L and 0.4 µg/L. X-ray contrast media detection limits were in the range of 0.01 µg/L - 1 µg/L and trialkyl phosphates had detection limits between 0.025 µg/L and 1.5 µg/L. For the analysis of the columns fed with filtrate of the MBR sample volumes were limited due to the performance of the experiment. The limits of detection increased to 100 ng/L, 10 ng/L and 50 ng/L, respectively for the pharmaceutical compounds, the endocrine disruptors and 4-*tert*-octylphenol and bisphenol A in the column influents. 4-*iso*-Nonylphenol had a detection limit of 250 ng/L and 130 ng/L in the influent and effluent of the columns, respectively, while pharmaceuticals, endocrine disruptors, 4-*tert*-octylphenol and bisphenol A had detection limits of 50 ng/L, 5 ng/L and 25 ng/L in the column effluents, respectively.

During the batch experiments at 10 µg/L detection limits for the different substances was as follows: 0.2 µg/L for pharmaceuticals, 0.08 µg/L for X-ray contrast agents and 0.02-0.5 µg/L for endocrine disruptors, including estrogens and Bisphenol A. After spiking the assays with 100 µg/L detection limits for X-ray contrast agents and pharmaceutical substances were 1 µg/L and 5 µg/L, respectively. The detection limit for hormones was in the range of 0.05 µg/L – 0.5 µg/L and for Bisphenol A 0.25 µg/L – 1 µg/L.

3.2. Detection of Microorganisms

Analysis for MS2 bacteriophages and viruses was done via the Polymerase Chain Reaction method (PCR) after enrichment by the cation coated filter method (Zawadsky et al. 2009). Viral RNA und DNA were extracted simultaneously using the ViralXpressTM nucleic acid extraction kit (Millipore), and cDNA was generated via the DyNAmo cDNA synthesis kit (Finnzymes): 4 µL of RNA were mixed with 1 µL of Random Hexamers, 2 µL M-MuLV RNase H⁺ Reverse Transcriptase, 10 µL RT-Buffer and 3 µL DEPC-treated water. Primer extension was done at 25°C for 10 min and for cDNA synthesis the mixture was incubated at 37°C for 30 min. The reaction was terminated at 85°C for 5 min. The resulting cDNA sample was applied to qualitative and real time PCR. Standard protocols were used for

the PCR mixtures. For qualitative PCR 3 μL of cDNA were mixed with 17 μL of a reaction mix containing 2 μL 10x PCR buffer (Molzym), 5 U/ μL Taq Polymerase (Molzym), 10 mM of each dNTP, 10 pmol/ μL forward primer and 10 pmol/ μL reverse primer. For real time PCR 2 μL cDNA were mixed with 23 μL of a reaction mix containing 12,5 μL 2xSensi Mix R-OX (Bioline), 10 pmol/ μL forward primer and 10 pmol/ μL reverse primer. PCRs were conducted at optimum temperatures. For real time PCR absolute quantification was performed giving the exact number of target DNA molecules by comparison with DNA standards. Qualitative PCR products were loaded on a 0.8% agarose gel stained with ethidium bromide and bands were detected via UV detection.

In case of the laboratory column experiment quantification of MS2 was done via the plaque-forming method. Thereby the MS2 concentration was assessed by the double layer agar method (EN ISO 10705-1:1995) with the bacterial host *Salmonella typhimurium* strain WG49. Host cells were prepared by inoculating 50 mL of broth (trypticase peptone, 10g; yeast extract, 1g; glucose, 1g; NaCl, 8g; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.3g; ultra pure water, 1L) with 0.5mL stock host culture. Broth cultures were grown for 3-4 h at 37°C to a concentration of 107-108 CFU/mL while shaking at 100 min^{-1} . A 1mL sample of the water to be assayed was mixed with 1mL of molten (45°C) top-Agar (trypticase peptone, 10g; yeast extract, 1g; glucose, 1g; NaCl, 8g; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.3g; agar, 7g; ultra pure water, 1L) and 1 mL of host culture in a test tube. The mixture was vortexed, poured onto a solid bottom-agar plate (trypticase peptone, 10g; yeast extract, 1g; glucose, 1g; NaCl, 8g; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.3g; agar, 15g; ultra pure water, 1L) and distributed evenly. The agar plate was incubated for 18±2h at 37°C. Duplicated plating was performed for each water sample, with countable numbers of plaques ranging from 30 to 300 per plate, and being reported as plaque forming units (pfu).

For the detection of coliforms and *E.coli*, Enterococci and *Pseudomonas aeruginosa* Colilert 18[®], Enterolert-DW[®] and Pseudalert[®] tests were used (IDEXX Laboratories, Portland, ME, USA). The tests are based on incubation of the eventually contained bacteria in 100 mL samples during 18 hours (Colilert-18[®]) and 24 hours (Enterolert-DW[®], Pseudalert[®]). Each test contains specific nutrients and adequate dyes for a colour change from which the concentration of bacteria in the sample can be deduced. In case of the Pseudalert[®] test the analysis of *Pseudomonas aeruginosa* is based on the detection of enzymatic activity.

4. Assessment of water quality during soil infiltration, aquifer storage and recharge (D 315)

Within this project, sampling campaigns were conducted to gain information on the occurrence and distribution of emerging pollutants in water samples of the Jordan Valley. In 2007 and 2008 waste water, surface water and groundwater samples have been taken along the Jordan Valley. As a following step biodegradation of a range of EPs, including 36 compounds was studied on laboratory scale in flow-through soil columns under unsaturated, aerobic conditions. The experiments were conducted in order to assess the risk of treated waste water to groundwater if used in artificial recharge for the replenishment of natural aquifers.

Therefore the fate of emerging pollutants and bacteria as well as viruses was first investigated in laboratory soil columns, simulating the soil passage. In a second stage analysis were performed within a pilot MBR treatment plant constructed by HUBER SE Company containing two soil columns supplied with MBR filtrate for demonstration of a subsequent soil passage treatment after membrane filtration. Results for the performance of the MBR with regard to emerging pollutants and microbiological parameters are given in the report for deliverable 316 (Lipp et al. 2012).

4.1. Soil column studies simulating soil infiltration

Laboratory column studies

For simulation of biodegradation processes during soil passage, column experiments under unsaturated conditions were conducted with treated waste water as feed over almost three years. Four columns of stainless steel, filled with natural vadose zone sediment, were irrigated with treated waste water from the municipal WWTP Neureut/Karlsruhe (Fig. 1). The sediment had a low organic content of 0.04 mg C/g with a grain size of 0.2 - 2 mm. Every two weeks reservoir bottles containing effluent of the WWTP were renewed and stored in a refrigerator at 2°C. Hydraulic retention time in the columns was 6 to 7 days. By comparing leachate concentrations of the 2 biologically inhibited (cooled down to 2°C) and the 2 bioactive columns (20°C), biodegradation of the trace organics during soil passage was assessed. Temperature and water content in the columns were controlled by temperature sensors and tensiometers. 5 µg/L of each substance were spiked into the reservoir bottles for one of the cooled and one of the 20°C columns, respectively. Thus the influence of concentration on the elimination behavior of the emerging pollutants was assessed, by comparing the performance of the columns with spiked waste water with the performance of the columns fed with original waste water.

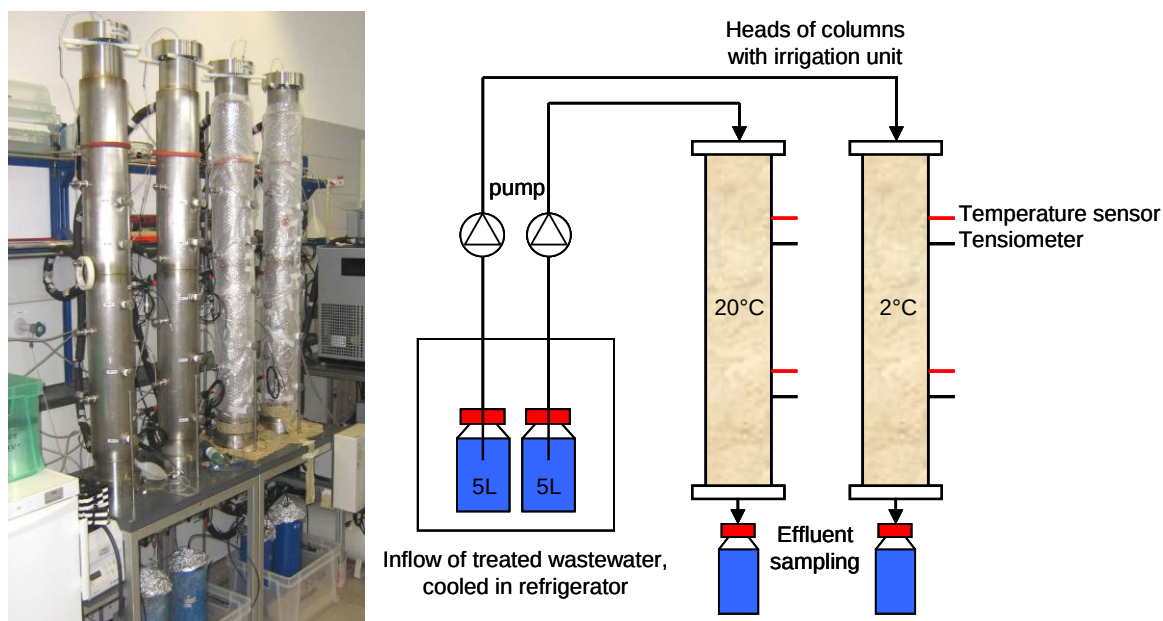


Fig. 1 Laboratory set-up and scheme of the column experiments

Furthermore the ammonium content in the treated waste water was varied. This was done in the light of recent literature suggesting an influence of the process of nitrification onto the elimination of some of the tested compounds as for instance diclofenac and 17 α -ethinylestradiol (Suarez et al. 2010, De Gusseme et al. 2009). In a first set of investigations the ammonium content remained unchanged with a natural median concentration of 2 mg/L contained in the waste water. In a second sampling campaign the ammonium content was increased to 10 mg/L by adding suitable amounts of a sterilized highly concentrated spiking solution (500 g/L ammonium in MilliQ water) to the treated waste water. A final set of samplings was done under exclusion of ammonium. This was achieved by aerating and stirring the reservoir bottles with the WWTP effluent for at least 24 hours, a time sufficient for the full oxidation of ammonium in the effluent to nitrite/nitrate. No ammonium was detected after one day of aeration.

Column influent and effluent were further investigated for MS2 bacteriophages, as surrogate for the study of viruses, at 2°C and 20°C. Therefore two columns were spiked with a stock solution of 10⁹ MS2 bacteriophages at the head of the columns. This single spiking event was repeated twice within 12 weeks and the phage concentration was measured once a week. The next spiking only took place, when no MS2 bacteriophages could be detected in the effluent anymore.

Column studies using MBR filtrate

Filtrate from a pilot scale membrane bioreactor by HUBER SE was used to feed two stainless steel columns filled with fresh quartz sand material. This was done in order to demonstrate the combination of MBR treatment and subsequent treatment during soil passage. As in the laboratory columns influent and effluent concentrations were compared in terms of emerging pollutants elimination, bacteriophages and additionally pathogenic

viruses' removal. The columns were run under saturated conditions with the filtrate flowing from the bottom to the top. Residence time in the columns was 12 days.

4.1.1. Behavior of emerging pollutants during soil passage

Emerging pollutants of the classes of endocrine disruptors (estrogens, alkylphenols and bisphenol A), beta blockers, analgesics, X-ray contrast media, lipid lowering agents, trialkyl phosphates and one antiepileptic were analyzed for their elimination behavior during soil passage. Thereby influencing parameters such as temperature, compound concentration and adaptation of biomass over time were considered. Additionally the influence of the auxiliary substrate ammonium, supplying energy to a certain group of microorganisms (e.g. nitrosomonas, nitrobacter) through its oxidation to nitrite and nitrate, was investigated. This process of nitrification is important in conventional waste water treatment. The enzyme involved in this processes however, the ammonium monooxygenase, is known to have a broad substrate range, oxidizing some organic compounds co-metabolically as well (Arp et al. 2002).

Tab. 1 gives a summary of the results from the laboratory column experiments, which were run for 35 months consisting of a run-in phase of five weeks which was followed by subsequent sampling after 7, 13, 29, 31, 34 and 35 months.

Tab. 1 Summary of the different emerging pollutants divided into four different groups with regard to their elimination in the 20°C soil column fed with spiked WWTP effluent and different amounts of ammonium: elimination <10% = -, 10% – 50% = +, 50% – 90% = ++, >90% = +++. Results of measurements were considered from month 7 on, avoiding misinterpretations due to lower elimination rates within the run-in phase.

		20°C					20°C		
		Ammonium [mg/L]					Ammonium [mg/L]		
		2	10	0			2	10	0
Estrogens, Alkylphenols and Bisphenol A	17-α-Ethinylestradiol	+++	+++	+++	X-ray contrast media	Diatrizoic acid	-	-	-
	17β-Estradiol	+++	+++	+++		Iohexol	+++	+++	+++
	Estriol	+++	+++	+++		Iomeprol	+++	+++	+++
	Estrone	+++	+++	+++		Iopamidol	+	+	+
	4-iso-Nonylphenol	+++	n.d.	+++		Iopromide	+++	+++	+++
	4-tert-Octylphenol	+++	+++	+++		Iothalamic acid	-	-	-
	Bisphenol A	+++	+++	+++			n=2	n=2	n=2
		n=2	n=1	n=1					
Beta blockers	Atenolol	+++	+++	+++	Lipid-lowering agents	Bezafibrate	+++	+++	+++
	Metoprolol	+++	+++	+++		Clofibric acid	-	-	-
	Propranolol	+++	+++	+++		Etofibrate	+++	+++	+++
	Sotalol	+++	+++	+++		Fenofibric acid	+++	+++	+++
						Fenofibrate	+++	+++	+++
						Gemfibrozil	+++	+++	+++
Antiepileptics	Carbamazepine	+	+	-	Trialkyl phosphates		n=2	n=2	n=2
		n=2	n=2	n=2					
Analgesics	Diclofenac	+++	+++	+++		Triethyl phosphate	+++	+++	+++
	Ibuprofen	+++	+++	+++		Tri-n-butyl phosphate	+++	+++	+++
	Naproxen	+++	+++	+++		Trikresyl phosphate (o-, m-, p-isomers)	+++	+++	+++
	Pentoxifylline	+++	+++	+++		Triphenyl phosphate	+++	+++	+++
	Phenazone	++	+++	+++		Tris(2-ethylhexyl) phosphate	+++	+++	+++
						Tris(2-chloroethyl) phosphate	+	+	++
						Tris(2-chloropropyl) phosphate	-	+	++
					n=2	n=1	n=2		

n.d. = not detected

The phase between 29 and 31 months was run with additional supply of ammonium (10mg/L) and the last two months of sampling were conducted without ammonium contained in the influent. The results presented in table 1 are taken from the 20°C column spiked with 5 μ g/L of each substance, not considering the results during the first 5 weeks.

Summarized eliminations for the different emerging pollutants tested are expressed with ‘-’, ‘+’, ‘++’ and ‘+++’, respectively. Where the elimination ranges differed between the two samplings considered for each ammonium concentration tested, the smaller elimination rate was considered. However, the elimination rates of the compounds in respect never differed by more than one category from each other. The estrogens already showed complete elimination during the run in phase and therefore only single measurements were conducted during the second and third sampling phase (10 mg/L and 0 mg/L ammonium). The summary shows that most substances are eliminated, independent of the process of nitrification. Only for five of the tested compounds marginal to no elimination during samplings within 35 months was found: the antiepileptic carbamazepine, the two ionic X-ray contrast media diatrizoic acid and iothalamic acid, the non-ionic X-ray contrast agent iopamidol and the lipid lowering agent clofibric acid.

According to their elimination behavior the compounds can be put into three groups by ranking them in group I with elimination being less than 50%, group II with elimination rates above 90% but with a clear dependence on certain conditions (e.g. temperature, adaptation time) and group III with more than 90% elimination rate irrespective of the conditions applied. Group I consists of the substances mentioned above. Clofibric acid is known to be very persistent but the usage of its parent compounds ethyl clofibrate, etofibrate und etofyllinclofibrate has declined over recent years. However, its prevailing occurrence in sea water, lakes and recharged ground water, used for drinking water production (Valcárcel et al. 2011, Weigel 2002, Heberer et al. 1997), makes it a most relevant substance and a possible tracer for anthropogenic pollution (Radke et al. 2010). Clofibric acid showed no removal during the whole time of the experiment. Other compounds were easily eliminated without evidence of a correlation to any of the influencing parameters considered. Among these substances are the estrogens, alkylphenols and bisphenol A as well as the analgesics ibuprofen and pentoxifylline, the beta blockers atenolol and propranolol, the trialkyl phosphates tri-n-butyl phosphate, trikresyl phosphate, triphenyl phosphate and tris(2-ethylhexyl) phosphate and the lipid lowering agents etofibrate and fenofibrate.

In between these two groups of substances, namely the easily removable (group III) and the persistent ones (group I), the rest of the examined compounds can be summarized as removable under certain conditions (group II). Temperature has an influence on the elimination, since some substances exhibited less elimination at 2°C. The concentration of the substance and adaptation of the biomass over time are further parameters influencing the elimination rate. In the original WWTP effluent some substances were detected only in the run-in phase or rarely to not at all over the whole experiment. Their elimination behavior could therefore only be analyzed after spiking. For these substances the influence of concentration on their elimination could not be investigated and they are discussed separately.

Spiking of the column influent resulted in an average concentration rise of one to two orders of magnitude for pharmaceutical substances, estrone and bisphenol A (Fig. 2). However some of the analyzed compounds exhibited higher concentrations already before spiking and thus the concentration after spiking was less than one order of magnitude

higher. Iomeprol was detected at 44 µg/L on average in the WWTP effluent, so that subsequent addition of 5 µg/L was almost negligible. Still for most of the substances a clear difference in concentration before and after spiking was measurable.

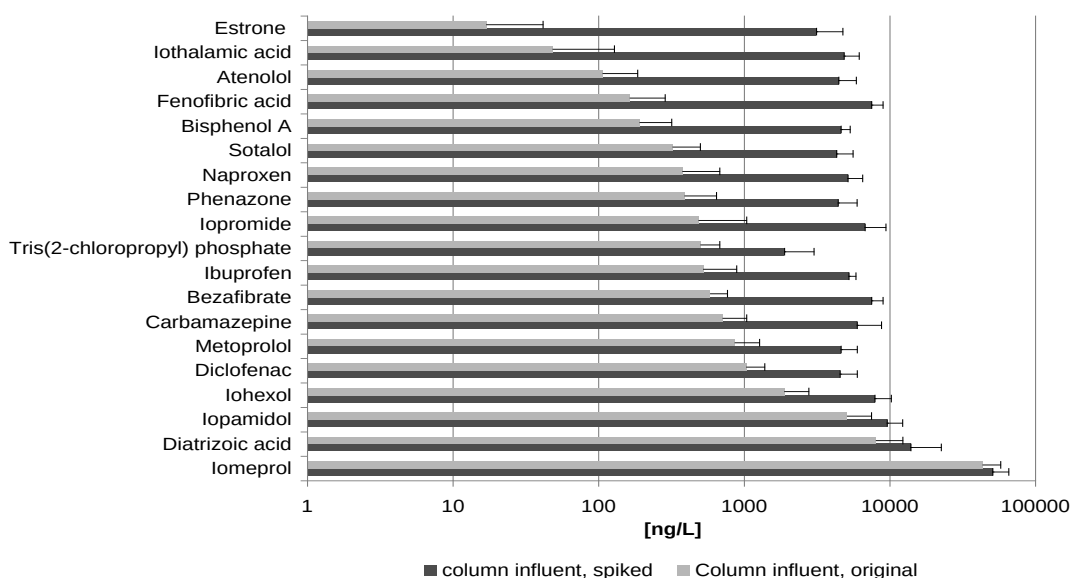


Fig. 2 Averaged concentrations of the different substances analyzed in the original and spiked column influent with error bars indicating standard deviations ($n=7$, for estrone and bisphenol A $n=5$)

The chlorinated trialkyl phosphates belong to category II but showed considerable removal only towards the last two samplings which is why they shall be discussed separately from the other compounds. In the original WWTP effluent only tris(2-chloroethyl) phosphate was detected and degradation was low at 20°C (24%, data not shown). At 20°C and spiked concentrations elimination of 17% and 66% for tris(2-chloroethyl) phosphate and tris(2-chloropropyl) phosphate in the beginning was followed by effluent concentrations exceeding influent concentrations, indicating a washout from the sediment contained in the column (Fig. 3, data below zero not shown).

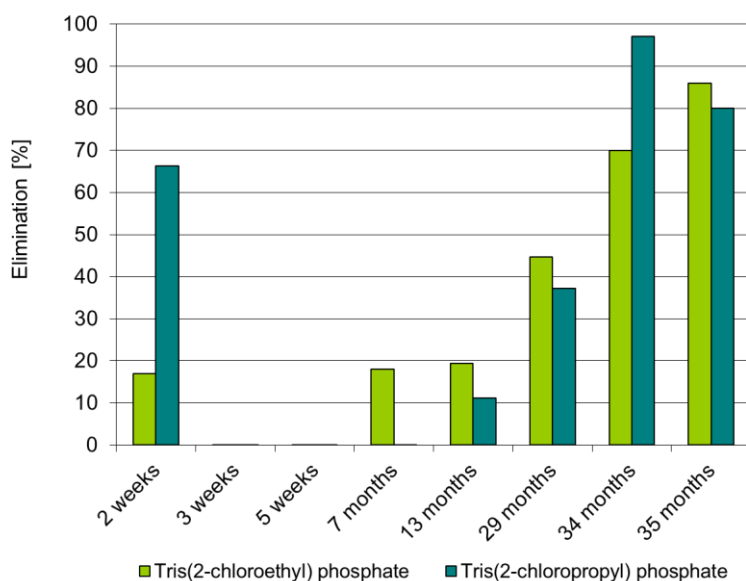


Fig. 3 Behavior of Tris(2-chloroethyl) phosphate and Tris(2-chloropropyl) phosphate in the 20°C column fed with spiked WWTP effluent during 35 months

This behavior is to be attributed to retardation of the compounds due to naturally occurring adsorption and desorption effects with the sediment matrix. Being described as rather recalcitrant in literature (Meyer and Bester 2004, Marklund et al. 2005) this behavior was not surprising.

However, within the last two months of sampling elimination reached up to 78% and 89% on average for tris(2-chloroethyl) phosphate and tris(2-chloropropyl) phosphate, respectively, as Fig. 3 shows. If the degradation of the chlorinated trialkyl phosphate was productive and bacteria could grow on it, at lower concentrations probably no adaptation could take place. This would explain the minimal removal in the column supplied with original WWTP effluent (data not shown). The results indicate that a constant application of these pollutants over a relatively long period of time can finally result in considerable removal rates through adaptation of biomass.

In the following the other compounds will be discussed by their behavior at two different temperatures and other influencing factors. For the averaged data presented, measurements from month 7 until month 31 are considered, excluding the run-in phase and the data obtained during the last phase without ammonium contained in the influent where only the spiked column at 20°C was investigated. Negative values for elimination depict a higher concentration measured in the effluent compared to the influent. Reasons for this are sorption/desorption effects and the specific retention times of the different compounds.

Substances detected at both concentrations

Elimination of the tested substances was followed at two different concentrations to find out if higher loads of the tested substances might reveal a different elimination behavior within the columns.

Tab. 2 Elimination of emerging pollutants at 2°C and 20°C and two different concentrations using original and spiked WWTP effluent: elimination <10% = -, 10% – 50% = +, 50% – 90% = ++, >90% = +++

		original		spiked				original		spiked	
		2°C	20°C	2°C	20°C			2°C	20°C	2°C	20°C
Estrogen	Estrone	+++	+++	+++	+++	Analgesics	Diclofenac	+	+++	+	+++
		n=2	n=2	n=3	n=3		Naproxen	+++	+++	+++	+++
Lipid-lowering agents	Fenofibric acid Bezafibrate	+++	+++	+	+++		Ibuprofen	+++	+++	+++	+++
		+++	+++	++	+++		Phenazone	+++	+++	++	++
		n=4	n=4	n=4	n=4	Beta blockers	Atenolol	+++	+++	+++	+++
X-ray contrast media	Iohexol Iomeprol Iopromide*	+++	+++	+++	+++		Metoprolol	++	+++	++	+++
		+++	+++	+++	+++		Sotalol	+++	+++	++	+++
		n=4	n=4	n=4	n=4			n=4	n=4	n=4	n=4

* original: n=2

As mentioned before the estrogen estrone, the analgesic ibuprofen and the beta blocker atenolol were eliminated within 90 to 100% right from the beginning of the experiment regardless of the temperature or concentration (Tab. 2). This is shown exemplarily for ibuprofen in Fig. 4 including also the run-in phase. From the averaged results presented in Tab. 2 a good elimination can be derived further for the X-ray contrast agents iohexol,

iomeprol and iopromide as well as for the analgesic naproxen with an average removal of more than 90% at both concentrations and temperatures.

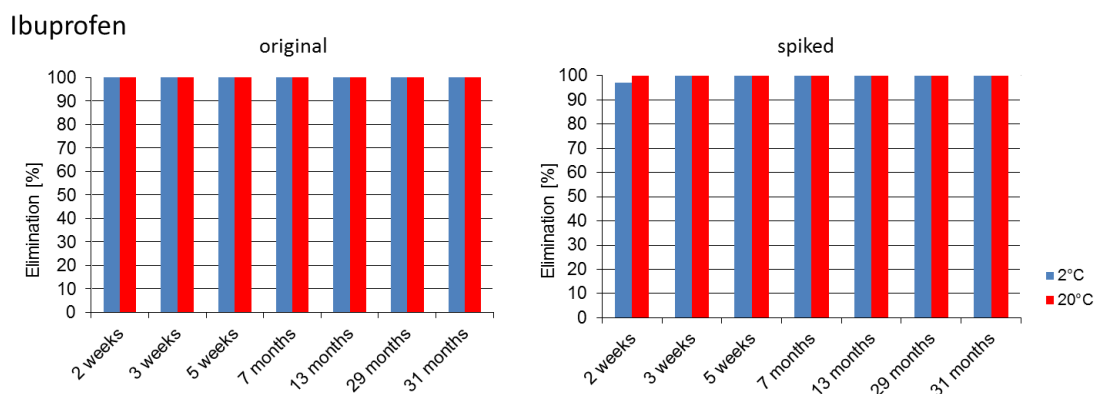


Fig. 4 Elimination of ibuprofen during the column experiment with original and spiked WWTP effluent

Elimination rates at 2°C improved for these substances either at both concentrations or only within the spiked tests as Fig. 5 and Fig. 6 show for naproxen and iohexol. The behavior of iomeprol and iopromide was similar to that of iohexol. This suggests adaptation of the biomass contained in the columns operated at 2°C.

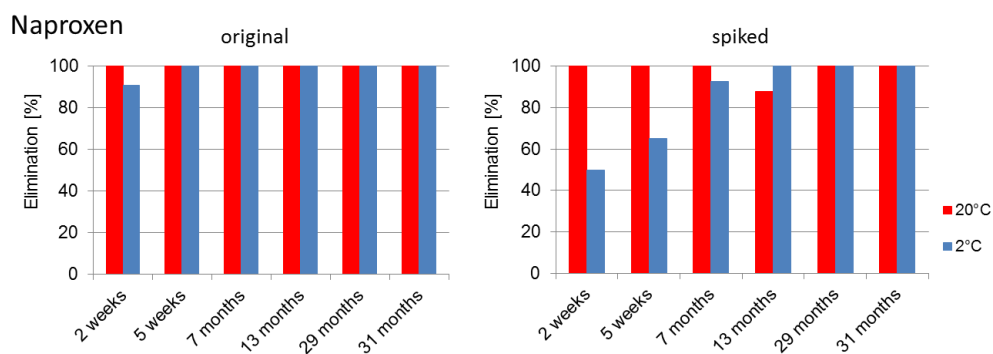


Fig. 5 Elimination of naproxen during the column experiment with original and spiked WWTP effluent

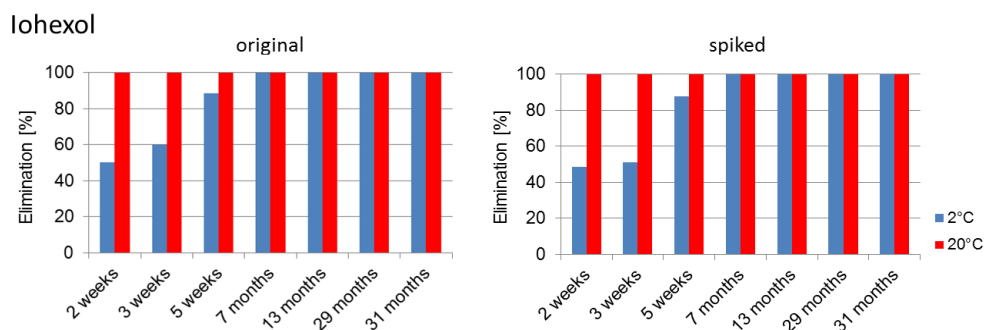


Fig. 6 Elimination of iohexol during the column experiment with original and spiked WWTP effluent

A primary decrease of the activity and most probably die off of some part of the microbial community due to the unfavorable temperature condition was obviously followed by a slow but steady microorganism's growth in these columns. The results for these

compounds show that even at adverse conditions degradation can occur if the biomass has enough time to adapt to less favorable conditions.

The beta blocker metoprolol and the analgesic diclofenac showed a distinct dependence of elimination on temperature. This is exemplarily shown for diclofenac where the difference in elimination at 2°C and 20°C was most pronounced (Fig. 7). On average diclofenac was removed by 95% and 96% in the 20°C columns run with original and spiked WWTP effluent, respectively. In contrast the elimination rate in the two columns at 2°C varied between 82% and -77% in the original column influent and 70% and -100% in the spiked influent. They did not show a development towards a more complete elimination as with naproxen or iohexol.

Diclofenac

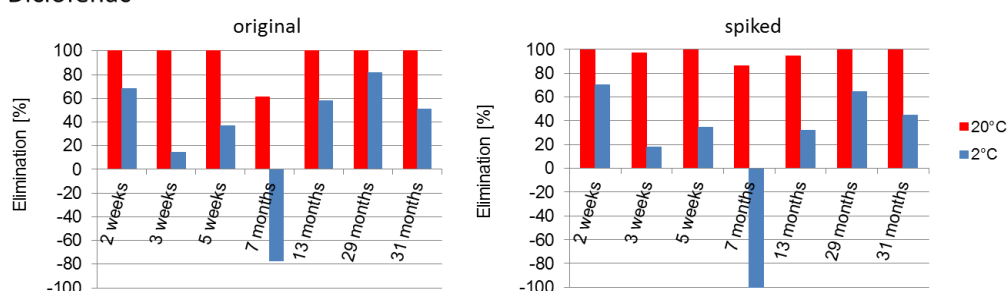


Fig. 7 Elimination of diclofenac during the column experiment with original and spiked WWTP effluent

The strong variations in elimination with the effluent of the columns exceeding influent concentrations after month 7 in both 2°C columns suggests desorption of diclofenac. The relatively stable and high elimination in the two 20°C columns points to biotic degradation processes occurring under these more favorable conditions for microbial activity.

For sotalol (beta blocker), fenofibric acid and bezafibrate (lipid lowering agents) elimination was mostly dependent on temperature at higher concentrations, which is exemplarily shown for bezafibrate in Fig. 8. While bezafibrate shows no elimination at 2°C within the first 5 weeks in the column fed with spiked influent, elimination was already at 66% after the first measurement in the column with original influent. Furthermore the elimination is complete from month 7 on, while it varies between 51% and 91% in the column operated with spiked influent. However in both 2°C columns again elimination rate rises over time.

Bezafibrate

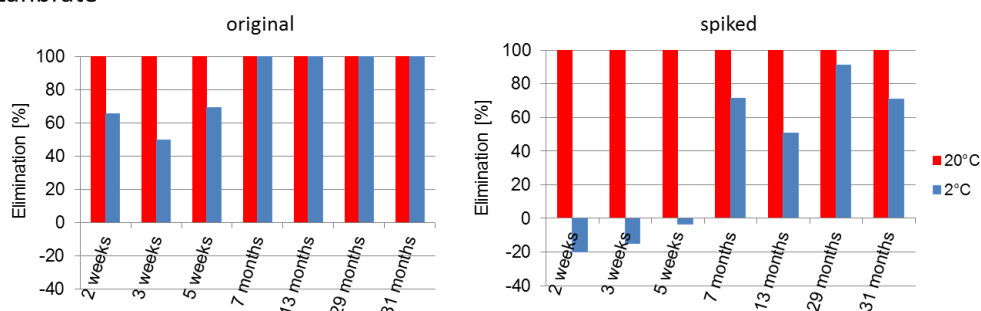


Fig. 8 Elimination of bezafibrate during the column experiment with original and spiked WWTP effluent

Elimination of phenazone in the columns fed with original influent is almost stable throughout the course of the experiment with the exception of the first measurement (Fig. 9). Concentration dependence was detected at 2°C and 20°C within the spiked column influent. Removal after soil passage is rather similar with minimal/maximal rates between -84% till 95% and -74% till 100% at 2°C and 20°C, respectively. However a general trend towards a more complete elimination can be derived from these results.

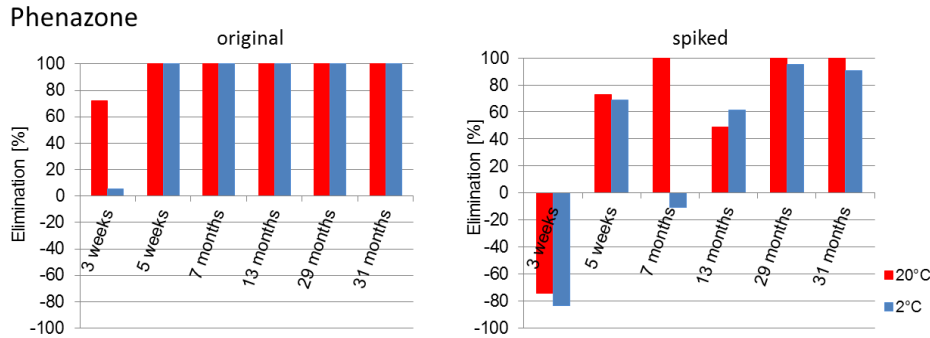


Fig. 9 Elimination of phenazone during the column experiment with original and spiked WWTP effluent

The bar charts show that with higher concentration loads adaptation times become relevant especially at lower temperatures or as in the case of phenazone at both low and higher temperatures. This is an important fact to be considered when thinking on managed aquifer recharge. It means that a relatively constant input of degradable compounds to an aquifer by means of soil passage can be handled with as long as no major and long term upwards concentration shifts occur. Consequently, in such an incidence, the soil biology would need considerable time to adjust to such a change. Furthermore these results suggest that a continuous operation is beneficial for long term pollutants elimination.

The DOC contained in the original influent before spiking was 10.7 mg/L on average and was used as substrate by the microorganisms leading to a decrease to less than 50 % (Fig. 10).

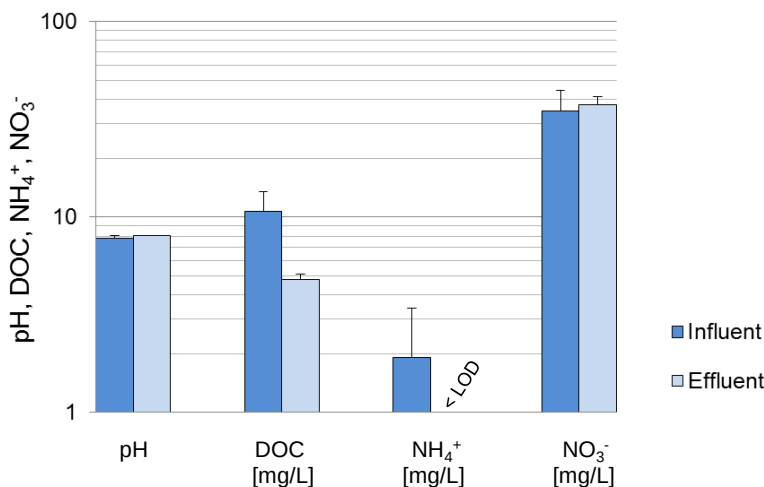


Fig. 10 pH, DOC, NH_4^+ and NO_3^- in the influent and effluent of the four columns during the first 13 months of the experiment. Influent and effluent values represent averaged results with error bars indicating standard deviations.

In column tests with aquifer material Rauch-Williams et al. (2010) demonstrated that naturally available organic matter can promote the degradation of e.g. naproxen and gemfibrozil by serving as a primary carbon substrate that stimulates biomass growth and enhances a co-metabolic degradation of emerging pollutants as secondary substrate. Similar DOC degradation in all columns was found, indicating good degradability of natural organic matter contained in the WWTP effluent irrespective of the temperature. At the same time biodegradation processes for the spiked organic matter might have been enhanced by higher temperature and the coherent development of a more differentiated microbial community, capable to degrade most of the tested compounds either productively or co-metabolically. This would explain why for bezafibrate and naproxen the difference in biodegradation between the cooled and the 20°C columns was more pronounced in the spiked experiments. The pH in the columns was slightly basic and a bit higher than in the influent as a result of the natural buffer capacity of the sediment. Ammonium was completely removed in each of the columns. Online measurement of 5.1 mg/L average oxygen content in one of the column effluents showed that aerobic conditions prevailed during the experiment.

Substances not detected in the original WWTP effluent

Non-detections in WWTP effluents give a hint to either a high removal potential for a substance or point towards changing prescription practices as in the case of clofibrilic acid. Tab. 3 gives an overview of the substances detected exclusively or predominantly in the spiked WWTP effluent used as feed for two columns operated at 2°C and 20°C.

Tab. 3 Elimination of emerging pollutants at 2°C and 20°C detected after spiking only: elimination <10% = -, 10% – 50% = +, 50% – 90% = ++, >90% = +++

		spiked				spiked	
		2°C	20°C			2°C	20°C
Estrogens, Alkylphenols and Bisphenol A	17β-Estradiol	+++	+++	Analgesic	Pentoxifylline	+++	+++
	Estriol	+++	+++			n=4	n=4
	17α-Ethinylestradiol	+++	+++	Beta blocker	Propranolol	+++	+++
	4-iso-Nonylphenol*	+++	+++			n=4	n=4
	4-tert-Octylphenol	+++	+++				
	Bisphenol A	+++	+++				
Lipid-lowering agents		n=3	n=3	Trialkyl phosphates	Triethyl phosphate	+	+++
	Etofibrate	+++	+++		Tri-n-butyl phosphate	+++	+++
	Gemfibrozil	+++	+++		Tricresyl phosphate (o-, m-, p-isomers)*	+++	+++
	Fenofibrate	+++	+++		Triphenyl phosphate	+++	+++
		n=4	n=4		Tris(2-ethylhexyl) phosphate	+++	+++
						n=3	n=3

*n=2

The removal of estrogens, alkylphenols and bisphenol A was complete from the beginning of the experiment. The natural hormones estrone (E1), 17β-estradiol (E2) and estriol (E3) and the synthetic hormone 17α-ethinylestradiol (EE2) are described as relatively hydrophobic and easily adsorbable to organic matter with logK_{OW} values between 3 and 4 (Lai et al. 2000, Ying and Kookana 2003, Yu and Huang 2005). 17α-Ethinylestradiol was detected only once in minor concentration in the effluent of one of the columns but still 99.7% elimination was found. Since in none of the samples of the column effluents any of the natural hormones were detected during 30 months of operation, sorption as the main

contributor to the retention of these substances is rather unlikely. It might be considered that sorption onto biofilm and subsequent degradation could be the dominating process as it was shown for EE2 in studies with conventional and membrane bioreactors (Yi et al. 2011).

High removal rates were also obtained for the analgesic pentoxifylline and the beta blocker propranolol as well as for the non-chlorinated trialkyl phosphates. Except for triethyl phosphate these compounds showed complete removal since the first measurement. This is in accordance with other findings in literature, where chlorinated trialkyl phosphates tend to pass through the WWTPs unaffected, while non-chlorinated species showed high elimination rates under aerobic conditions (Meyer and Bester 2004, Marklund et al. 2005). Further on hydrolysis, resulting in the formation of the respective active metabolites clofibric acid and fenofibric acid is probably responsible for the complete elimination of etofibrate and fenofibrate since the start of the experiments, as it has been previously described in literature (Daughton & Ternes 1999).

The lipid lowering agent gemfibrozil and the trialkyl phosphate triethyl phosphate however, showed a distinct dependence on adaptation time (Fig. 11). The elimination rates of both substances increased over time. While no elimination was found for triethyl phosphate in the first measurement it was completely eliminated after 7 months at 20°C supposedly due to an adaptation of the biomass between the measurement after 5 weeks (10% elimination) and the measurement after 7 months. At 2°C the final degradation rate after 29 months reached 100% with a delayed elimination compared to the 20°C column which set in between 7 and 13 months. The pattern of elimination of Gemfibrozil is similar to that of iohexol or bezafibrate with elimination increasing in the 2°C column over time, while complete elimination was found from the start of the experiment in the 20°C column.

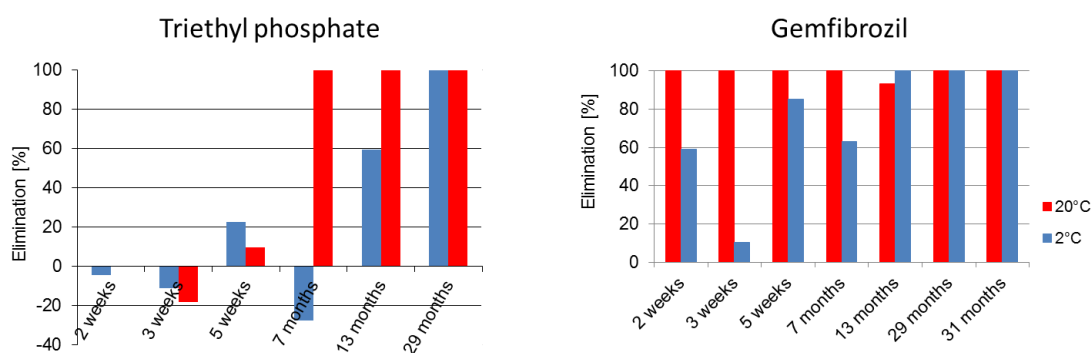


Fig. 11 Elimination of triethyl phosphate and gemfibrozil in the columns fed with spiked WWTP effluent at 2°C and 20°C

In general the substances which could only be analyzed in the spiked effluent, showed good elimination right from the beginning of the experiment. After a sufficient adaptation time also triethyl phosphate and gemfibrozil showed complete removal at both temperatures. The increase in removal at 2°C indicates biological removal mechanisms to be responsible for the elimination of these compounds. As a consequence the persistent compounds detected in this study do not seem to have the potential to serve as direct or indirect (co-metabolism) substrate to the biomass.

Removal during soil passage fed with MBR filtrate

Fig. 10 shows the results for the two soil columns that were run in parallel using membrane filtered water as feed. The concentration scheme in both columns is very similar. The substances detected in the influent of the two columns are representative for the spectrum contained in the MBR effluent after membrane filtration. Apart from trialkyl phosphates, which were not analyzed, some substances like atenolol or gemfibrozil showed good removal and were not detected in the filtrate. Other compounds like ibuprofen, propranolol and phenazone were sometimes detected in minor concentrations in the effluent of the MBR. For a detailed discussion of the results see the report for the deliverable 316 (Lipp et al. 2012).

With regard to the subsequent treatment of the filtrate in the two columns the results in Fig. 12 show that after membrane filtration none of the remaining compounds were eliminated in the two soil columns. Phenazone was not detected in the effluent of column 2. The fact that all other compounds showed similar concentrations in both columns suggests that the concentration of phenazone in column 2 was below the detection limit, which was 50 ng/L in these measurements. Diatrizoic acid and iopamidol showed up only in the effluent samples, which can be attributed to logistic reasons. The influent sample had to be taken after the effluent sample in order to avoid any drawbacks concerning sample volume and time. Therefore measurements of influent and effluent of the two columns represent random samples and do not correspond to each other. Additionally, since X-ray contrast agents will only be used in hospitals, daily shifts can occur.

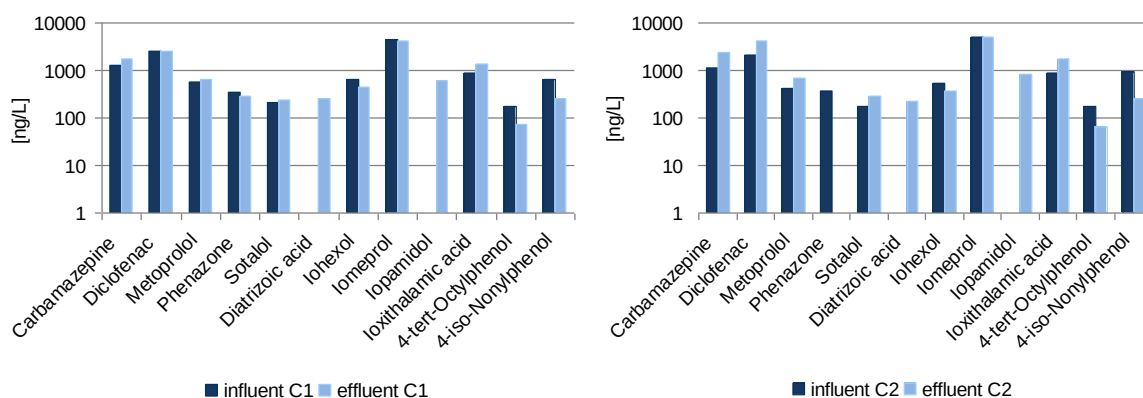


Fig. 12 Concentrations of pharmaceutical substances, X-ray contrast agents and alkylphenols in the influent and effluent of two soil columns (C1 and C2) supplied with filtrate from a MBR

Comparing the results with the elimination rates gained out of the laboratory column experiments it would have been expected that the compounds out of groups II and III, namely diclofenac, metoprolol, iohexol and iomeprol and for the easily removable compounds 4-tert-octylphenol and 4-iso-nonylphenol, will be eliminated. The reason for this distinct difference in removal rates is probably to find in the different microbial communities of a WWTP effluent used in the laboratory columns and of the membrane filtrate used in the pilot plant columns. The membranes usually retain most of the microorganisms of bacterial size and above, resulting in a different microbial matrix than WWTP effluent. In addition to that the pilot plant columns were filled with fresh quartz

sand in contrast to the sediment used in the laboratory columns, with microorganisms already growing on it. Three months of run-in period might have been too short for the establishment of a biofilm on the quartz sand capable of degrading these specific micropollutants. It has to be seen whether a longer period of growth and adaptation of the biomass will change the concentration pattern of column influents and effluents. Further tests are currently conducted at the test site Fuheis/Jordan. Thereby the same pilot plant together with the columns is used, but with different soil material and higher temperatures due to the climatic conditions in Jordan. The filling material for the columns consists of natural top soil mixed with sand to increase the hydraulic conductivity. First samples are planned for autumn 2012 after a sufficient run-in period.

4.1.2. Behavior of bacteria and viruses during soil passage

MS2 in laboratory column experiments

During the 12 weeks experiment with two of the laboratory columns at 2°C and 20°C (Fig. 1) spiked with MS2 bacteriophages, the model virus was detected via qualitative PCR and plaque assay in the effluent at 2°C (breakthrough after 1 week of dosing), but not at 20°C (Fig. 13). Complete MS2 removal was observed in the 20°C column. In the cooled column an increasing retention of the bacteriophages was observed with increasing operation time.

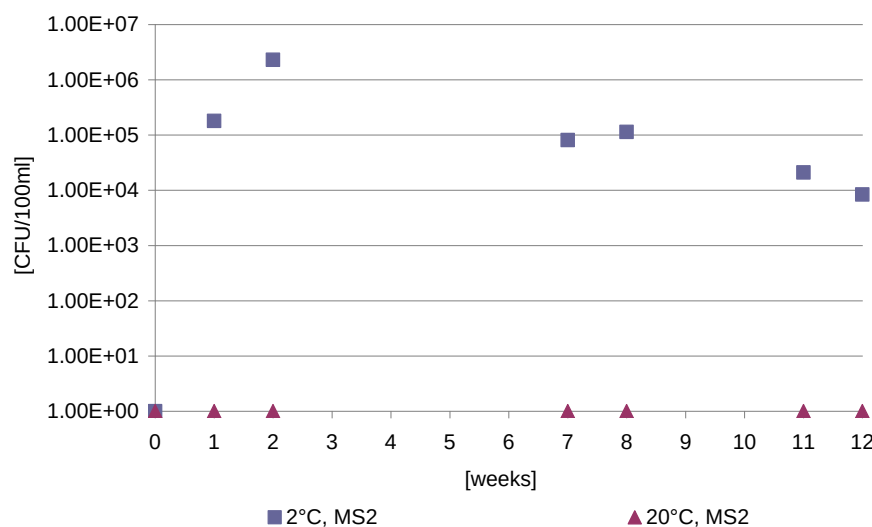


Fig. 13 Concentration development of MS2 bacteriophages after soil passage at 2°C and 20°C ($x = 0$: sample taken before spiking of MS2 bacteriophages)

Schijven & Hassanizadeh (2000) stated temperature as the most important factor to influence virus inactivation during soil passage and showed that inactivation rates for MS2 phages significantly increase with temperature. However, also other parameters, such as oxygen level and nutrient supply, indirectly affect virus elimination by influencing the activity of indigenous groundwater microorganisms which in turn can act as predators to viruses (Gordon & Toze, 2003). Maximum elimination for MS2 was observed under aerobic conditions at 28°C and removal was low at 4°C even in the presence of groundwater microorganisms. Also Sobsey et al. (1995) found a higher removal for MS2 at 25°C compared to 5°C. Thermal inactivation, adsorption, predation, and antiviral microbial

activity have to be considered as the main elimination processes (Grabow 2001, Wolf et al. 2004, Chibani-Chennoufi et al. 2004).

Removal of bacteria and viruses in columns operated with MBR filtrate

Fig. 14 and Fig. 15 show the elimination of *E. coli*, coliform bacteria, enterococci and *Pseudomonas aeruginosa* as well as of adenoviruses and MS2 bacteriophages. Data have been obtained through random sampling. It can be derived from the comparison of waste water with conventional waste water treatment plant effluent (light grey) and filtrate (after membrane filtration, blue) that the latter treatment improves the water quality in terms of bacteria by several orders of magnitude compared to the conventional waste water treatment (Fig. 14). In none of the two samplings for the column filtrate bacteria were found, except for one coliform at one measurement. Further investigations are planned at the Fuheis test site taking more regular samples at a higher frequency.

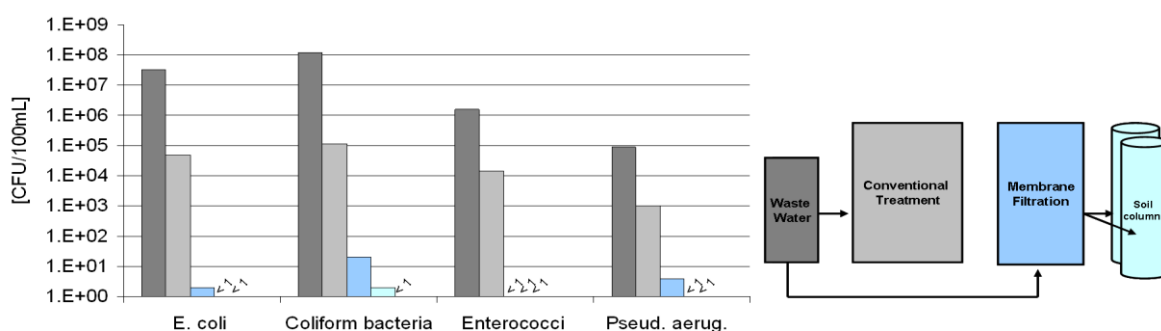


Fig. 14 Removal of *E. coli*, coliform bacteria, enterococci and *Pseudomonas aeruginosa* within conventional waste water treatment, after membrane filtration and after subsequent soil passage in the two soil columns

On the other hand viruses' removal could not be detected after passage of filtrate through the columns (Fig. 15). Interestingly MS2 bacteriophages were not removed after soil passage either although temperature was around 20°C as in the laboratory columns.

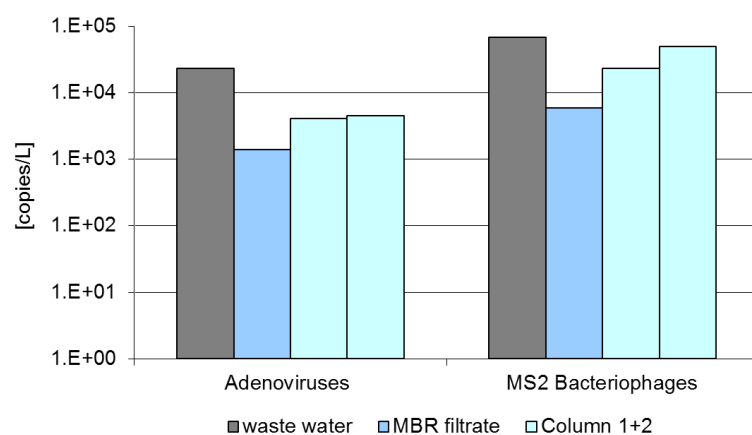


Fig. 15 Concentration of adenoviruses and MS2 bacteriophages measured in waste water, after MBR filtration and after soil passage in the two columns (LOQ = 10² copies/L)

One explanation for this might be the probably less diverse and less adapted microbial community compared to the laboratory columns in case predation is considered responsible for MS2 bacteriophage removal.

Overall the elimination of viruses during soil passage was demonstrated by the removal of the bacteriophage MS2 in the laboratory column experiment. Due to a probably less adapted microbiology in the columns fed with membrane filtrate elimination of viruses could not be shown in these experiments. As already mentioned in chapter 4.1.1 with regard to the pharmaceuticals and the other micro-pollutants further research is being conducted for the elimination of viruses at the Fuheis test site in Jordan.

5. Identification of biodegradation processes (D311)

5.1. Long term batch studies under different redox conditions

Additionally to the soil column experiments emerging pollutants were examined in long term batch studies to assess their behavior during aquifer storage under different redox conditions. Thereby original river bank filtrate without the addition of auxiliary substrates was used. Thus the direct degradation of the substances could be studied when in a mixture with a whole range of emerging pollutants and some of them with high persistency in nature. The exclusion of easily degradable organic matter (auxiliary substrates) allowed studying degradation processes under environmentally relevant conditions.

The anaerobic samples were taken at the riverbank filtration test site Insel Hengsen situated in the River Ruhr valley and serving as one of the water catchment areas of the Dortmund water works (Dortmunder Energie- und Wasserversorgung GmbH). Aerobic samples originated from a riverbank filtration site at the River Rhine operated by the Duisburg water works (Stadtwerke Duisburg AG). Residence times in the field are between five to 20 days.

At two different concentrations microbial adaptation to the compounds was investigated. A mix of the pharmaceuticals with 10 µg/L of each substance was added to the assays. After following their behavior for at least 15 months, another 100 µg/L of each of the pharmaceutical substances were added, examining their fate for another 10 months. The anaerobic batch assays were incubated under different redox conditions with the addition of different terminal electron acceptors (TEA) (NO_3^- , Mn(IV), Fe(III), SO_4^{2-}). Next to pharmaceutical compounds the natural estrogens, 17 α -ethinylestradiol and bisphenol A were analyzed. Alkylphenols and trialkyl phosphates were not considered for these experiments.

All anaerobic measurements were done under nitrogen air in an anaerobic box. Each redox condition was examined with two batch assays, which were sampled in turns during the first phase of the experiment to facilitate the long experimental phase. In the first measurement of each of the two experimental phases all assays were sampled and the results of the double assays for each redox condition were averaged. Next to the concentration of the pollutants the following parameters were followed: pH, O_2 , redox potential, nitrite, nitrate, sulfate, Fe(II), total iron, Mn(II) and total manganese. Fe(III) and Mn(IV) concentrations were calculated as deviations from total iron and manganese and the respective measurements of their reduced forms, Fe(II) and Mn(II).

5.2. Redox conditions and overview of results

The set-up of the assays under different redox conditions resulted in different redox potentials that were measured during each of the sampling events (Fig. 16). Typically for aerobic conditions the redox potential was above 400 mV, while it declined steadily from

NO_3^- - and Mn(IV) -reducing conditions to Fe(III) - and SO_4^{2-} -reducing conditions. In the assays under Mn(IV) -reducing conditions the redox potential dropped towards the end of the first phase. The original riverbank filtrate from the anaerobic zone contained iron but no further increase of Fe(II) due to Fe(III) reduction was measured since all iron was already reduced at the start of the experiment. Sulfate values were below the detection limit. Most probably the redox potential declined due to a slight but steady increase of Mn(II) within the first phase (data not shown) that resulted in a decreased concentration difference between Mn(IV) and Mn(II) species generating a lower potential difference. Due to the ongoing removal of the analgesic naproxen (Fig. 19) however, manganese reduction was estimated to be still active and no further Mn(IV) was supplied to the assay.

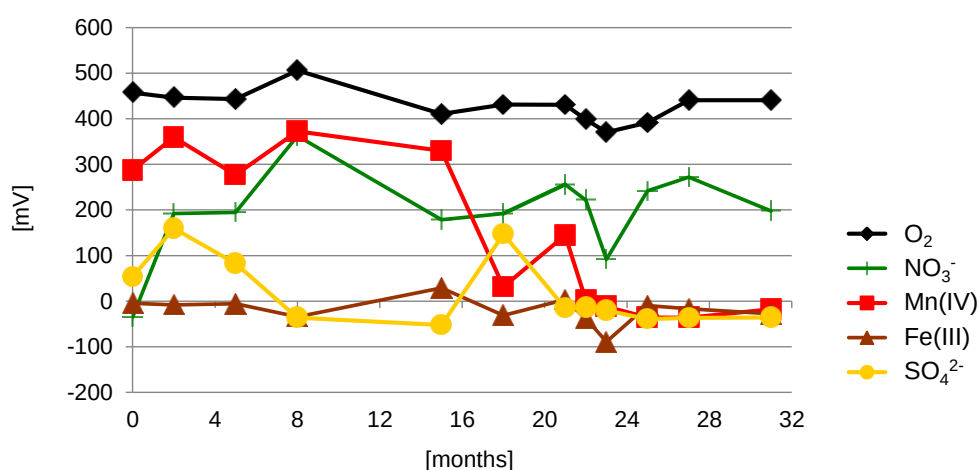


Fig. 16 Redox potentials for the different batch assays with river bank filtrate

Most of the substances were eliminated under aerobic conditions (Tab. 4). However, the concentration of some pharmaceuticals (diclofenac, ibuprofen, bezafibrate, fenofibric acid) and the synthetic hormone 17α -ethinylestradiol remained unchanged in contrast to a removal above 90% during the column tests. This recalcitrant behavior was also detected during the second phase of the batch experiments after subsequent addition of another $100 \mu\text{g/L}$. The recalcitrance of ibuprofen, whose degradation during waste water treatment is known to be good from the literature, is surprising. Also the column experiments have shown a very good elimination for it. The reason for its stability in these tests is most probably to find in the different microbial community between a WWTP or the soil column experiments and the groundwater used for these tests. It is supposed that due to its good removal during conventional waste water treatment and resulting minor relevance in surface water and riverbank filtrate the biomass contained in riverbank filtrate is not adapted to this compound. However it is remarkable that no adaptation has taken place over the time of the experiment. Possibly the mixture of the emerging pollutants tested here has led to some adaptations for specific substances (e.g. naproxen, pentoxifylline) while other substances like for instance ibuprofen were not favored. Iopamidol and iothalamic acid reached similar elimination rates in both phases, which were always below 50%, while less than 10% elimination or none at all was measured for carbamazepine.

Tab. 4 Overview of the elimination of pharmaceutical substances in the batch studies with riverbank filtrate under different redox conditions, with elimination <10% = -, 10% – 50% = +, 50% – 90% = ++, >90% = +++

		O ₂		NO ₃ ⁻		Mn(IV)		Fe(III)		SO ₄ ²⁻	
		10 µg/L	100 µg/L	10 µg/L	100 µg/L	10 µg/L	100 µg/L	10 µg/L	100 µg/L	10 µg/L	100 µg/L
Analgesics	Diclofenac	-	+	-	+	-	+	-	+	+	-
	Ibuprofen	+	-	+	+	+	-	-	-	+	-
	Naproxen	+	+++	+	-	+++	+++	+++	+++	+++	+++
	Phenazone	+++	+	-	+	+	-	-	+	-	-
	Pentoxifylline	+++	++	+	++	-	+	+	+	+	-
Lipid-lowering agents	Bezafibrate	-	-	-	-	-	+	-	-	-	-
	Gemfibrozil	+++	++	+	-	+	-	+	-	++	-
Antiepileptic	Carbamazepine	-	-	-	-	-	-	-	-	-	-
Beta blockers	Atenolol	++	+++	++	+++	+	+	+	++	-	+
	Metoprolol	++	+++	-	+	-	+	-	-	-	-
	Propranolol	++	+++	+	++	-	+	-	+	-	-
	Sotalol	+	++	-	+	-	+	-	-	-	-
X-ray contrast media	Diatrizoic acid	++	+	+	+	++	-	+	-	-	-
	Iohexol	+++	+++	-	-	+	-	-	-	-	+
	Iomeprol	+++	+++	+	-	+	-	-	-	-	-
	Iopamidol	+	+	+	+	+	+	+	+	+	-
	Iopromide	+++	+++	+	-	+	-	-	-	+	-
Estrogens and Bisphenol A	17α-Ethinylestradiol	+	-	+	-	-	-	+	-	+	-
	17β-Estradiol	+++	+++	+++	+++	+	+	+++	+++	+++	+++
	Estriol	+++	+++	+++	+++	-	+	-	+	+	-
	Estrone	+++	+++	+++	+++	-	-	-	-	+	+++
	Bisphenol A	-/-	+++	+	+	-	-	-	+	-	+

In the following chapters results for the elimination behavior of the emerging pollutants are presented and discussed by each redox condition.

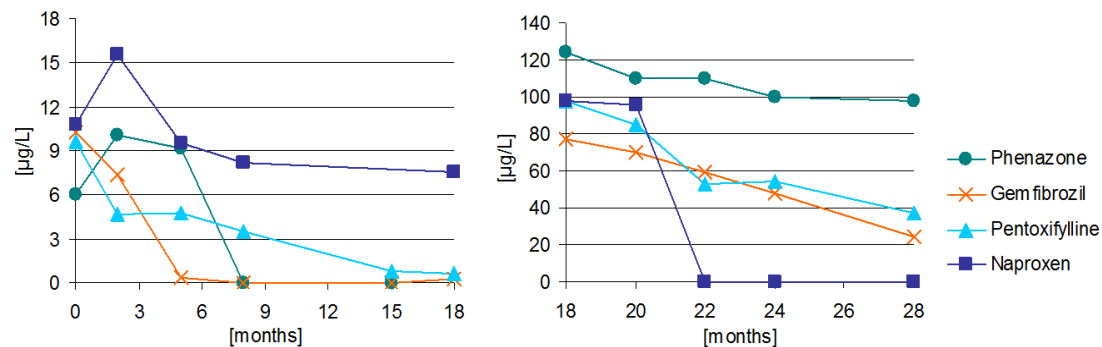
5.2.1. Aerobic conditions

Analgesics & Gemfibrozil

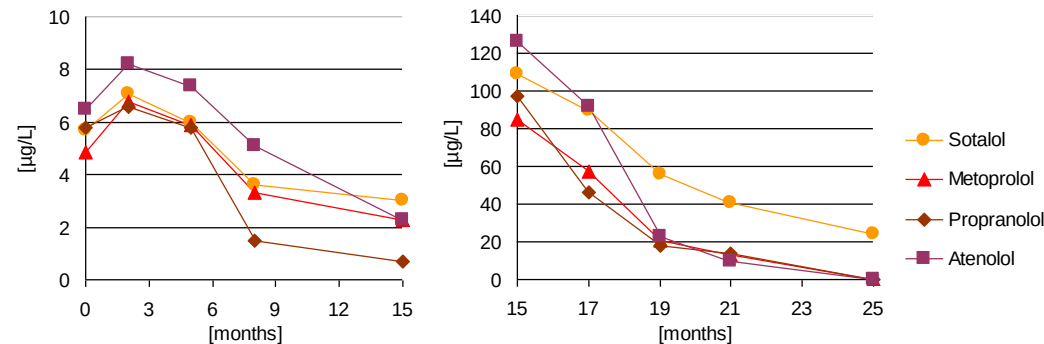
Most of the substances that showed elimination were removed under aerobic conditions. After a lag period of at least two months naproxen was completely removed within two months in the second phase while removal was less pronounced in the first phase (30%), (Fig. 17). Aerobic conditions contributed to a complete elimination of naproxen only in the second phase where higher loads of the pollutants might have enhanced metabolic biodegradation processes. Pentoxifylline and gemfibrozil showed a diminished removal rate during the second phase of the batch experiments under aerobic conditions. Within eight months gemfibrozil was removed almost completely down to 3% within the first phase, however only down to 31% within the second phase after 10 months. A similar behavior was found for pentoxifylline with a removal down to 6% and 38% in the first and second phase, respectively. Still the absolute elimination within the first 2 months of each of the two spiking events showed a 2.4- and 2.7- fold increase in removal for gemfibrozil and pentoxifylline. Maeng et al. (2011) suggested co-metabolic degradation for gemfibrozil in batch studies with different amounts of biodegradable DOC (BDOC) under oxic conditions. For pentoxifylline 50% to 97% were found with the least elimination under BDOC poor conditions (0.5 ± 0.2 mg/L). Though DOC in the beginning was 1.6 mg/L it would not have been sufficient to promote co-metabolic growth during the whole length of the experiment. Phenazone showed a rather exponential progress of

concentration, when it was completely removed in the first phase. However this was not the case in the second phase with only 21% of elimination.

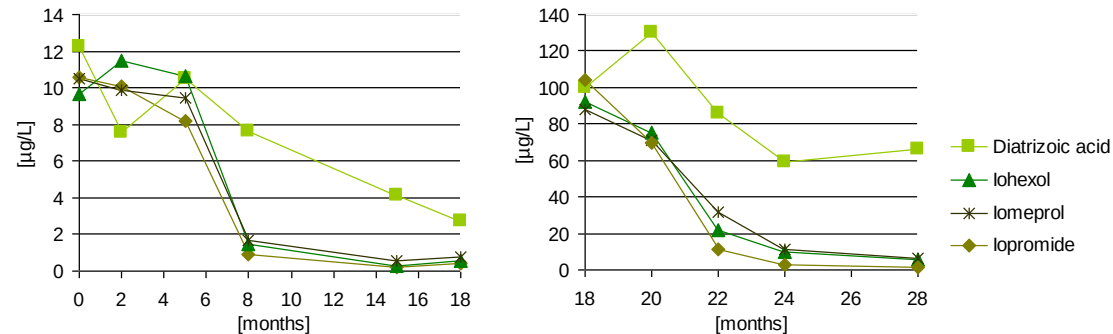
Analgesics & Gemfibrozil



Beta blockers



X-ray contrast media



Estrogens & Bisphenol A

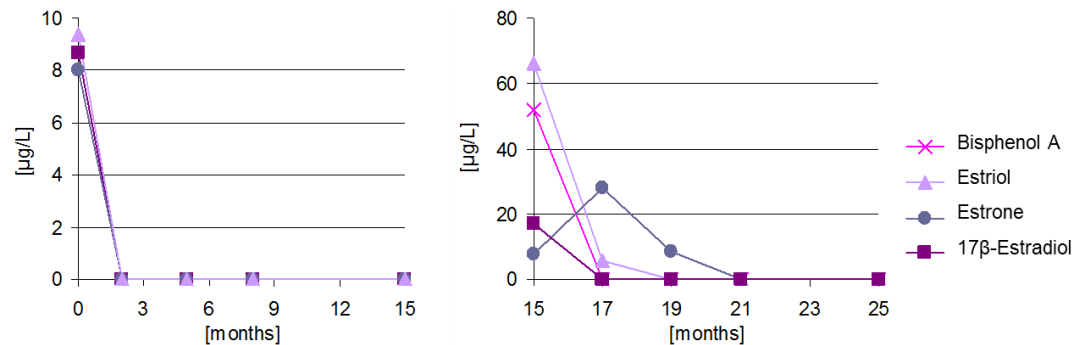


Fig. 17 Behavior of selected analgesics, X-ray contrast media and estrogens, the lipid lowering agent gemfibrozil, the endocrine disruptor bisphenol A and the beta blockers under aerobic conditions

It might be possible that phenazone was not favoured by the microbial degraders anymore after adaptation to other substances. Zühlke et al. (2007) tested groundwater with and without phenazone pollution. Since no degradation was found in the unpolluted water it was concluded that phenazone degradation is conducted by specialized organisms. In the present studies growth of other microorganisms, not capable of degrading phenazone, might have been enhanced due to the increase in concentration of e.g. naproxen or the beta blockers.

Beta blockers

The behavior of the beta blockers atenolol, metoprolol, propranolol and sotalol indicated adaptation of the biomass due to a complete and faster removal in the second phase compared to the first phase (Fig. 17). Within five months in the first phase no elimination was detected for any of the beta blockers, while in the second phase they were removed down to 18%, 25%, 19% and 51% of their original concentration, respectively, within four months. Until month 19 elimination of the beta blockers followed a rather exponential progress which indicates biodegradation. The linear degradation in the first phase points towards a degradation behavior controlled by maintenance metabolism. From month 19 on the concentration of atenolol for instance is rather in the range of the concentration in the first phase, which could explain the decreased linear degradation. Still, most probable as a result of the enhanced biomass, degradation of 23 µg/L over the following six months was faster compared to the degradation of 8 µg/L down to 2 µg/L within 15 months.

X-ray contrast agents

High removal rates were also measured for the X-ray contrast agents iohexol, iomeprol and iopromide. Again removal rates were faster in the second phase with 0%, 10% and 22% within five months and 76%, 64% and 89% within four months, respectively. Elimination of diatrizoic acid was 78% in the first phase and 49% in the second phase, if the measurement after 20 months is considered as the starting point (delayed dissolution). Biodegradation can be considered in case of iohexol, iomeprol and iopromide, since the curve progression is rather exponential in both phases. The same goes for diatrizoic acid in the second phase. However, linear degradation in the first phase and stagnation at 67 µg/L in the second phase could point towards co-metabolism with other substances which have already been utilized to that time (e.g. iohexol). However, this has to be studied separately and so far no such degradation process for diatrizoic acid has been described in literature.

Estrogens & Bisphenol A

The estrogens were completely eliminated in both phases of the experiment, except 17 α -ethinylestradiol (Fig. 17, Tab. 4). In general start concentrations in the second phase did not reach 100 µg/L due to the low water solubility of the estrogens. The natural hormones are described as easily degradable under aerobic conditions (Sarmah & Northcott 2008). In the present studies elimination took place within two months, in the first phase and four to six months in the second phase. It is rather likely that 17 β -estradiol

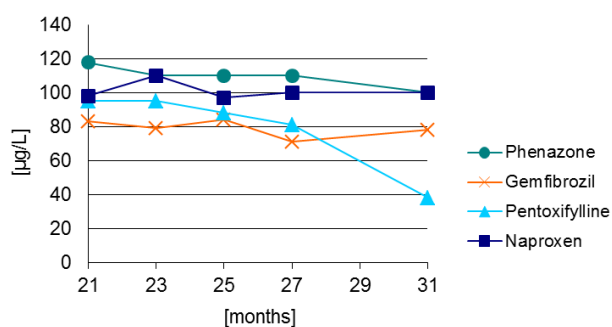
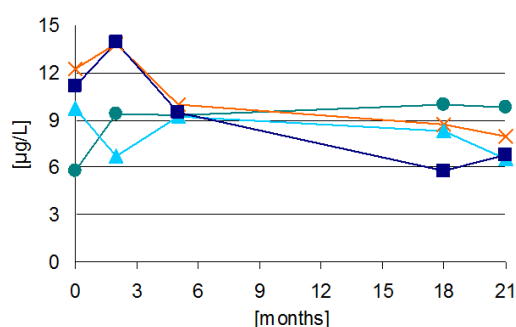
was hydroxylated to estrone, as it is known to be a metabolite of it (Ternes & Joss 2006). Estriol is a metabolite of E2 and E1 and known to be easily degradable under aerobic conditions, which could explain, why no build-up of this natural hormone was detected during the experiment. However, the higher concentration load of the hormones could not be fully compensated by adaptation of the biomass as it was the case with other substances (e.g. atenolol, iohexol) which resulted in a longer time span compared to the first phase until all natural estrogens were fully degraded. The industrial chemical bisphenol A was completely eliminated during aerobic conditions and was not detected within the first phase, presumably due to its rapid degradation after the first spiking event (Fig. 17, Tab. 4).

5.2.2. Nitrate-reducing conditions

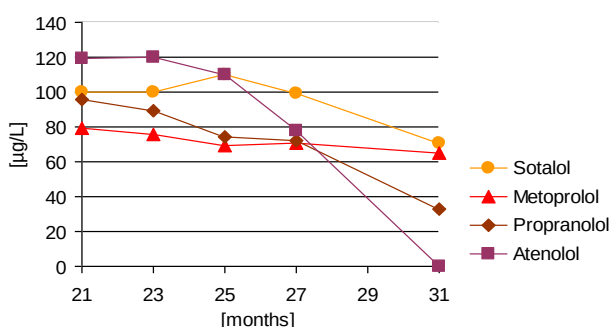
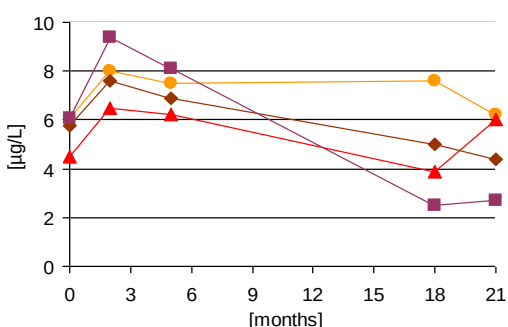
Analgesics & Gemfibrozil

Naproxen could not be degraded under nitrate-reducing conditions and these were the only conditions under which it showed recalcitrance (Fig. 18). While naproxen was removed down to 61% of its original concentration in the first phase no such behavior was found in the second phase within ten months. In an activated sludge reactor under denitrifying conditions Suarez et al. (2010) could not detect any significant degradation for naproxen either. In wetland experiments naproxen was found to be better degradable under denitrifying conditions than under SO_4^{2-} reducing conditions (Matamaros & Bayona 2006, Garcia et al. 2005). In the present study a preferred degradation of other organic compounds might be responsible for the persistence of naproxen. One of these substances could be pentoxifylline, which was removed down to 40% of its original concentration in the second phase although its concentration only varied by $\pm 3 \mu\text{g/L}$ in the first phase. In the second phase however the curve for removal is rather exponential than linear which suggests biodegradation to be responsible for the decline of pentoxifylline. Gemfibrozil showed a decline of 33% from $12 \mu\text{g/L}$ down to $8 \mu\text{g/L}$ in the first phase. However, due to an analytical error of 30% and the fact that its concentration remained unchanged during the following 10 months the decline is not considered significant. So far literature for the degradation of gemfibrozil and pentoxifylline under specific anaerobic conditions is rare and further research in that area is needed. Maeng et al. (2011) suggested co-metabolic degradation for gemfibrozil in batch studies with different amounts of BDOC under anoxic conditions. It would be interesting to investigate the fate of gemfibrozil under the given parameters but with the supply of sufficient easily degradable organic matter. Finally, no considerable removal of phenazone was found under anaerobic conditions, which is in consistency with its behavior in the field where removal was closely linked to aerobic conditions (Massmann et al. 2008). Other substances that showed a concentration decline or have been completely removed under nitrate-reducing conditions are the beta blockers atenolol and propranolol as well as the natural hormones.

Analgesics & Gemfibrozil



Beta blockers



Estrogens & Bisphenol A

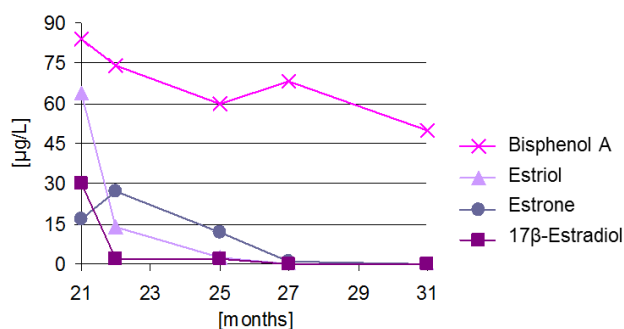
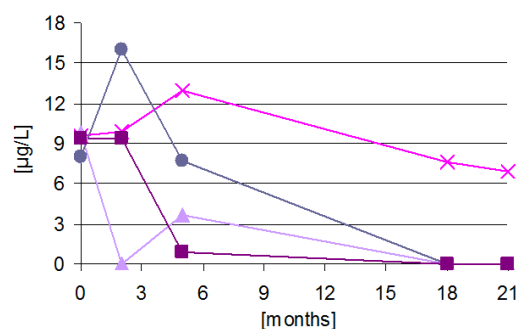


Fig. 18 Behavior of selected analgesics and estrogens, the lipid lowering agent gemfibrozil, the endocrine disruptor bisphenol A and the beta blockers under NO_3^- -reducing conditions

Beta blockers

Most probably due to the time intervals for measurement degradation in the first phase appears to be linear for propranolol and atenolol. The curve progression in the second phase looks rather exponential. Sotalol and Metoprolol did not show a clear elimination under anoxic conditions in the first phase but for sotalol 26 % of its concentration at start were eliminated within the following ten months. From the whole experimental course of both spiking events it can be derived that degradation increased after the second addition. Within this phase 66% of propranolol and a complete elimination of atenolol were performed. In contrast only 24 % and 55 % were degraded in the first phase, respectively.

Estrogens & Bisphenol A

The natural hormones showed complete elimination in the first and second phase. As in the aerobic assays mainly the build-up of estrone as a transformation product was visible. A

shift from 17 β -estradiol to estrone is clear in the second phase. In the first phase an increase of estrone is not coinciding with a decrease of 17 β -estradiol (2 months). This could be due to delayed dissolution of E2 and its conversion occurring in between the first and the second sampling. The plasticizer bisphenol A shows a rather linear removal of 84 $\mu\text{g/L}$ down to 50 $\mu\text{g/L}$ (41% elimination) in the second phase and elimination of 27% was reached in the first phase.

5.2.3. Mn(IV)-reducing conditions

Analgesics & Gemfibrozil

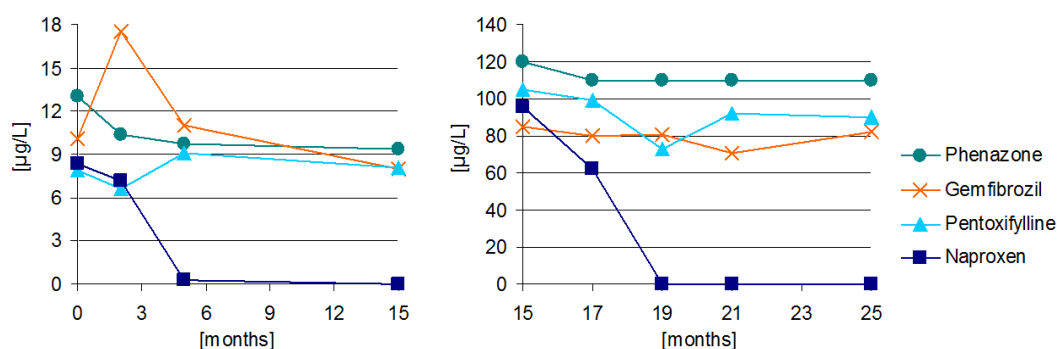


Fig. 19 Behavior of selected analgesics and the lipid lowering agent gemfibrozil under Mn(IV)-reducing conditions

Naproxen showed an almost complete elimination of 8 $\mu\text{g/L}$ within the first 5 months which was followed by another complete elimination of 96 $\mu\text{g/L}$ within 4 months after the second dose (Fig. 19). Comparing the second measurement after each spiking event it becomes clear that naproxen was eliminated faster after the second dosage compared to the first spiking event. It can be derived that naproxen was degraded by microbial activity through manganese reducing microorganisms. Though some information on the removal of naproxen under anoxic and anaerobic conditions exists to our knowledge the fate of this analgesic has not been tested under Mn(IV)-reducing conditions before.

Under Mn(IV)-reducing conditions no considerable removal of endocrine disruptors took place (Tab. 4). It was the only redox conditions which did not show any impact on the estrogens or bisphenol A.

For diatrizoic acid and iothalamic acid removal was not as good as in the first phase (Tab. 4). Since it is not clear which microorganisms are responsible for the degradation of each of the substances tested it cannot be ruled out that the 96 $\mu\text{g/L}$ naproxen acted as a competitor to the degradation of other organic substances like the X-ray contrast agents. A full mineralization by means of Mn(IV) reduction would result in 6.2 times the amount of Mn(IV) used for the reduction compared to the amount of naproxen (96 $\mu\text{g/L}$). That is 662.4 $\mu\text{g/L}$ or 0.66 mg/L. The amount of Mn(II) was 6.6 mg/L at the start of the second spiking event and increased to 6.8 mg/L - 7.1 mg/L, a difference of 0.2 mg/L - 0.5 mg/L which is in the range of the amount of Mn(IV) needed for a complete mineralization of naproxen. Hence, a degradation of other compounds in the same order of magnitude could have been limited. However, considering a 5% error of measurement for the detection of

the manganese species concentration differences in this range are susceptible to high uncertainties.

5.2.4. Fe(III)-reducing conditions

Analgesics & Gemfibrozil

Fig. 20 shows a similar behavior of naproxen as in the assays under aerobic and Mn(IV)-reducing conditions.

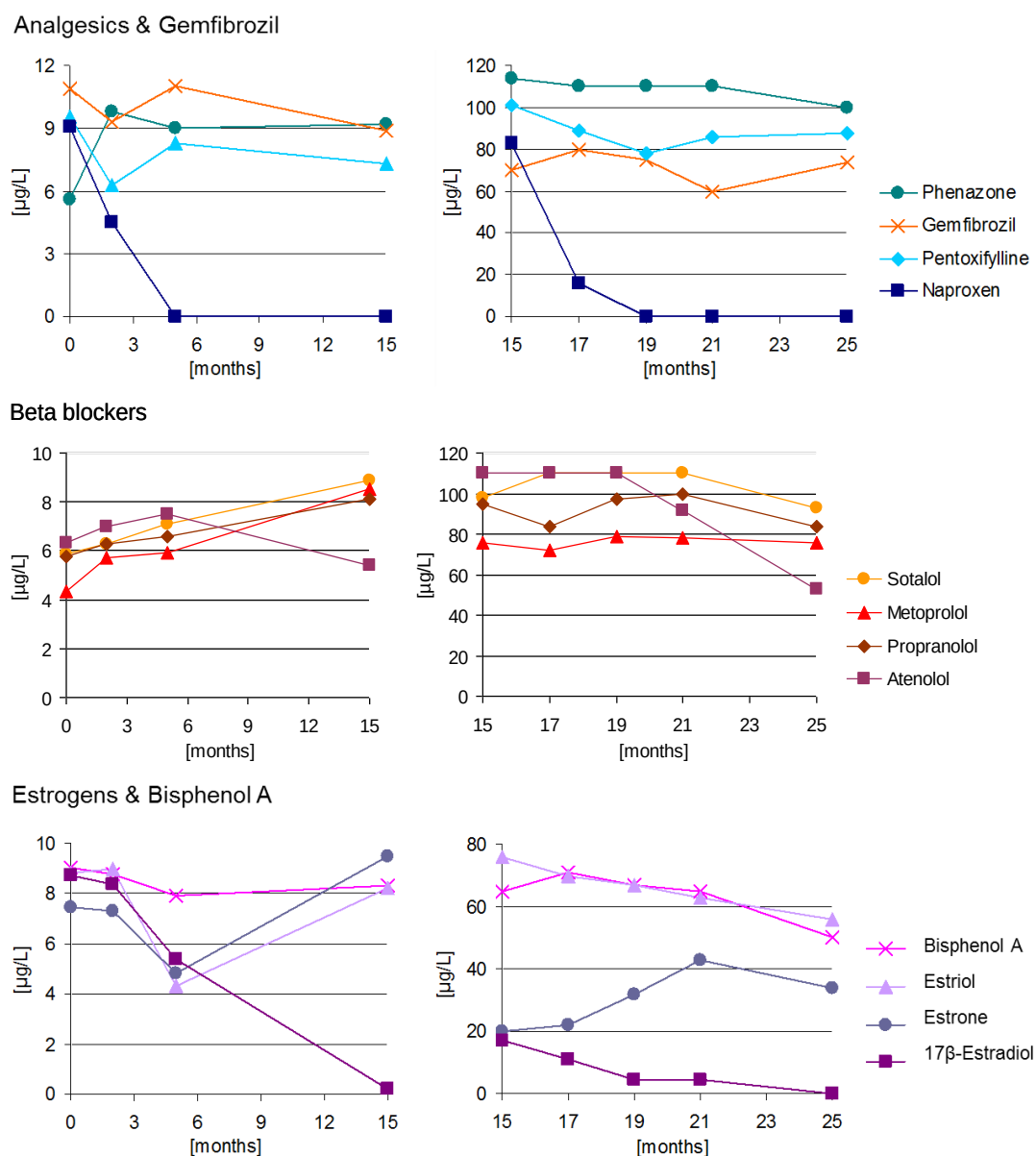


Fig. 20 Behavior of selected analgesics and estrogens, the lipid lowering agent gemfibrozil, the endocrine disruptor bisphenol A and the beta blockers under Fe(III)-reducing conditions

Again the analgesic was found to decrease completely within 5 months after the addition of 9 µg/L and a faster removal was even found after addition of 83 µg/L with an elimination of 67 µg/L (81%) compared to 5 µg/L (51%) in the measurement after 2 months. Overall concentration of the other analgesics remained unchanged.

Beta blockers

For atenolol a removal of 52% was detected under Fe(III)-reducing conditions, with removal rates increasing from the first to the second phase of the experiment. However degradation was found to be rather linear, which points towards slower degradation activity in these assays compared to the aerobic and NO₃⁻-reducing conditions.

Estrogens & Bisphenol A

The hormonal pattern shows a linear decrease of estriol and a shift from 17 β -estradiol to estrone in the second phase. Estriol is removed linearly to 74% of its start concentration and bisphenol A is eliminated down to 50 μ g/L from 65 μ g/L. In the first phase the concentrations of estriol and estrone vary due to analytical issues and probably degradation of 17 β -estradiol. But from the results obtained during the second phase it can be derived that estriol and estrone seem to be less degradable under Fe(III)-reducing conditions than under aerobic and NO₃⁻-reducing conditions.

5.2.5. Sulfate-reducing conditions

Analgesics & Gemfibrozil

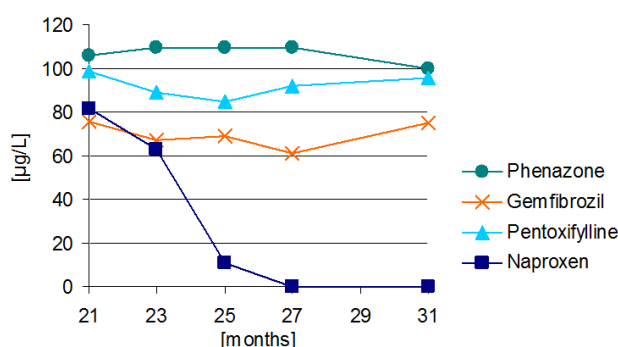
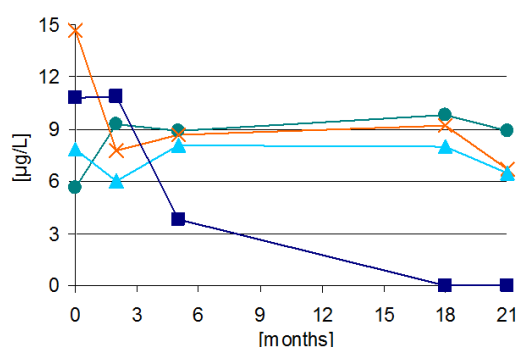
Naproxen showed a complete degradation, which was again faster in the second phase (Fig. 21). While in the first phase no degradation took place within the first two months, already 19 μ g/L (23%) were degraded in the second phase. Gemfibrozil showed 54% elimination under sulfate-reducing conditions, which could not be repeated however, in the second phase.

In anaerobic batch experiments with natural sediment from a groundwater recharge site 1 μ g/L of atenolol were shown to be well degradable within 200 days at maximum and fastest degradation under sulfate-reducing conditions (Barbieri et al. 2011). However DOC in the experiments exceeded the DOC in the present study by one order of magnitude facilitating higher biomass growth at the start of the experiment. Further on no direct correlation of a decrease in sulfate and atenolol concentration was shown. Further research will be beneficial for a better understanding of degradation processes under different redox conditions.

Estrogens & Bisphenol A

Estriol did not decrease in the assays under sulfate-reducing conditions but estrone and 17 β -estradiol both were removed to below the detection limit within six and four months in the second phase, respectively. The shift from estrone to estriol as the transformation product can be seen clearly in the first phase. While estriol and estrone both proved to be recalcitrant under Fe(III)-reducing conditions, only estriol appeared to be non-degradable under SO₄²⁻-reducing conditions.

Analgesics & Gemfibrozil



Estrogens & Bisphenol A

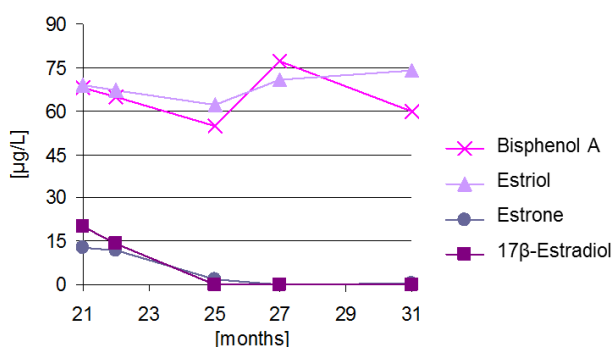
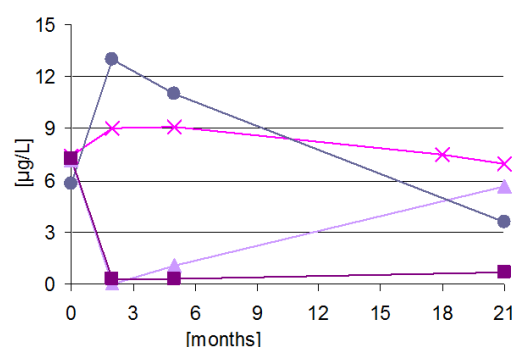


Fig. 21 Behaviour of selected analgesics and estrogens, the lipid lowering agent gemfibrozil and the endocrine disruptor bisphenol A under SO_4^{2-} -reducing conditions

5.3. Summarized discussion and conclusions

In general under aerobic conditions many of the substances show a high elimination in the batch tests with riverbank filtrate. Compounds which have not been eliminated or showed marginal elimination rates were clofibric acid, fenofibric acid, carbamazepine, diatrizoic acid, iothalamic acid and iopamidol. Their persistency in the environment is well known and has been shown in different column and field studies before (Patterson et al. 2011, Scheytt et al. 2007, Schmidt et al. 2007). Surprisingly also the analgesics diclofenac, ibuprofen and the lipid-lowering agent bezafibrate, which showed a good removal in the soil column studies, behaved recalcitrant irrespective of the redox condition. All other compounds showed elimination rates above 90% regardless of the concentration applied.

In aerobic batch studies using a soil/sediment – water mixture Kormos et al. (2010) found complete degradation and the build-up of several transformation products for iohexol and iomeprol. In field studies the non-ionic X-ray contrast agents iomeprol, iohexol and iopromide were found to be very good degradable regardless of the redox potential but with an enhanced mineralization rate towards anaerobic conditions measured by an increasing removal of adsorbable organic iodine (AOI) (Grünheid et al. 2005, Wiese et al. 2011). Due to these findings removal under anaerobic conditions would have been expected as well. Exclusion of sediment in the present study might have had an influence on the microorganism community resulting in different removal schemes. Additional tests with sediment would help to enlighten this question and furthermore add valuable input on

the so far scarce literature considering the fate of these pollutants under specific redox conditions.

Iopamidol reached a similar elimination rate in both phases, which was always below 50%. Together with clofibric acid which did not show any concentration decline either these two substances proved to be as persistent as in the column tests. Diatrizoic acid showed an improved elimination in the batch tests compared to the column experiments. But its removal rate decreased when higher concentrations were applied. It cannot be ruled out that naproxen, which was completely eliminated under manganese reducing conditions, acted as a competitor to the degradation of diatrizoic acid, while naproxen together with other compounds did so in the aerobic assay. Carbamazepine remains unchanged over the whole time of the experiment under aerobic as well as anaerobic conditions (tab. 1). Although it is generally known for its high stability in the environment there has been evidence for its removal potential during riverbank filtration under anaerobic conditions (Schmidt et al. 2007). Since the riverbank filtrate used in this study originated from the same site a similar behavior for carbamazepine in the batch tests would have been expected. In wetland studies Matamaros et al. (2005) compared several pharmaceuticals for their suitability as tracers and decided against carbamazepine, due to its high retention, which they referred to its sorption onto organic surfaces ($\log K_{OW}$ 2.45). If sorption is considered as a contributor to a concentration decrease of carbamazepine, then this could explain its concentration decline under anaerobic conditions in the field on the one hand and its persistency in the batch assays on the other hand. Still degradation of carbamazepine could have taken place in the field since in the long run sorption alone unlikely sustained complete retention. Batch experiments with sediments would be helpful to clarify this matter.

In Tab. 5 the results for the average oxygen contents and the start and end concentrations of the TEAs in each of the batch assays are listed. The results for NO_3^- , Mn(VI), Fe(III) and SO_4^{2-} are shown for the second phase of the experiment. On average O_2 concentration was 7.7 mg/L in the aerobic assays due to a regular aeration and shaking of the flasks while anaerobic conditions were maintained in the other assays with max. 0.11 mg/L O_2 .

Tab. 5 Average oxygen contents (including standard deviations in brackets) and start/end concentrations of the terminal electron acceptors (TEA) in the batch assays under different redox conditions. TEA concentrations are depicted for the second phase only.

	O_2	NO_3^-	Mn (IV)	Fe (III)	SO_4^{2-}
O_2	7.69 (1.16)	0.09 (0.05)	0.07 (0.05)	0.07 (0.05)	0.11 (0.10)
TEA	-	11.4 / 10.4	2 / 2	28 / 27	7.2 / 7.6

Although some compounds showed elimination under anaerobic conditions, only for the assay under nitrate-reducing conditions a clear difference in TEA concentration between the start and the end of the second phase could be measured, resulting in a consumption of 1 mg/L nitrate. Under nitrate-reducing conditions the analgesic pentoxifylline, the beta blockers atenolol and propranolol and the natural hormones 17β -estradiol, estriol and estrone have been partly (>50%) or completely eliminated (Fig. 18). Taking all compounds

together would make up 1.2 mg/L of theoretical nitrate consumption, which is close to the measured nitrate consumption. For oxidizing conditions 1.8 mg/L of theoretical oxygen consumption were calculated. This was confirmed by CSB measurement resulting in a decline by 3 mg/L between the first and the last measurement, which is again close to the calculated value. The concentrations of Mn(IV), Fe(III) and SO_4^{2-} did show some variation throughout the experiment but overall no considerable concentration decline was observed. Calculation of the theoretical TEA consumption due to pollutants elimination makes up 0.9 mg/L Mn(IV), 2.4 mg/L Fe(III) and 0.4 mg/L SO_4^{2-} . Due to the detection limit of the IC a sulfate consumption of this concentration was not measurable. Considering a 5% error of measurement for the detection of the manganese and iron species quantification of concentration differences of these TEAs was not possible, either. In order to assess a direct correlation of the elimination of the pollutants and a decrease of the respective TEA concentration even higher pollutants concentrations have to be applied. However, it was decided to increase the pollutants concentration only by one order of magnitude in these studies, to avoid any inhibitory effects by these pollutants due to a sudden and drastic concentration increase.

Within the first two months removal rates for naproxen were faster after the second spiking with 22%, 38% and 80% removal under SO_4^{2-} -, Mn(IV)- and Fe(III)-reducing conditions compared to 0%, 8% and 50% in the first phase. In both phases removal efficiency increased by the following order: SO_4^{2-} < Mn(IV) < Fe(III) with complete elimination rates reached within both phases.

The anaerobic conditions were less favorable for the removal of the natural hormones. E2 was completely eliminated under SO_4^{2-} und Fe(III) reducing conditions but its metabolites behaved more recalcitrant under these conditions. E3 proved to be persistent and partly accumulated in the SO_4^{2-} - and Fe(III)-reducing assay and E1 was only completely degraded in the second phase of the experiment under SO_4^{2-} -reducing conditions. A removal of E2 under sulfate, iron and nitrate-reducing conditions has been observed in other studies as well (Czajka & Londry 2006, Sarmah & Northcott 2008, Ying et al. 2008).

Contradictory findings for the behavior of EE2 under aerobic conditions have been documented. Ying et al. (2008) detected no degradation, while Sarmah and Northcott (2008) and Ying et al. (2003) determined a concentration decline. In these studies the synthetic estrogen showed no considerable degradation under any of the redox conditions. Degradation of EE2 under anaerobic conditions was investigated by Ivanov et al. (2010) with respect to iron reducing bacteria but the hormone turned out to be persistent.

In summary a good removal was found for many of the substances. In terms of MAR applications the studies show the importance of the prevalent redox conditions and the retention time. Under field conditions with the contribution of sorption processes onto sediment and enhanced biodegradation through biofilm attached to it a faster and increased removal is assumed.

6. Water quality analysis in waste water and recharged groundwater (D317)

Due to lacking information on the occurrence of the investigated micropollutants in the model region water sampling campaigns have been conducted between 2007 and 2012. Chapter 6.1 lists the spectra and concentrations obtained within this period.

6.1. Occurrence of pharmaceutical residues in the Lower Jordan Valley

To gain an overview of relevant trace organics and pathogenic microorganisms, along the Lower Jordan Valley in total 181 samples from 87 different locations were taken between March 2007 and March 2012. 55 samples were taken in the West Bank and 126 samples were collected east of the Jordan Valley, between Wadi Kufrinja in the north and Wadi Kafrein in the south. Surface water samples were taken at the King Abdullah Canal, the Wadi Shueib, and the Wadi Kafrein. Waste water samples were taken from effluents of four different WWTPs in Palestine and four WWTPs in Wadi Shueib and Wadi Kafrein in Jordan. Surface water samples consisted either of surface runoff or of a mixture with WWTP effluents. Additionally, groundwater samples from springs and wells were analyzed at both sides of the Jordan Valley.

Limits of detection for pharmaceuticals were 100-200 ng/L in waste water samples and 50 ng/L in surface water and groundwater samples. X-ray contrast agents had detection limits of 200-500 ng/L in waste water samples and 100 ng/L and 20-40 ng/L in surface water and groundwater samples, respectively. Limits of detection for trialkyl phosphates were 130 ng/L (waste water) and 75 ng/L (surface water and groundwater). In waste water estrogens, alkylphenols and bisphenol A had detection limits of 10 ng/L, 250 ng/L (only 4-*iso*-nonylphenol) and 50 ng/L (bisphenol A and 4-*tert*-octylphenol), respectively. In surface water and groundwater samples their limits of detection were 2 ng/L (estrogens), 10 ng/L (4-*tert*-octylphenol and bisphenol A) and 50 ng/L (4-*iso*-nonylphenol).

Averaged data were calculated on the basis of the results from all sampling campaigns. Where concentrations were below the detection limit half of the detection limit was included in the calculations.

Figures 22 and 23 give an overview of substances that were detected in water samples from Jordan. Pharmaceutical compounds and X-ray contrast agents were at present in most of the collected waste water and surface water samples in concentrations of ng/L to several µg/L (Fig. 22).

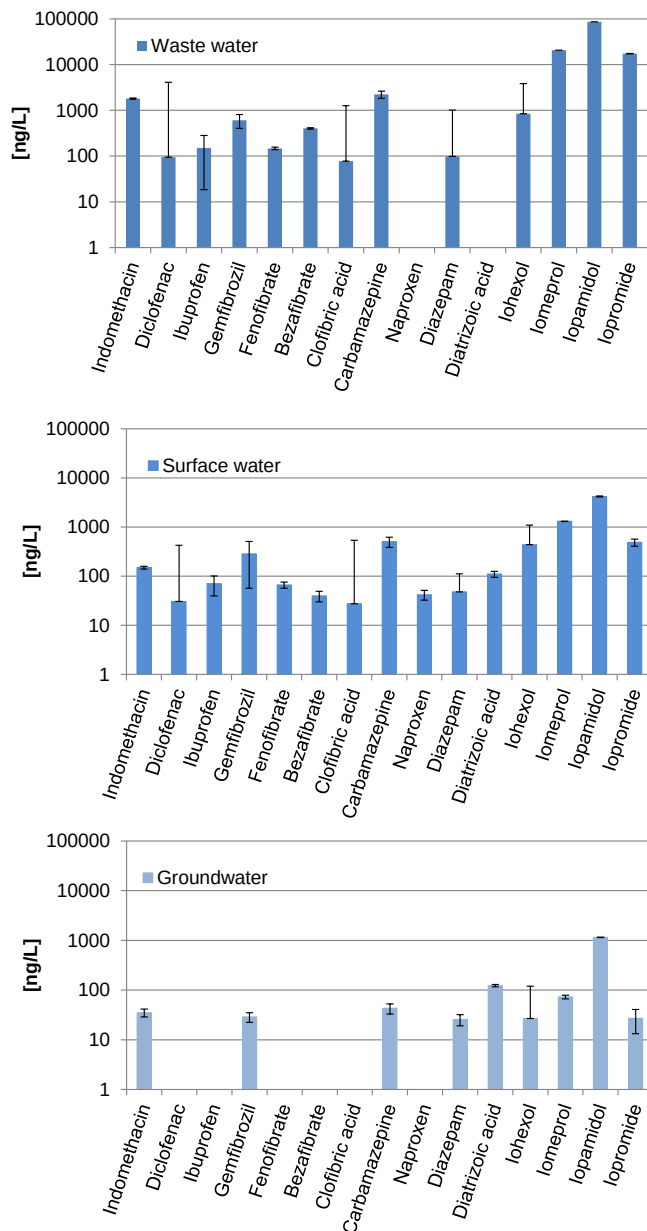


Fig. 22 Mean concentrations of pharmaceuticals and X-ray contrast agents detected in waste water, surface water and groundwater samples from Jordan ($n_{\text{waste water}} = 18$, $n_{\text{surface water}} = 36$, $n_{\text{groundwater}} = 65$ for pharmaceuticals and 72 for X-ray contrast agents; error bars indicate standard deviations)

Waste water samples were always taken at the effluent of a WWTP. Concentrations in surface water samples in general were one order of magnitude below the concentrations in the WWTP effluents (e.g. indomethacin, diclofenac, iopamidol). Attenuation of these micropollutants can be attributed to dilution effects, sorption onto sediments and naturally occurring biodegradation processes. Substantial attenuation during infiltration into the underground can be derived from the results for groundwater samples. Still some of the pharmaceuticals were detected with several ng/L and especially the X-ray contrast agents show relatively high concentrations of up to 1.2 $\mu\text{g/L}$ on average (iopamidol). The concentrations measured are comparable to other studies from the US or Europe (e.g. Hoppe-Jones et al. 2010, Rabiet et al. 2006, Sacher et al. 2001).

Next to pharmaceutical residues estrogens, alkylphenols and trialkyl phosphates were investigated in the field samples (Fig. 23).

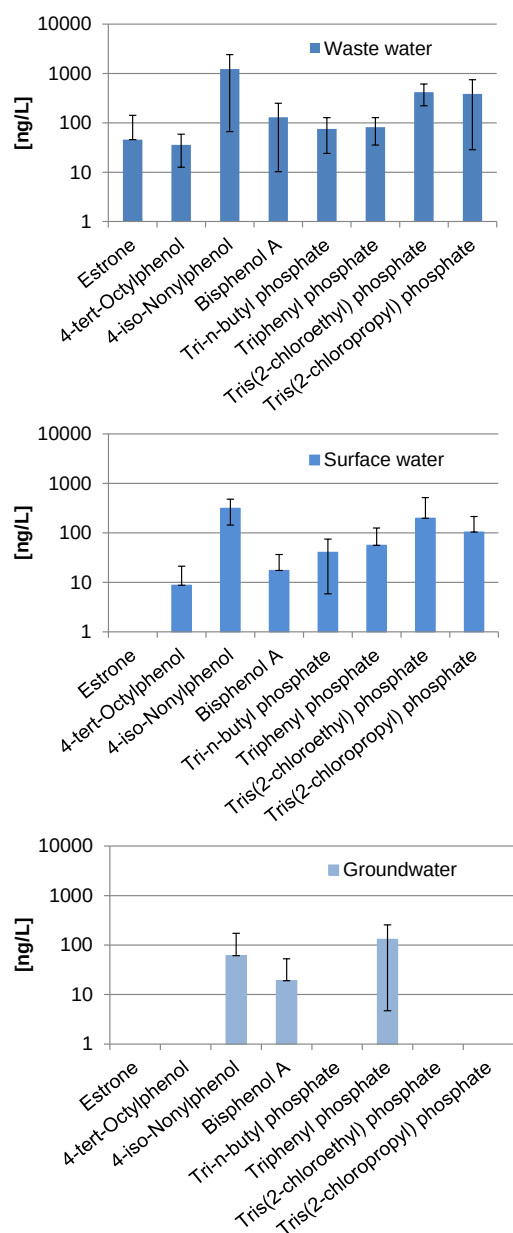


Fig. 23 Mean concentrations of estrogens, alkylphenols and trialkyl phosphates detected in waste water, surface water and groundwater samples from Jordan ($n_{\text{waste water}} = 6$ for estrogens and alkylphenols and 5 for trialkyl phosphates, $n_{\text{surface water}} = 12$, $n_{\text{groundwater}} = 15$; error bars indicate standard deviations)

Alkylphenols are under suspicion to mimic estrogenic activity and carcinogenic effects have been reported for some trialkyl phosphates (Soto et al. 1991, InChem 1991, 1998). Among the natural estrogens only estrone was detected with 46 ng/L on average in waste water. Within the group of alkylphenols 4-iso-nonylphenol exhibited the highest concentrations with a mean value of 1.2 $\mu\text{g/L}$. Concentrations of trialkyl phosphates were between 76 ng/L (tris-n-butyl phosphate) and 416 ng/L (Tris(2-chloroethyl) phosphate). In surface water samples 4-iso-nonylphenol and the chlorinated trialkyl phosphates still exhibited concentrations of some hundred ng/L. Although they are known for their persistency only the non-chlorinated triphenyl phosphate was detected in groundwater samples together with Bisphenol A and 4-iso-Nonylphenol.

With regard to the samples taken in the West Bank differences in the detected spectrum of substances were noticed (Fig. 24). Thus the lipid lowering agents fenofibrate and clofibrate

acid as well as the anxiolytic and antiepileptic diazepam were only detected in samples from Jordan and in none of the five samples from Palestine. On the other hand the X-ray contrast agent ioxithalamic acid was detected in the waste water samples from the West Bank only.

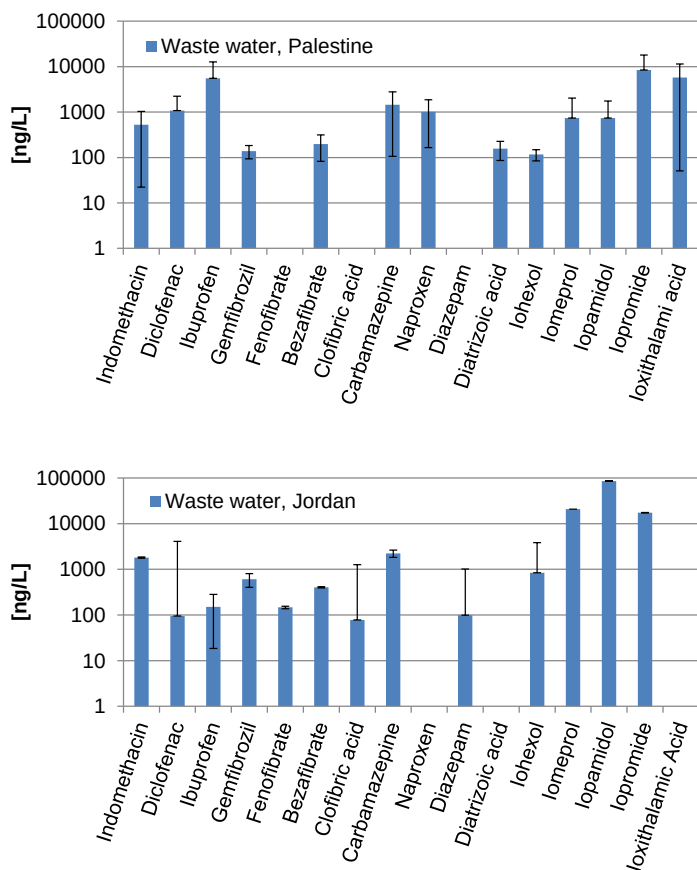


Fig. 24 Mean concentrations of pharmaceuticals and X-ray contrast agents analyzed in waste water samples from Palestine (top) and Jordan (bottom) (samples from Palestine $n = 5$, samples from Jordan $n = 18$; error bars indicate standard deviations)

In the sampling campaigns for waste water the effluent of four different WWTPs west and east of the Lower Jordan Valley were sampled. Naproxen and diatrizoic acid were sometimes detected in samples from Jordan but due to the larger amount of samples ($n = 18$) their mean concentrations were below the detection limit, while ioxithalamic acid was found in only one groundwater sample with 10 ng/L. A later measurement in one of the WWTPs resulted in the same spectrum with neither fenofibrate, nor clofibric acid or diazepam detected in the effluent. Since clofibric acid and ioxithalamic acid are both known for their persistence during waste water treatment, leaving the plants often without a substantial concentration decline, it is assumed that different prescription practices are applied in the two regions.

Next to these obvious differences in the spectra of water samples taken west and east of the Jordan Valley concentration shifts from one drug to another occur. The anti-inflammatory indomethacin and the lipid lowering agent gemfibrozil are found in higher concentrations in Jordanian than in Palestinian waste water, while diclofenac and ibuprofen exceed concentrations by one order of magnitude in the West Bank compared to Jordan. This

could be the reason why indomethacin and gemfibrozil have been detected in groundwater samples in Jordan but not in Palestine (Fig. 25).

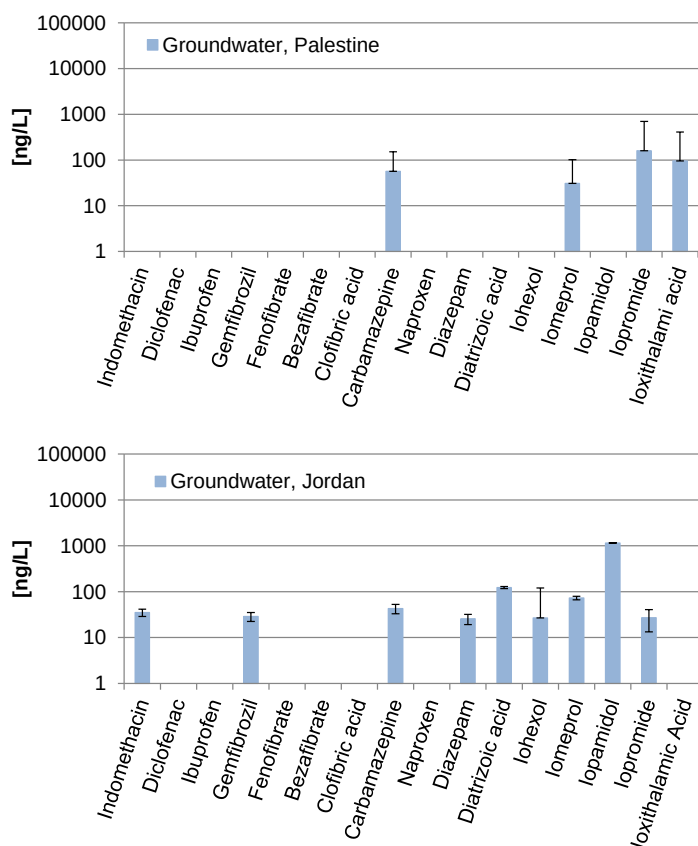


Fig. 25 Concentrations of pharmaceuticals and X-ray contrast agents detected in groundwater samples from Palestine (top) and Jordan (bottom) (samples from Palestine $n = 50$, samples from Jordan $n = 65$ and 72 for pharmaceuticals and X-ray contrast agents, respectively; error bars indicate standard deviations)

Different substances spectra in the groundwater as presented here could be useful for the identification of hydrogeological conditions if the substances turn out to be recalcitrant to environmental impacts. The antiepileptic carbamazepine is already under discussion for the use as a tracer (Nakada et al. 2008, Daneshvar et al. 2012) and its high persistency is once more confirmed with the present groundwater samples.

7. Assessment of groundwater risk by treated waste water (D503)

The studies presented in this report were conducted to gain further understanding of the fate of emerging pollutants and microorganisms of hygienically importance in the realm of wastewater reuse for groundwater recharge. In terms of microbiological parameters the data from the laboratory and pilot plant analysis gave a first impression of the treatment capacity of the soil passage. MS2 bacteriophages were shown to be eliminated under certain conditions, depending on temperature and the type of water (waste water and MBR filtrate). However, more studies are required to understand the fate of pathogens under field conditions. Therefore, continuous measurements at an infiltration basin at the test site Fuheis are foreseen to study e.g. the fate of viruses during a longer time span. Thereby more time for biomass growth is given, which could have a positive effect on the viruses removal.

In the following chapter the focus for the assessment of groundwater risk by treated waste water is set on the organic compounds investigated in the batch and column studies. Their emerging significance was already presented in chapter 2. Based on the experimental results a classification was derived which is presented in chapter 7.1.

7.1. Classification

In the following the results from the column and batch experiments in terms of emerging pollutants are summarized and a tentative classification of the substances tested will be given. To assess the risk for groundwater supplemented with treated waste water many parameters have to be considered and a sound knowledge of the boundary conditions and the water quality of the aquifer in question is essential. Therefore this summary and the classification can only answer a part of this question, namely considering the emerging pollutants, tested under the defined conditions. To apply the results to operations in the field, data need to be obtained from running MAR projects with the results being compared and integrated into an advanced classification scheme that considers both laboratory and field data.

Tab. 6 summarizes the results for the batch experiments under anaerobic and aerobic conditions. Out of 22 substances tested 14 were either completely or to more than 50% removed. This is indicated by the letter 'b' under the column named 'classification'. All 14 substances were removed under aerobic conditions, while naproxen, pentoxifylline, atenolol, propranolol and the natural estrogens estrone, 17 β -estradiol and estriol showed distinct removal under anaerobic and/or anoxic conditions as well. Letter 'a' stands for substances that either did not show any elimination, were only eliminated partly (< 50%), or that showed a distinctly different behavior in the two phases of the experiment (at 10 μ g/L and 100 μ g/L). This was the case with phenazone, which was completely degraded at 10 μ g/L but showed only 21% elimination at 100 μ g/L under aerobic conditions. Also diatrizoic acid showed nearly 80% degradation in the first phase but less than 50% in the

second phase in the aerobic assays and is therefore classified under ‘a’. However in the same assay the elimination of sotalol increased from 48% to 78% and it was therefore ranked into group b. Bisphenol A was not detected within the first phase, presumably due to its rapid degradation within the first hours after the spiking of 10 µg/L to the aerobic assay. However degradation of 100 µg/L within the first 2 months was detected in the second phase, which classifies it under ‘b’. With less than 50% elimination in any of the assays during the whole experiment diclofenac, ibuprofen, bezafibrate, clofibrac acid, carbamazepine, iopamidol and finally the synthetic estrogen 17 α -ethinylestradiol all belong to group a. The two ionic X-ray contrast agents iothalamic acid and ioxithalamic acid behaved similar to diatrizoic acid under Mn(IV)-reducing conditions. While more than 50% removal was measured in the first phase, elimination decreased substantially in the second phase, ranking these two substances under ‘a’ as well.

Tab. 6 Summary of the results from the batch experiments with riverbank filtrate under anaerobic and aerobic conditions and 10 µg/L and 100 µg/L start concentration of each of the substances (elimination <10% = -, 10% – 50% = +, 50% – 90% = ++, >90% = +++; ‘a’ = removal <50%, ‘b’ = removal >50%)

		O ₂		NO ₃ ⁻		Mn(IV)		Fe(III)		SO ₄ ²⁻		Classification
		10 µg/L	100 µg/L	10 µg/L	100 µg/L	10 µg/L	100 µg/L	10 µg/L	100 µg/L	10 µg/L	100 µg/L	
Analgesics	Diclofenac	-	+	-	+	-	+	-	+	+	-	a
	Ibuprofen	+	-	+	+	+	-	-	-	+	-	a
	Naproxen	+	+++	+	-	+++	+++	+++	+++	+++	+++	b
	Phenazone	+++	+	-	+	+	-	-	+	-	-	a
	Pentoxifylline	+++	++	+	++	-	+	+	+	+	-	b
Lipid-lowering agents	Bezafibrate	-	-	-	-	-	+	-	-	-	-	a
	Gemfibrozil	+++	++	+	-	+	-	+	-	++	-	b
Antiepileptic	Carbamazepine	-	-	-	-	-	-	-	-	-	-	a
Beta blockers	Atenolol	++	+++	++	+++	+	+	+	++	-	+	b
	Metoprolol	++	+++	-	+	-	+	-	-	-	-	b
	Propranolol	++	+++	+	++	-	+	-	+	-	-	b
	Sotalol	+	++	-	+	-	+	-	-	-	-	b
X-ray contrast media	Diatrizoic acid	++	+	+	+	++	-	+	-	-	-	a
	Iohexol	+++	+++	-	-	+	-	-	-	-	+	b
	Iomeprol	+++	+++	+	-	+	-	-	-	-	-	b
	Iopamidol	+	+	+	+	+	+	+	+	+	-	a
	Iopromide	+++	+++	+	-	+	-	-	-	+	-	b
Estrogens and Bisphenol A	17 α -Ethinylestradiol	+	-	+	-	-	-	+	-	+	-	a
	17 β -Estradiol	+++	+++	+++	+++	+	+	+++	+++	+++	+++	b
	Estrilol	+++	+++	+++	+++	-	+	-	+	+	-	b
	Estrone	+++	+++	+++	+++	-	-	-	-	+	+++	b
	Bisphenol A	-/-	+++	+	+	-	-	-	+	-	+	b

Addressing the issue of a risk assessment for managed aquifer recharge the table illustrates the importance of the redox condition for the elimination of the tested substances. Even naproxen, which showed a ready biodegradability, behaved recalcitrant under nitrate-reducing conditions. Similarly 17 β -estradiol showed an overall good removal except under Mn(IV)-reducing conditions. Other substances behaved even more specific and were only degraded under aerobic and nitrate-reducing (pentoxifylline and propranolol) and sometimes only under aerobic conditions (e.g. gemfibrozil, iohexol). Next to the redox conditions present in the recharged aquifer the composition of the microbial community is an important factor for the degradation of the trace organic chemicals. It depends on the redox condition but also on the water quality that the microorganisms are adapted to, basically a low or high organic content and the specific mixture of organic substances. If the biological community of the aquifer is not adapted to water containing a certain composition of micropollutants it will take up to several months to adapt. This was shown

in the batch experiments, running for over 2 ½ years. The degradation of the beta blockers for example increased from the first phase to the second phase in both the anoxic and aerobic assays. For atenolol such an increase was also detected under Fe(III)-reducing conditions. However the elimination rate of other substances like the ionic X-ray contrast agents and iopamidol did not increase. Furthermore due to their high persistency and the frequency with which they have been detected in the environment the recalcitrant substances clofibric acid and carbamazepine are rather unlikely to be degraded during underground storage, even after long time spans (Heberer 2002, Scheytt et al. 2004, Arye et al. 2011).

Another aspect which has to be considered in managed aquifer recharge is the hydraulic retention time of the recharged water, needed to reach considerable degradation rates. As an example Fig. 26 shows the degradation of 17 β -estradiol under the different redox conditions over time. The natural estrogen is completely degraded within 2 months under aerobic conditions which is followed by a complete degradation under sulfate- and nitrate-reducing conditions within another two to four months. Finally complete degradation is reached under Fe(III)-reducing conditions within 10 months, while under Mn(IV)-reducing conditions a removal of 20 μ g/L was measured within 8 months after an increased concentration within the first two months due to delayed dissolution.

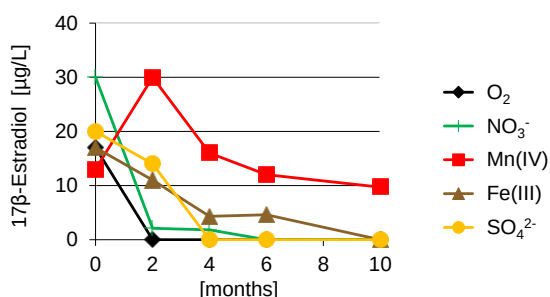


Fig. 26 Overview of 17 β -estradiol degradation under different redox conditions with 100 μ g/L start concentration

In case a substance is degraded under more than one redox potential, aerobic conditions often accounted for the fastest degradation as it is depicted in Fig. 27. Since no measurement was carried out in between the first and the second sampling after 2 months an even faster degradation for 17 β -estradiol and estriol cannot be ruled out. Other substances like the beta blockers atenolol, metoprolol and propranolol needed between six and ten months for a complete degradation. Finally pentoxifylline, gemfibrozil, sotalol and the x-ray contrast agents still exhibited final concentrations of 38%, 31%, 22%, 6%, 7%, 1%, respectively, after 10 months under aerobic conditions. For pentoxifylline, atenolol, propranolol and estrone, final concentrations under the respective anaerobic and anoxic conditions were 40%, 48%, 34% and 2%. It can be summarized that the estrogens and Bisphenol A as well as the analgesic naproxen are being completely eliminated the fastest, depending on the redox potential. Subsequently atenolol, metoprolol and propranolol show complete elimination under aerobic and anoxic conditions, respectively. At the same time considerable degradation has taken place for instance in case of pentoxifylline with 51 μ g/L and 67 μ g/L removal under aerobic and nitrate-reducing conditions.

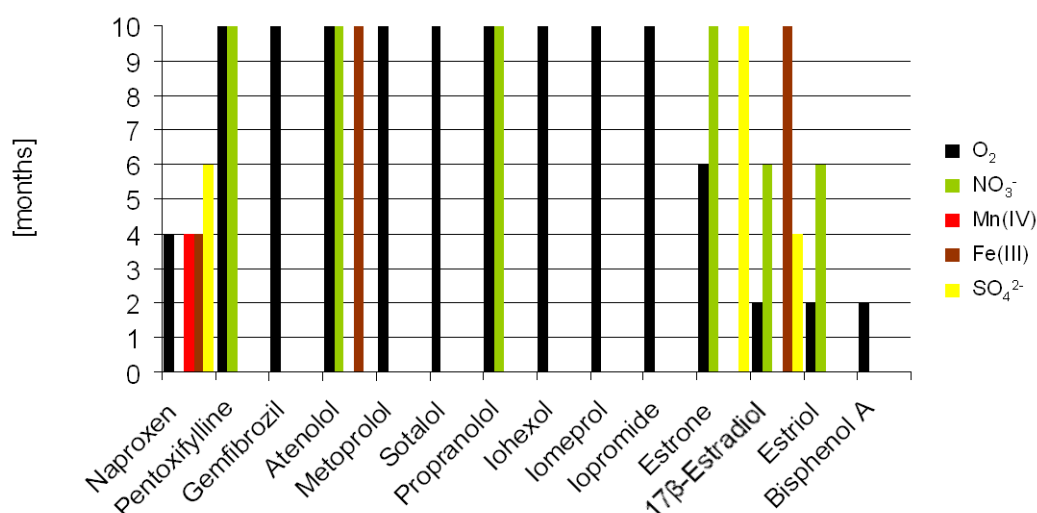


Fig. 27 Overview of the time spans for either total or partial (>50%) elimination of the emerging pollutants classified biodegradable with regard to the different redox conditions in the batch assays at 100 µg/L start concentration

Since pharmaceuticals in the environment are generally detected in the ng/L and low µg/L range, which is tenth the part of the experimental concentration, a complete degradation of environmental concentrations of these substances is to be expected of this adapted biomass. However, it needs to be considered that in general threshold values for substances that serve as carbon and energy source for microorganisms exist, covering a range of 2 µg/L up to 790 µg/L for substrates like glucose and starch or 2,4-dichlorophenol, benzoate and quinoline (Alexander 1999). It cannot be ruled out that a complete degradation of e.g. atenolol under aerobic conditions only occurred in the second phase of the experiment due to an increase in biomass, favored by a higher substrate concentration. In contrast to that it has been recently suggested that due to the low concentrations observed for these kinds of pollutants microorganisms adapted to oligotrophic environments are able to sustain growth within such low concentration ranges (Rauch-Williams et al. 2011, Egli 2010). Biologically available carbon compounds are usually in the range of 10 – 100 µg/L in groundwater. Thereby the simultaneous utilization of many different carbon/energy sources is thought to be the mechanism by which heterotrophic microorganisms maintain sufficient nutrient uptake and growth in oligotrophic environments (Egli 2010).

In conclusion a degradation of environmental concentrations of the substances classified under ‘b’ can be expected in an aquifer with an adapted biomass. The amount to which a substance is degraded and the hydraulic retention time needed for this decrease comply predominantly with the composition of the microbial community and its affinity towards the trace organic compounds. These are variables that have to be identified by either studying the respective aquifer in the field or taking soil and water samples for laboratory tests (e.g. batch assays or soil columns). The present experiments were conducted with water only to avoid sorption and changes of the redox conditions by the influence of sediments. Degradation in natural environments containing also sediment however is estimated to be even faster. Since soil passage and aquifer storage are subsequent processes, Tab. 7 combines the classification derived from the batch experiments with the ranking drawn from the column experiments.

Tab. 7 Classification of emerging pollutants by their biodegradation behavior during soil passage through the laboratory soil columns and in the batch studies with riverbank filtrate under aerobic and anaerobic conditions

		low Degradability high				
		Ia	Ila	Ilb	IIla	IIlb
Analgesics	Diclofenac		•			
	Ibuprofen				•	
	Naproxen			•		
	Pentoxifylline					•
	Phenazone		•			
Lipid-lowering agents	Bezafibrate		•			
	Gemfibrozil			•		
Antiepileptic	Carbamazepine	•				
Beta blockers	Atenolol					•
	Metoprolol			•		
	Propranolol					•
	Sotalol		•			
Hormones and Bisphenol A	Bisphenol A					•
	17 β -Estradiol					•
	Estriol					•
	Estrone					•
	17- α -Ethinylestradiol				•	
X-ray contrast media	Diatrizoic acid	•				
	Iohexol			•		
	Iomeprol			•		
	Iopamidol	•				
	Iopromide			•		
	Iothalamic acid	•				
	Ioxithalamic acid		•			

As already mentioned in chapter 4.1.1 on the elimination of emerging pollutants during soil column experiments the substances tested can be ranked into three different groups. Group I with the substances eliminated to less than 50%, group II with substances eliminated to more than 50% under certain conditions, and group III with substances that show a removal rate above 90% regardless of any conditions. Substances of group I mostly showed no removal at all and were not eliminated in the batch experiments. Therefore they are classified under Ia and consequently no class Ib exists. Bezafibrate is one example of the class IIa category. It is eliminated completely in the soil columns, but with a dependence on temperature and substance concentration. In the batch experiments however

its concentration remained unchanged. Naproxen on the other hand showed a good degradability in most of the batch assays and was degradable as well in the soil columns, depending on the factors temperature and time. It is therefore ranked in IIb. Ibuprofen however showed very good removal within the column tests irrespective of any influencing parameters but remained unchanged in the batch assays, which puts it into class IIIa. Bisphenol A and the natural estrogens belong to the substances ranked in class IIIb. Like ibuprofen they were always completely eliminated under any condition. Furthermore their behavior during the batch experiments indicated easy degradability under aerobic and anaerobic conditions, respectively.

Tab. 8 shows substances that could not be assessed during the batch experiments and therefore were grouped into one of the three main classes derived from the column experiments.

Tab. 8 Classification of the substances, not measured in the batch experiments

		I	II	III
Lipid-lowering agents	Etofibrate			•
	Clofibric acid	•		
	Fenofibrate			•
	Fenofibric acid		•	
Alkylphenols	4-iso-Nonylphenol			•
	4-tert-Octylphenol			•
Trialkylphosphates	Triethylphosphate		•	
	Trikresylphosphate (o-, m- u. p-Isomer)			•
	Tri-n-butylphosphate			•
	Triphenylphosphate			•
	Tris-(2-chloroethyl)-phosphate		•	
	Tris-(2-chloropropyl)-phosphate		•	
	Tris-(2-ethylhexyl)-phosphate			•

No elimination was found for clofibric acid, which is therefore listed under group I. The elimination of fenofibric acid and three of the trialkyl phosphates showed dependence on time, temperature or the substance concentration, which ranks them under group II. Except for clofibric acid the compounds listed in Tab. 8 show a ready degradability during the soil passage. However, on the basis of the experimental results substances like diatrizoic acid and carbamazepine are expected to be eliminated neither during soil passage nor during storage in the aquifer. However, conventional waste water treatment does not remove these substances sufficiently and consequently they will be contained in the treated waste water used for recharge. Thereby it needs to be considered that the composition of waste water can differ, depending on the pharmaceuticals prescribed in each country and not all substances mentioned here necessarily have to be in a certain WWTP effluent. This needs to be analyzed case by case. Still the possibility to find some of the substances listed under Ia is high, as the usage of carbamazepine and the X-ray contrast agents is widely spread (Kümmerer et al. 2004). Advanced waste water treatment as reverse osmosis,

nanofiltration or the application of powdered activated carbon has shown to eliminate these recalcitrant substances (Drewes et al. 2002, Ternes & Joss 2006). An easy and more economic step however would be to change the usage of pharmaceuticals and switch to substances of category II, by using e.g. iohexol instead of diatrizoic acid. These are substances of the class II categories. Together with the substances from the class III categories they pose less a risk to the groundwater due to their high degradability during soil passage. In fact the usage of fibrates, with clofibric acid being one of the active transformation products, has ceased over recent years in Europe while its prescription in the US was always of minor importance (Sedlak et al. 2004). Due to reports of either low or contradictory effects of fibrates on high blood lipid levels, statins have been prescribed as alternative drugs. The main advantage of a statin over a fibrate is the effective dose of mg compared to g, respectively (Mutschler 2008). However the high stability of clofibric acid in nature which was confirmed by the current experiments shows the urgency for action in this field.

Furthermore the boundary conditions during soil passage have to be known (e.g. temperature, retention time, redox conditions). Since the column experiments were run under unsaturated conditions and no distinct nitrate reduction occurred it can be deduced that aerobic conditions prevailed along the 1.10 m passage through the columns. Therefore the results of the degradability of the emerging pollutants tested in the columns have to be estimated based on aerobic conditions. If others than aerobic conditions predominate the soil passage, the outcome for the elimination rate of the emerging pollutants might be different.

It can be concluded that the adoption of a tentative classification is a first step towards a risk assessment of emerging pollutants in terms of groundwater recharge with treated waste water. Thereby the substances were ranked into three main classes subdivided into two categories, according to their behavior in soil column and batch experiments. The classification can support field experiments by giving a first hint as to what to expect from the investigations, e.g. the elimination of these compounds during soil passage and the subsequent storage in the underground. However differing hydrodynamics and biochemical boundary conditions of aquifers may result in different outcomes with respect to the elimination behavior of the trace organics. Another question is the fate of eventual metabolites, which might occur during degradation processes. Laboratory tests have been run already in this direction and results are being evaluated. Next to the fate of emerging pollutants future investigations will concentrate on the elimination capacity of the soil passage in terms of microbiological parameters, where already promising data could be gained so far. Further on it will be important to obtain field data on the fate of emerging pollutants, pathogens and indicator organisms during MAR in order to reassess the classification derived from the laboratory results.

8. Publications

Parts of the results presented in this report have been already published in the following papers and conference proceedings.

Journal papers

- Schmidt, N., Lipp, P., Paris, S., Tiehm, A., (2011). Sustainable Use of Water Resources - emerging pollutants and viruses in the Jordan Valley. Bluefacts 38-43.
- Tiehm, A.; Schmidt, N., Lipp, P., Zawadsky, C., Marei, A., Seder, N., Ghanem, M., Paris, S., Zemmann, M., Wolf, L., (2012). Consideration of emerging pollutants in groundwater based reuse concepts. Water Science & Technology 66 (6): 1270 – 1276.
- Tiehm, A., Schmidt, N., Stieber, M., Sacher, F., Wolf, L., Hoetzel, H., (2011). Biodegradation of pharmaceutical compounds and their occurrence in the Jordan Valley. Water Resources Management 25 (4), 1195-1203.

Conference proceedings

- Schmidt, N., Tiehm, A., (2012). Classification of Emerging Pollutants for Managed Aquifer Recharge in the Lower Jordan Valley. Accepted for presentation at the IWRM conference (Integrated Water Resources Management) in Karlsruhe, 21. – 22.11.2012.
- Zawadsky, C., Schmidt, N., Tiehm, A., (2011). Detection of viruses in the Jordan valley and elimination in laboratory soil columns. In: Biospektrum, VAAM-Jahrestagung, Karlsruhe 3.-6. April 2011: 104.
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