Behavior of selected organic micropollutants in horizontal subsurface-flow constructed wetlands operating at high organic load

Dissertation

zur Erlangung des

Doktorgrades der Ingenieurwissenschaften (Dr.-Ing.),

des

Zentrums für

Ingenieurwissenschaften

der Martin-Luther-Universität

Halle-Wittenberg,

vorgelegt

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geb. am 30. März 1982 in Mexiko

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Tag der Verteidigung: 16.02.2015

Declaration of Academic Honesty

I declare that I have carried out this PhD research project on my own, having used only the listed resources and tools. This thesis was done at the Helmholtz Center for Environmental Research-UFZ-Leipzig. Furthermore, I declare that I did not apply for a doctor degree in any other place.

M.Sc. Otoniel Carranza Díaz Leipzig, 29.10.2014

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M.Sc. Otoniel Carranza Díaz Leipzig, 29.10.2014

Dedidated to Dr. Peter Kuschk (in memoriam)

We have a big responsibility for the new generations.

L’viv, Ukraine, October 2013

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Abstract

In the last decades, Horizontal Subsurface-Flow Constructed Wetlands (HSSFCWs) have been used for the treatment of municipal wastewater and firstly in 2005, the removal of organic micropollutants (OMPs) contained in municipal wastewater was reported. In some cases, the operation of HSSFCWs is suboptimal due to the overdose of wastewater above the recommendation of 6 g BOD_5 m^{-2} d^{-1}. The condition of high organic load and its influence on removal of OMPs has not been studied in HSSFCWs research although several systems operate worldwide under such undesired modus. Thus, the objective of this thesis was to study the removal behavior of 11 selected OMPs occurring in real municipal wastewater in a planted and an unplanted HSSFCWs operating under high organic load. The research was focused on (i) elucidation of the role of plants on the mass removal and (ii) understanding the removal behavior of the OMPs at different sampling locations along the flow path of the HSSFCWs. The OMPs were selected on the basis of their environmental relevance, high consumption and frequently detection in the aquatic environment. The investigations were conducted over a twelve month period. Conventional wastewater parameters were also considered to elucidate the potential removal mechanisms of the OMPs within the HSSFCWs. Analytical methods for the detection of the OMPs were set by means of Solid Phase Extraction, derivatization and Gas Chromatography-Mass Spectrometry for detection and quantification of the selected OMPs.

The studied OMPs occurred in the real municipal wastewater in the range of 1-100 µg L^{-1}. Load-based data predicted OMPs treatment efficiencies more accurately than concentration data. The planted HSSFCW performed better than the unplanted in terms of mass removals for all OMPs except of caffeine which was similarly removed in both HSSFCWs (average of the 20 sampling events). The stimulant caffeine was the investigated OMP which showed the highest removal with an average efficiency of 66%. The average removal efficiency of the other OMPs was below 30%. In addition, temporal dependence of the removal efficiency of the OMPs was observed. OMPs were better removed during the warm months than during the cold ones. Negative removals were observed in both HSSFCWs (planted and unplanted). Carbamazepine showed the same percentage of negative removal in the planted and in the unplanted HSSFCW whereas higher number of negative removals was found for polar compounds than for the neutral ones in the planted HSSFCW. Moreover, in the unplanted HSSFCW higher number of negative

removals was found for the EDCs and the lipophilic compounds oppose to the polar compounds. The origin of this phenomenon remains obscure, but from the results of this investigation it was hypothesized that the high organic load condition of the HSSFCWs and the inflow variation of the studied OMPs were involved in the occurrence of this phenomenon.

The behavior of the OMPs along the flow path of the HSSFCWs indicated an increase of some of the OMPs (Bisphenol A and 4-nonylphenols). In contrast, caffeine showed exponential concentration decrease. The anti-inflammatory drugs ibuprofen, naproxen, ketoprofen and diclofenac fluctuated along the flow path and showed significant concentration reductions with respect to the inflow concentrations at the internal sampling locations of 0.5 and 0.75 fractional distance from the inlet (sampling depth of 0.13 m). The redox indicator sulfide (S^{2-}) and the total nitrogen (TN) were investigated at the CW system scale (macrogradients in flow direction and depth). Sulfide showed a positive correlation with the removal behavior of naproxen and diclofenac at the sampling location 0.75 fractional distance from the inlet (sampling depth of 0.13 m). This finding suggested that the removal of this two anti-inflammatory compounds was associated with the dynamics of sulfate-reducing bacteria probably present in the planted HSSFCW. Contrary, in the unplanted HSSFCW no correlations between sulfide, total nitrogen and the OMPs were found. The partially aerobic conditions measured in the unplanted bed suggested that aerobic conditions were involved in the removal of naproxen and diclofenac probably regulated by the sulfide concentrations in the pore water. From these observations, sulfide was proposed as an indicator of the removal behavior of naproxen and diclofenac. The results of this thesis are relevant for those HSSFCWs operating above the recommended areal-loading rate of 6 g BOD_5 m^{-2} d^{-1}. This investigation highlights the potential of CWs to be used for the removal of OMPs in municipal wastewater although further optimization or testing of more engineered variants can be helpful to improve their potential to remove organic, bioactive trace contaminants.

Zusammenfassung

Seit Jahrzehnten werden horizontal durchströmte bepflanzte Bodenfilter [Horizontal Subsurface-Flow Constructed Wetlands (HSSFCWs)] zur Behandlung von kommunalem Abwasser eingesetzt. Dabei wurde zum ersten Mal im Jahr 2005 über die Entfernung von organischen Mikroverunreinigungen [organic micropollutants (OMPs)] berichtet. In einigen Fällen werden HSSFCWs unter suboptimalen Bedingungen einer Überfrachtung mit Abwasser oberhalb der empfohlenen Flächenbelastung von 6 g BSB_5 m^{-2} d^{-1} betrieben. Obwohl eine Vielzahl von Systemen weltweit unter diesen unerwünschten Bedingungen arbeiten, gab es bisher noch keine Forschung mit dem Ziel, in HSSFCWs die Entfernung von OMPs unter Bedingungen einer hohen organischen Fracht zu untersuchen.

Aus diesem Grund war es das Ziel der vorliegenden Arbeit, die Entfernung von 11 abwassertypischen OMPs (endokrin aktive Stoffe-endocrine disrupting compounds ECDs, Arzneimittelreststoffe, Duftstoffe), in einem bepflanzten sowie einem unbepflanzten horizontal durchströmten Bodenfilter unter den Bedingungen hoher organischer Abwasserfracht zu untersuchen. Die Forschungstätigkeit konzentrierte sich darauf, (i) die Rolle der Pflanzen in Hinsicht auf die Eliminierung der OMPs aufzuklären und (ii) die Entfernung der OMPs an verschiedenen Probenahmepunkten entlang der Fließrichtung der HSSFCWs zu verstehen. Die OMPs wurden auf Grundlage ihrer Umweltrelevanz, ihres hohen Verbrauchs und ihres häufigen Nachweises in der aquatischen Umwelt ausgewählt.

Die Untersuchungen wurden über einen Zeitraum von zwölf Monaten durchgeführt. Um mögliche Entfernungsmechanismen der OMPs in HSSFCWs aufzuklären, wurden ebenfalls konventionelle Abwasserparameter berücksichtigt. Für den Nachweis und die Quantifizierung der ausgewählten OMPs wurden die analytischen Methoden der Festphasenextraktion und Gaschromatographie-Massenspektrometrie genutzt. Die Ergebnisse zeigen, dass die untersuchten OMPs in Konzentrationen im Bereich von 1-100 µg L^{-1} im Abwasser auftraten. Die Ermittlung der Effizienz der Entfernung der OMPs war unter Einbeziehung frachtbasierter Daten akkurater als anhand von Konzentrationsdaten. Der bepflanzte Bodenfilter war der unbepflanzten Anlage in Hinblick auf die Eliminierung der ausgewählten OMPs überlegen. Für Koffein wurde in beiden Anlagen zu gleichem Maßen die höchsten Entfernungsraten mit durchschnittlich 66%

ermittelt. Die durchschnittliche Eliminierungseffizienz der anderen OMPs lag unterhalb von 30%. Zusätzlich wurde eine jahreszeitliche Abhängigkeit der Effizienz beobachtet: Die OMPs wurden während der warmen Monate besser entfernt als während der kalten Jahreszeit.

Negative Eliminierungsraten wurden in der bepflanzten sowie der unbepflanzten Anlage beobachtet. Für Carbamazepin wurden ähnliche negative Eliminierungsraten in beiden Anlagen ermittelt. In der bepflanzten Anlage wurde eine größere Anzahl negativer Eliminierungsraten für polare als für neutrale Verbindungen gefunden. Im Gegensatz zu den polaren Verbindungen, wurden für EDCs and andere lipophile Verbindungen höhere Eliminierungsraten in der unbepflanzten HSSFCW beobachtet. Während der Ursprung dieses Phänomens noch nicht geklärt ist, konnte aufgrund der Ergebnisse dieser Forschungsarbeit die Hypothese aufgestellt werden, dass die Bedingungen der hohen organischen Belastung in den HSSFCWs und die Variation der Zusammensetzung der OMPs im Rohabwasser am Auftreten dieses Phänomens beteiligt sind. Im Hinblick auf das interne Verhalten der OMPs, stand einer Zunahme von einigen der OMPs (Bisphenol A und 4-Nonylphenol) eine exponentielle Konzentrationsabnahme des Koffeins entlang des Strömungsweges der HSSFCWs gegenüber. Die Konzentrationen der entzündungshemmenden Arzneimittel Ibuprofen, Naproxen, Ketoprofen und Diclofenac schwankten entlang des Strömungsweges und zeigten signifikante Konzentrationsabnahmen gegenüber den Zulaufkonzentrationen an den inneren Probenentnahmestellen (0.5 und 0.75 des fraktionierten Abstands vom Einlass, Probentiefe von 0,13 m).

Der Redoxindikator Sulfid (S^{2-}) und der Gesamtstickstoff (TN) wurden in Strömungsrichtung und in verschiedenen Tiefen untersucht. Sulfid zeigte eine positive Korrelation mit dem Entfernungsverhalten von Naproxen und Diclofenac bei der Messstelle 0,75 des fraktionierten Abstand vom Einlass (Probentiefe von 0,13 m). Dieser Befund zeigt, dass die Entfernung dieser zwei pharmazeutischen Verbindungen mit der Dynamik der wahrscheinlich im bepflanzten HSSFCW vorkommenden sulfatreduzierenden Bakterien zusammenhängt. Im Gegensatz dazu wurde in der unbepflanzten HSSFCW keine Korrelationen zwischen Sulfid, dem Gesamtstickstoff und den Eliminierungraten der OMPs gefunden. Die teilweise aeroben Bedingungen, die im unbepflanzten Filterbett gemessen wurden, suggerieren, dass aerobe Bedingungen einen Einfluss auf die Entfernung von Naproxen und Diclofenac haben, wahrscheinlich reguliert durch die Sulfidkonzentrationen im Porenwasser. Auf Grundlage dieser

Beobachtungen wurde Sulfid als ein Indikator für das Entfernungsverhalten von Naproxen und Diclofenac vorgeschlagen. Die Ergebnisse dieser Arbeit sind für solche HSSFCWs relevant, die über der empfohlenen Flächenbelastung in Höhe von 6 g BSB_5 m^{-2} d^{-1} betrieben werden. Diese Untersuchung unterstreicht das Potenzial von Bodenfiltern zur Entfernung von OMPs im kommunalen Abwasser. Jedoch wäre eine weitere Optimierung und Prüfung neu entwickelter Varianten empfehlenswert, um die Leistungsfähigkeit naturnaher und technisch kostengünstiger, dezentraler Abwasserbehandlung hinsichtlich der Entfernung von organischen, biologisch aktiven Spurenverunreinigungen zu verbessern.

Chapter 1

Introduction

1.1 Sources of organic micropollutants in the aquatic environment

The annual production of organic chemicals increased over the last years (Schwarzenbach et al., 2006). Thousands of chemicals are produced worldwide in order to satisfy industrial and human needs. The advances in analytical chemistry during the last decades have permitted the detection of even trace amounts of organic and inorganic chemicals in different environmental compartments, including air, water and soil (Luo et al., 2014). In the aquatic environment, these traces currently called as micropollutants have been found in the ranges of ng L^{-1} and µg L^{-1} (Bell et al., 2011). The organic micropollutants (OMPs) cover several groups of substances for instance pharmaceuticals, additives in personal care products, plasticizers and agrochemicals like pesticides and herbicides (Ternes and Joss, 2006; Osenbrück et al., 2007; Calderón-Preciado et al., 2011a).

Sewage, and effluents from wastewater treatment plants (WWTPs) are recognized as a main source for OMPs entering the aquatic environment (Luo et al., 2014). Due to their chemical nature and environmental conditions they are diluted, can be persistent or transformed and spread finally in the environmental compartments. Persistent OMPs can reach groundwater and even drinking water when river bank filtrates or contaminated groundwater is used for drinking water production (Bradley et al., 2014).

The biosludge produced by WWTPs contains a wide range of lipophilic and semi polar OMPs. In fact, sewage sludge applied to land has the potential to leach OMPs into soil and groundwater or to be taken up by plants and animals (Carballa et al., 2007). For instace, the uptake of OMPs by plants as well as their occurrence in groundwater has been documented (Osenbrück et al., 2007; Calderón-Preciado et al., 2011a; Macherius et al., 2012). Although the short term risk for humans due to the exposure to this traces seem to be low (Gröning et al., 2007), OMPs have been found in human blood and urine samples (Trejo-Acevedo et al., 2009; Orta-García et al., 2014) confirming to enter the human food chain. To date, the long term effects of mixtures of organic trace pollutants on human health remain unknown. Moreover, the investigation of the fate of OMPs in the complex environment is a challenge as well as the identification of their biological effects on organisms (Cleuvers, 2003).

The occurrence and environmental impact of OMPs has increased during the last years (Schwarzenbach et al., 2006, Kümmerer, 2009; Fromme et al., 2014). While in the early 70´s, pesticides were discovered to be an environmental issue, nowadays complex structures such as flame retardants, pharmaceuticals and surfactant metabolites are found in several environmental compartments including plants (Goldstein et al., 2014), air dust (Fromme et al., 2014) and wastewater (Carranza-Diaz et al., 2014).

Compounds have been priorized as potentially harmful based on their suspected negative biological effects on organisms and their occurrence and persistency in the environment. Thus, the production and marketing of some chemicals has been restricted in different countries. For instance, the European Union (EU) has established a list of 33 priority substances (see Table A1) included in the Water Framework Directive (Annex II of Directive 2008/105/EC) for achieving good water quality standards. Recently, on Tuesday 2th July 2013, 15 more substances were added to the 33 priority pollutant list (http://europa.eu/rapid/press-release_IP-12-88_en.htm). These new substances are, Aclonifen, Bifenox, Cypermethrin, Dicofol, Heptachlor and Quinoxyfen (used for crop and plant protection); Cybutryne, Dichlorvos, Terbutryn (used in biocidal products); Perfluorooctane sulfonic acid (PFOS), Hexabromocyclododecane (HBCDD) (industrial chemicals); Dioxin and Dioxin-Like PCBs (combustion by-products) and for the first time three phramaceutical substances were added in the first watch list. They are, Diclofenac, 17-beta-estradiol (E2) and 17-alpha-ethinylestradiol (EE2) (EU, 2013). The member states are committed to monitor these substances regularly and to force their reduction from the environment. Similarly, the US EPA has catalogued 129 substances as priority pollutants (US EPA, 40 CFR 423).

1.1.1 Pharmaceuticals and personal care products (PPCPs)

Pharmaceuticals summarize a group of biological active chemicals used to improve human health against diseases. Moreover, pharmaceuticals are used in veterinary medicine to maximize growth of livestock (Ternes and Joss, 2006). Additives in personal care products such as biocides, fragrances, plasticizers, antioxidants or UV-filter compounds are present in products of daily use such as cosmetics, tooth paste, washing lotion, among others (Ternes et al., 2004). Many of these compounds are suspected for endocrine disrupting effects on living organisms and support the formation of bacteria resistances (Schwarzenbach et al., 2006). The sources of PPCPs in the environment are wastewater from households, the runoff from agricultural fields, industrial and hospital effluents (Bell et al., 2011; Kovalova et al., 2012; Verlicchi and Zambello, 2014). Conventional wastewater treatment plants (WWTPs) are generally designed to reduce carbon, ammonia and phosphorous load from wastewater but not OMPs like PPCPs (Margot et al., 2013). Thus, PPCPs are less removed in conventional WWTPs or even not removed at all ending finally in the environment (Joss et al., 2005; Bernhard et al., 2006; Suárez et al., 2008).

During the last decade, the production and consumption of PPCPs increased worldwide. In Germany alone, 408 tons analgesics, 278 tons antiphlogistics, 125 tons beta-blockers and 81 tons of antiepileptic drugs were consumed in 2005, among additional other drugs with special biological actions such as antibiotics or cancerogenic chemicals (Adam, 2010). The sold rates of PPCPs depends on the demands of social and demographic changes as well as and new trends in consumers lifestyle and environmental regulations (Kümmerer, 2009; EU, 2013).

Among PPCPs, the concentrations of pharmaceuticals in the environment are generally higher than those released from personal care products. This is attributed to the consumption of pharmaceuticals at high daily doses (mg/pill) and the fact that some pharmaceuticals are sold in the market without medical prescription (Ternes and Joss, 2006). Due to the biological-directed mode of action of pharmaceuticals, aquatic organisms are expected to be exposed to the active fraction of pharmaceuticals (Fent et al., 2006; Nakada et al., 2007). Moreover, transformation products and metabolites formed during biotic and abiotic transformation processes of PPCPs are increasingly recognized as a potential risk to aquatic organisms but their toxicological properties are not largely investigated (Lienert et al., 2007; Kümmerer, 2009; Veldhoen et al., 2014). After the consumption of pharmaceuticals, a certain amount is excreted unchanged from the human or animal organism. Additionally, after the intake, the active fraction of the compound is converted into metabolites which are released from the body through skin (sweat), urine (the renal system) or faeces (biliary system) (Jjemba, 2006; Stülten et al., 2008). The wastewater containing those compounds reaches the aquatic environment containing both the active compound and their metabolites (Metcalfe et al., 2010; Jelic et al., 2011). Furthermore, some conjugated forms of parent compound can be converted back to the parent form (Stadler et al., 2012) by hydrolysis or microbial activity which are typical processes occurring in raw wastewater and during the treatment in WWTPs (Vieno et al., 2007; Kovalova et al., 2012). Thus, the balance between the amount of active fraction and their metabolites in the environment is hardly to predict.

The fate of the OMPs in the aquatic environment depends on their physicochemical properties as well as on the efficiency of the wastewater treatment conditions (Luo et al., 2014). Thus, the characteristics of each compound such as its polarity and sorption affinity (expressed as partition coefficients e.g. the octanol-water-partition coefficient or Log K_{ow} value), acidic or basic properties (pK_a), water solubility, chemical structure (e.g. reactive functional groups) and volatility (Henry coefficient) determine their partition in different environmental compartments as well as their potential to be degraded/transformed by microorganisms (Tixier et al., 2003; Cirja et al., 2008; Ergüder and Demirer, 2010). For instance, polar PPCPs with high water solubility will preferentially be removed from wastewater by microbial processes whereas semi-polar and lipophilic compounds will be sorbed onto carbon reach surfaces such as sludge, soil or plant roots (Dordio and Carvalho, 2010; Luo et al., 2014).

For some of the PPCPs, negative effects on organisms have been proved in laboratory experiments but also in real ecosystems (Vethaak et al., 2005; Veldhoen et al., 2014). For instance, the polycyclic musk compounds, galaxolide® and tonalide®, widely used as fragrance in cosmetic and household products and suspected for endocrine disrupting effects on aquatic organisms (Chase et al., 2012) have been shown to inhibit efflux transporters in gills of the marine mussel *Mytilus californianus* (Luckenbach et al., 2005). Another example is triclosan, one of the most produced antibacterial agents in the world (Kim et al., 2011) which was found to cause an increase of bacterial mortality at environmentally relevant concentrations 0.21 $\mu g\ L^{-1}$ (Ricart et al., 2010) and to force the formation of resistant bacteria (Halden, 2014).

The among top five most produced pharmaceuticals in Europe, ibuprofen, carbamazepine, diclofenac, naproxen, ketoprofen, and caffeine belong to the most found pharmaceuticals in municipal wastewater (Ternes and Joss, 2006; Luo et al., 2014). Monitoring of these compounds is essential to understand their impact and to protect ecosystems and natural resources. Considering their widespread occurrence in environment, their potential negative effects to organisms even at low concentrations, a long term exposure becomes probable.

A prediction methodology was developed by Adler et al., (2006) to estimate the maximum possible concentrations of pharmaceuticals in the wastewater based on their consumption. The mathematical expression is as follows:

$$\mathrm{PEC}_{\mathrm{STPin}} = \frac{\mathrm{F}_{\mathrm{API}} \times 10^{12} \times \mathrm{E}}{365 \times \mathrm{Pop} \times \mathrm{AWW}}$$

where, $\mathrm{PEC}_{\mathrm{STPin}}$ is the predicted concentration in the raw sewage (ng L^{-1}). $\mathrm{F}_{\mathrm{API}}$ is the consumption of active pharmaceutical ingredient per year in the “catchment” area of influence (kg a^{-1}). E is the fraction excreted without metabolization in urine and faeces. Pop is the population within the area (cap). AWW is the amount of wastewater per capita and day (200 to 400 L cap^{-1} d^{-1}). However, any effort for calculating PPCPs in wastewater should consider the local climatic conditions and the seasonal changes as they vary from site to site. In addition, economic issues such as the access to medicaments and cultural habitudes (i.e. self-medication) in the different societies need to be considered too (Leyva-Flores et al., 2001; Santos et al., 2008). These factors are not considered in the equation of Adler et al., (2006) but may greatly change the predicted amount of PPCPs in the raw sewage.

Ecological consequences related from the traces of PPCPs in environment are still under discussion. Due to the limited data on chronic biological effects of these compounds, regulations defining the maximum residue levels in water are missed as usually found in food quality control.

1.1.2 Endocrine disrupting chemicals (EDCs)

Endocrine disrupting compounds (EDCs) are defined as “exogenous substances or mixtures that alter the function(s) of the endocrine system and consequently cause adverse health effects in an intact organism or its progeny or (sub) populations” (Frische et al., 2013). Products of daily use such as fragrances, soups, plastic products, epoxy resins, among others have been identified as their sources (Belmont et al., 2006; Baker et al., 2009; Chase et al., 2012).

Some EDCs used as biocides, pesticides, plasticizers and detergents have been banned from production and use such as DDT (EPA, 1975). Other chemicals such as the 4-nonylphenols were reduced in their production voluntarily due to their proved estrogenic effects to organisms (RPA, 1999). Nowadays, the EU Water Framework Directive included 4-nonylphenols and diclofenac in the list of priority pollutants and further compounds such as bisphenol A are still under discussion to be considered as ecotoxic and risk for human health (Vethaak et al., 2005; WHO, 2012). Among the negative effects of these EDCs, feminization of fish species and interruption of reproduction cycles of amphibians have been observed (Tetreault et al., 2011; Jia et al., 2014). Moreover, decline in population male fertility, thyroid-related disorders and interfering of the pubertal timing and menopause are suspected to be related with human contact to environmental EDCs (WHO, 2012; Hotaling and Patel, 2014).

EDCs enter into the environment by discharges of WWTPs, agricultural application, and diffuse input from runoff and leachates (e.g. from deposits) (Liu et al., 2009). Additionally, natural and synthetic hormones are introduced in environment along with sewage effluents (Cai et al., 2012). While biodegradation of natural hormones such as the estrogens estrone or 17β-estradiol are removed with high efficiency in WWTPs, synthetic hormones such as ethynylestradiol are more persistent and pose higher estrogenic potency than the natural ones (Liu et al., 2009). In the aquatic environment EDCs have been found in concentrations ranging between ng L^{-1} to µg L^{-1} (Osenbrück et al., 2007; Calderon-Preciado et al., 2011b; Luo et al., 2014).

Bisphenol A, applied in polycarbonate plastic and epoxy resins and 4-nonylphenols are well known as EDCs with estrogen-like effects (Staples et al., 1998; Klecka et al., 2010). They occur in environment due to their large scale production and use over years (Fromme et al., 2002; Belmont et al., 2006). Nonylphenols (NPs) have been used for the production of alkylphenol ethoxylate detergents and other chemical products. In addition, NPs can be also generated from these ethoxylates during wastewater treatment (Stasinakis and Gatidou, 2010). Although, non-ionic surfactants are well eliminated by conventional biological wastewater treatment (Klecka et al., 2010), the formation of treatment-resistant metabolites out of the parent surfactants remains a problem of WWTPs. It has been estimated by Petrović et al., (2003) that between 60-65% of all nonylphenolic compounds entering into WWTPs are discharged into the aquatic environment.

Bisphenol A is better removed under aerobic than anaerobic conditions (Flint et al., 2012). Removals of bisphenol A in conventional WWTPs are reported up to 75% (Mohapatra et al., 2011). However, the relative high concentrations (in the range of $\mu g\ L^{-1}$) of bisphenol A found in surface and groundwater (Latorre et al., 2003; Osenbrück et al., 2007) reveal an enormous production and use of this chemical. So, the threat of exposure to humans and aquatic organisms is highly probable. Currently, traces of bisphenol A have been determined in human blood and urine samples (WHO, 2012; Liao and Kannan, 2012).

The Figure 1 summarizes the main sources of OMPs entering in the aquatic environment. As observed, the reduction of the OMPs in the environment can be realized by decreasing the absolute inputs. For instance, reducing production and use of chemicals or the replacement of hazardeous chemicals by less toxic substances will reduce the risks for living organisms. Both strategies are in discussion, either banning substances from production or replace them by other less risky. A good example is the case of the phthalates known as endocrine disrupting compounds. These plasticizers are increasingly being substituted by trimellitates or adipates, particularly in medical applications (Bernard et al., 2014). Even though, the mode of action of the several OMPs has been investigated during the last decades, still the understanding of long term effects on living organisms exposed to these substances needs further research (WHO, 2012). Figure 1 shows different sources of entering OMPs in the aquatic environment. They include untreated wastewater, treated wastewater, biosolid applications and industrial discharges.

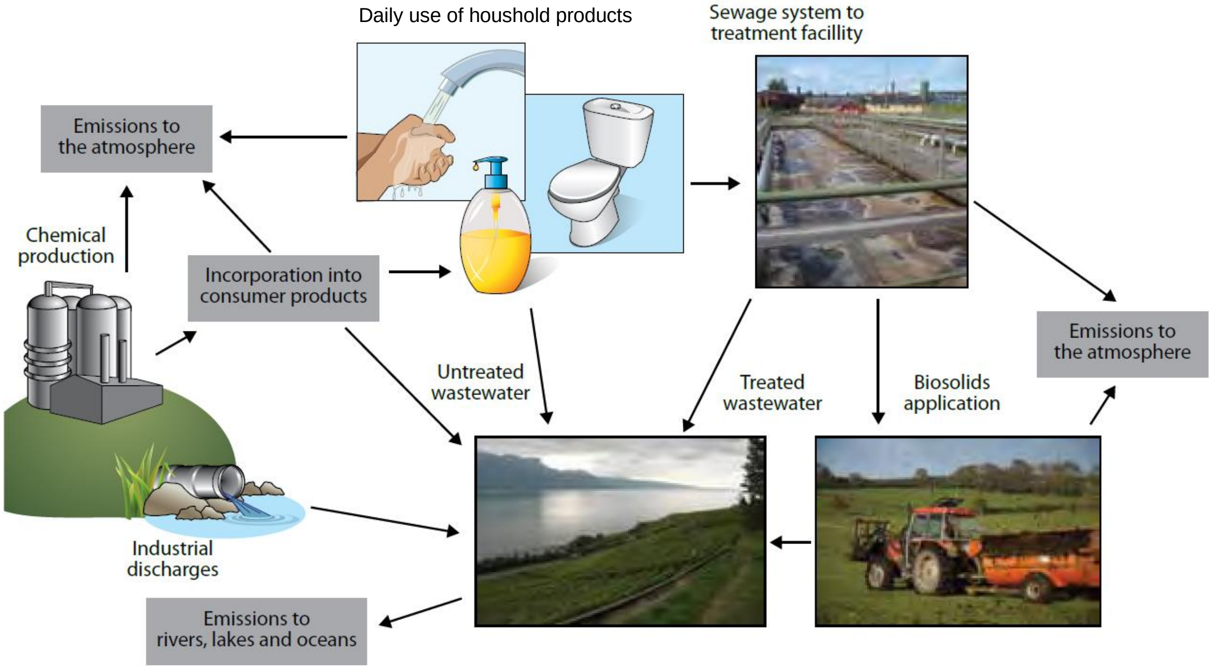


Figure 1 Environmental releases of OMPs from the manufacturing stage to the environment (The figure was modified from WHO, (2012) figure 3.4).

1.2 Ecotoxicological effects of organic micropollutants in the aquatic environment

Ecotoxicological investigations deal with understanding and prediction of the effects of chemicals on natural communities under realistic exposure conditions (Chapman, 2002). Because, OMPs like pharmaceuticals are designed to pose biological effects on humans and animals, negative effects of some of these traces on aquatic organisms are expected to occur (Fent et al., 2006; Nakada et al., 2007). The state of the art in ecotoxicology still reveals a lack of information regarding identification, assessment, management and predicting effects of OMPs in living organisms (Frische et al., 2013; Kühnel and Nickel, 2014). Regarding acute toxic effects on aquatic organisms, some recognized ecotoxicological effects on aquatic organisms include: bacteria resistance to antibiotics (Sidrach-Cardona et al., 2014), declining of natural population due to the exposure to micropollutants (Esteban et al., 2013), and early life stage endocrine disrupting effects in fishes (Duffy et al., 2014), feminization and other reproduction and developmental disturbances (Flint et al., 2012).

In ecotoxicology, “target” organisms from different trophic levels, taxonomic groups and life forms are used to test the toxicity of chemicals (Vernouillet et al., 2010). The selection of the “target” organisms requires in some cases the approval of ethical committees for animal welfare prior investigation. In practice, the most used organisms in ecotoxicology are: fish (e.g. Atlantic salmon or Zebrafish embryos), crustaceans (e.g. *Daphnia magna*, *Hyalella azteca*, *Thamnocephalus platyurus)*, algae (e.g. *Raphidocelis subcapitata*, *Dunaliella tertiolecta*), and bacteria (e.g. *Vibrio fischeri* commercially known as Microtox® assay) (Hallare et al., 2004; Heckmann et al., 2007; Dussault et al., 2008; DeLorenzo et al., 2008; Vernouillet et al., 2010; Tang et al., 2013; Duffy et al., 2014). Apart from selecting the right test organism, the time of exposure of the chemicals to the target organisms is an important issue to distinguish between effects caused by acute or chronic exposure (Quinn et al., 2008). While in the environment, acute toxicity of OMPs is hardly probable to occur due to the low concentrations of the compounds, chronic toxicity may be of higher importance for risk assessment. Thus, long term exposure of chemicals occurs at low concentrations (Fent et al., 2006).

Presently, strategies have been developed to control the influence of chemicals to the environment and several regulations must be fulfilled before chemicals enter to the market. For instance, the European Union (EU) has created the “REACH” program (Registration, Evaluation, Authorization and Restriction of Chemicals) to control and to estimate the risk of a chemical in the environment (Regulation (EC) No 1907/2006). In Europe, new chemicals (e.g. pharmaceuticals, pesticides, etc.) have to be registered in the REACH program before they are placed on the market.

1.2.1 Biological effects of selected PPCPs

Biological effects on living organisms due to exposition of PPCPs have been reported in literature. The undergoing metamorphosis and the biological status of the Bullfrog (*Rana catesbeiana*) tadpoles was observed to be affected by exposition of ibuprofen at concentration relevant to municipal wastewater effluents (15 μg L^{-1}) (Veldhoen et al., 2014). Furthermore, the decay of vulture population and other birds like Gyps vultures were attributed to renal failure associated to diclofenac uptaken with food (Oaks et al., 2004; Fent et al., 2006). Ferrari et al., (2003) investigated in laboratory experiments the acute and chronic toxicity of carbamazepine, clofibric acid and diclofenac. The authors found acute toxicity of diclofenac higher than carbamazepine and clofibric acid for ceriodaphnid and daphnid tests. However, the opposite trend was found for chronic toxicity where carbamazepine displayed higher toxicity than clofibric acid and diclofenac.

In humans, metabolites formed from diclofenac included 4´-hydroxy (OH), 3´-OH and 5-OH metabolites (Dorado et al., 2003). These metabolites have been found to be more toxic to the bacteria *Vibrio fischeri* (Microtox®) than diclofenac as parent compound (Yu et al., 2013). Toxicity tests of diclofenac indicated effect concentrations are fare from environmental concentrations: For instance, EC_{50} of 5.3 mg L^{-1} (*Danio rerio*, time of exposure of 72 h) (Van den Brandhof and Montforts, 2010), EC_{50} of 11.4 mg L^{-1} (Microtox®, time of exposure of 0.5 h) (Ferrari et al., 2003) and EC_{50} of 39.9 mg L^{-1} and 44.7 mg L^{-1} (*Daphnia magna,* time of exposure of 48 h) (Haap et al., 2008). However, in the aquatic environment, great numbers of bioactive compounds with similar modes of action are present and may act in unknown manner to organisms.

Other compound with environmental relevance is the bactericide triclosan which is associated with several environmental and health issues (Halden, 2014). Toxic effects of triclosan in the environment includes morbidity of biofilm bacteria communities (Ricart et al., 2010), algae and invertebrates (Dussault et al., 2008) and bacteria *Vibrio fischeri* (Microtox®) (Farré et al., 2008). In addition, metabolites of triclosan have been found to be more toxic than the parent compound (Kim et al., 2011). Toxicity of triclosan is reflected by EC_{50} of 390 µg L^{-1} (*Daphnia magna,* time of exposure of 48 h) (Orvos et al., 2002), EC_{50} of 200 µg L^{-1} (*Hyalella azteca,* time of exposure of 240 h) (Dussault et al., 2008) and EC_{50} of 3.55 µg/L (*Dunaliella tertiolecta*, time of exposure of 96 h) (DeLorenzo et al., 2008). Compared to diclofenac, *Daphnia magna* responds much more sensitive to triclosan at a concentration level which is not so far from that in raw wastewater (Orvos et al., 2002; Haap et al., 2008).

For the anti-inflammatory drugs ibuprofen and naproxen, toxic effects to aquatic organisms (Quinn et al., 2008) have been reported for *Hydra attenuate* with EC_{50} of 2.62 mg L^{-1} for naproxen and for ibuprofen EC_{50} of 1.65 mg L^{-1} (time of exposure: 96 h). Other toxicity data of naproxen include EC_{50} of 66.3 mg L^{-1} (*Ceriodaphnia dubia*, time of exposure: 48 h) (Isidori et al., 2005) and for ibuprofen EC_{50} of 19.1 mg L^{-1} (Microtox®, time of exposure: 0.25 h) (Farré et al., 2001).

To which organisms, chemicals can have adverse effects depends also on their partition between the environmental compartments (e.g. water, soil, biomass). Generally lipophilic substances (Log $K_{ow} > 3$) are favorably adsorbed on surfaces and can be bio-accumulate in the tissue of microorganisms (Sanderson et al., 2003). Dordio et al., (2010) points that pharmaceuticals with moderate lipophilicity (corresponding to $0.5 < \text{Log } K_{ow} < 3$) like carbamazepine, clofibric acid and ibuprofen have adequate properties to be easily taken up by plants. OMPs uptaken by plants can so enter into the food chain although the concentration are low as found by Macherius et al., (2012) in their plant-uptake-experiments with carrots grown in triclosan-polluted soils. According to Cleuvers, (2003), the potency of a chemical to induce toxicity is entirely dependent on its hydrophobicity generally expressed by the Log K_{ow}. According to this hypothesis, fragrances with lipophilic nature pose a major concern regarding biological effects to organisms. Not surprisely, galaxolide and tonalide, two fragrance precursors frequently found in the wastewater and effluents of WWTPs, are suspected to have endocrine disrupting effects (Chase et al., 2012).

1.2.2 Biological effects of selected EDCs

Environmental EDCs can interfere with the endocrine system of humans and aquatic organisms (Lewis and Ford, 2012; Hotaling and Patel, 2014). Bisphenol A and nonylphenol have been found to pose endocrine disrupting effects to several type or organisms regardless the trophic level (Staples et al., 1998). Negative effects of bisphenol A and 4-nonylphenols include, estrogenic potency to induce feminization of aquatic organisms like Rainbow trout (*Oncorhynchus mykiss*), tadpoles (*Xenopus laevis*) (Levy et al., 2004) and Atlantic salmons (Duffy et al., 2014). In addition, 4-nonylphenols have been found to affect sperm motility in animals such as the Pacific oyster (Nice, 2005).

Bisphenol A is considered as moderately toxic and toxic to aquatic biota depending on the EC_{50} ranging from 1.0 to 10 mg L^{-1} (Flint et al., 2012). Acute toxicity data on bisphenol A have reported EC_{50} of 20.5 mg L^{-1}, (*Daphnia magna*, time of exposure of 48 h) (Jemec et al., 2012). Mixtures of several OMPs from six different WWTP effluents which contained bisphenol A and 4-nonylphenols were evaluated by Lundström et al., (2010). The lowest EC_{50} (% of growth inhibition) at 29.1 days was found when an effluent from an activated sludge process including biological nitrogen removal supplemented by a sand filter and ozone treatment combined with moving bed biofilm reactor was studied with the organism *Ceramium tenuicorne*. In this case the time of exposure was ≤ 168 h.

1.3 Treatment of organic micropollutants in wastewater

Several technologies have been used for removing OMPs from wastewater. For instance, conventional WWTPs employing biological reactors in their treatment chains have been found to remove some PPCPs (e.g. naproxen, diclofenac and ibuprofen) in the range of 50 up to 90% (Joss et al., 2005). The most common implemented biological treatments in WWTPs are “activated sludge reactors” (ASRs) (Oulton et al., 2010). The removal of OMPs in biological WWTPs has been found to depend on the operational settings of the ASRs as well as on the physicochemical properties of the OMPs (Ergüder and Demirer, 2010). Among the parameters found to influence the removal of OMPs in ASRs, the organic loading rate, the temperature of the water in the ASRs, the sludge age and the hydraulic retention time are essential for the degradation of these traces (Onda et al., 2003; Joss et al., 2005; Cirja et al., 2008; Suárez et al., 2008; Fernandez-Fontaina et al., 2012).

For optimizing the performance and understanding removal mechanisms of OMPs in such reactors, apart of the technical parameters of the WWTP, also the properties of the OMPs need to be considered (Ergüder and Demirer, 2010). In ASRs, sorption and biodegradation of OMPs are the most important removal mechanisms (Luo et al., 2014) whereas volatilization is negligible for polar and semi-polar OMP and their mostly polar transformation products (Verlicchi et al., 2012). Because OMPs are present in the wastewater at concentration of ng L^{-1} to µg L^{-1}, the limited bioavailability to microorganisms could be associated with limited removal of OMPs when passing through WWTPs (Bell et al., 2011). Thus, explaining the commonly observed incomplete removals of OMPs in full scale treatment facilities (Clara et al., 2005; Nakada et al., 2007). Researchers have recently investigated other types of biological technologies which may result in better treatment performances than ASRs. They include: membrane biofilters, anaerobic reactors, biofilters, biological trickling filters and sand filters (Bernhard et al., 2006; Matamoros et al., 2009b; Rattier et al., 2014). Furthermore, eco-technologies like Constructed Wetlands (CWs) have been moved into the focus of interest as low cost technology, with sustainability issues, and additional removal mechanisms e.g. rhizospheric processes and different redox zones which can enhance the elimination of OMPs (White et al., 2006).

Currently, the most effective technologies to remove OMPs from wastewater are those based on physicochemical processes like ozonation, ultra-violet irradiation, advanced oxidation processes and soption on activated carbon materials (Suárez et al., 2008; Chen et al., 2012a; Ruhl et al., 2014). Although these techniques have shown high removal efficiencies of several types of OMPs, they require large investments on infrastructure, maintenance, operation safety and energy consumption (Jones et al., 2007a). Moreover, particularly those processes achieved by disinfection processes (either chlorination or ozonation) may lead to several transformation products which can be more toxic than parent compounds (Petrović et al., 2003). Due to the extra costs, actually these advanced techniques have not been widely implemented in large scale WWTPs (Margot et al., 2013). Furthermore, the formation of OMP metabolites to evaluate the water quality of effluents from water treatment disinfection processes as well as their possible biological effects towards aquatic organisms has been poorly studied. In this sense, constructed wetlands seem to be a promising technology. Ávila et al., (2014) found removals > 90% for triclosan and bisphenol A and moderate removals from 50 to 90% for ibuprofen and diclofenac in a hybrid CW system. In addition, the authors found elimination above 90% of the initial generic toxicity after passing through the CW system employing *Daphnia magna* and *Zebra fish* embryo toxicity assays.

1.3.1 Constructed wetlands (CWs)

Constructed wetlands (CWs) are wastewater treatment facilities used to improve the quality of several types of wastewaters including municipal sewage, industrial effluents, agricultural run offs and stormwater (Kadlec and Wallace, 2009). Because CWs have proven their efficiency during the last decades, they are nowadays worldwide used (Vymazal and Kröpfelová, 2008). Kadlec and Wallace, (2009) classified four types of constructed (treatment) wetlands as follows: Free Water Surface Wetlands (FWS), Stormwater Wetlands, Horizontal Subsurface Flow Wetlands (HSSFCW) and Vertical Flow Wetlands (VF). Combinations of them are named Hybrid CW systems (Vymazal, 2005). Planted CWs and unplanted variants rely on biotic and abiotic processes for the decontamination of the wastewater (Imfeld et al., 2009). In CWs, the removal of the contaminants occurs as a result of the interactions between water, soil, plants and microorganisms (Kadlec and Wallace, 2009). In terms of hydraulics, CWs are transient systems with micro and macro gradients in flow path and depth. These conditions let CWs to create areas with different redox status that co-exist simultaneously at both micro and macro scales (García et al., 2010). This characteristic of CWs allows different physicochemical and biological processes to occur simultaneously (Wu et al., 2013). Thus, the establishment of heterogeneous aerobic and anaerobic microbial communities is achieved in CWs and different contaminant removal pathways are enabled.

Contaminant gradients in the CWs depend on the wastewater characteristics which are influenced by temporal (diurnal and seasonal) fluctuations. Thus, the removal of contaminants in CWs is expected to be quite dynamic. Depending on the wetland type and hydraulic conditions, the removal efficiency in CWs may significantly vary.

Matamoros et al., (2005) and Chapman, (2003) assessed for the first time the removal of PPCPs in HSSFCWs and EDCs in FWS, respectively. By comparing a shallow HSSFCW (0.30 cm depth) against a conventional HSSFCW (50 cm depth), Matamoros et al., (2005) found higher removal of ibuprofen in the shallow wetland (81%) than in the deep one (48%). This was attributed to the oxidized conditions in the shallow CW suggesting that some OMPs can be preferentially removed by aerobic processes.

At time, most of the research on OMPs removal in CWs has been done in order to determine the optimum conditions which favor the removal of those organic trace substances. These investigations included for instance:

- The influence of different inflow compositions (e.g. aerobic or anaerobic) (Ávila et al., 2010, 2013).
- Seasonal influences (summer/winter) on the removal of OMPs (Reyes-Contreras et al., 2012).
- The role of the substrate media on the removal of the OMPs (Dordio et al. 2009).
- The benefit of the plants (e.g planted CWs vs unplanted variants) and the different species (Hijosa-Valsero et al., 2010)
- The effects of different operation conditions of the systems (Ávila et al., 2013) and
- Different loading modes (e.g. batch vs continuous operation mode) (Zhang et al., 2013a).

In most cases, controlled conditions e.g. injection experiments and laboratory scale studies have been preferred over real wastewater conditions (Ávila et al., 2010; Dordio et al., 2010). In practice, laboratory experiments assumed conditions which are far away from the real environmental and technical situation influencing the behavior of the OMPs in full or pilot-scale CW systems. Despite the number of studies in CWs research has increased during the last years, still few papers can be found regarding process understanding of contaminant removal.

1.3.2 Horizontal Subsurface Flow Constructed Wetlands (HSSFCWs)

Horizontal Subsurface Flow Constructed Wetlands (HSSFCWs) are types of CWs where the water flows horizontally below the surface through porous media under saturated conditions from upstream to downstream of the facility (Figure 2). HSSFCWs are the most common type of CW built worldwide due to its technical simplicity, low costs of implementation, low energy consumption and low maintenance requirements (Vymazal and Kröpfelová, 2008). HSSFCWs have been used for the removal of organic load (García et al., 2005), pathogenic germs (Reinoso et al., 2008), nitrogen and phosphorus (Akratos and Tsihrintzis, 2007), and organic micropollutants (Matamoros and Bayona, 2006). In Germany, there is a long tradition with the use of CW for wastewater treatment. Actually, the technology of CWs was developed in Germany in the 1960s and nowadays many HSSFCWs are used for private households in

Germany. In 2003 more than 50,000 small CWs were estimated to be in operation in Germany (Vymazal and Kröpfelová, 2008).

Conventional HSSFCWs have usually a water depth of 0.5 m and water retention times between 4-7 days (Matamoros et al., 2005; DWA-A 262, 2006). HSSFCWs can operate as planted treatment system or as an unplanted variant (Carranza-Diaz et al., 2014). The different features of a HSSFCW are shown in Figure 2 and described in the following bullets:

- The inlet and the outlet zones where the water enters and leaves the system,
- The root zone representing an important part for efficient treatment of the contaminated water,
- The geo-membrane which enables insulation of the systems and avoids infiltration of wastewater into the groundwater,
- The control tank which allows the control of the water level in the HSSFCW.

With respect to other CWs types, HSSFCWs are the most simple and economic variants of CWs available. VF systems are indeed more efficient than HSSFCWs for the removal of nutrients (Kadlec and Wallace, 2009). However, VF systems often required special technical devices such as effluent collectors and pumps which are expensive and not available everywhere. Thus, at time VF systems are less implemented than HSSFCWs. HSSFCWs has also advantages over other CW variants like FWS. Although HSSFCWs lack of the additional treatment of the water provide by photodegradation in FWS, it has the huge advantage to limit the development of mosquitos as the contaminated water flows below the surface of the CW. This is of particular advantage in regions where diseases transmitted by mosquitos can be a serious health problem (Kengne et al., 2003).

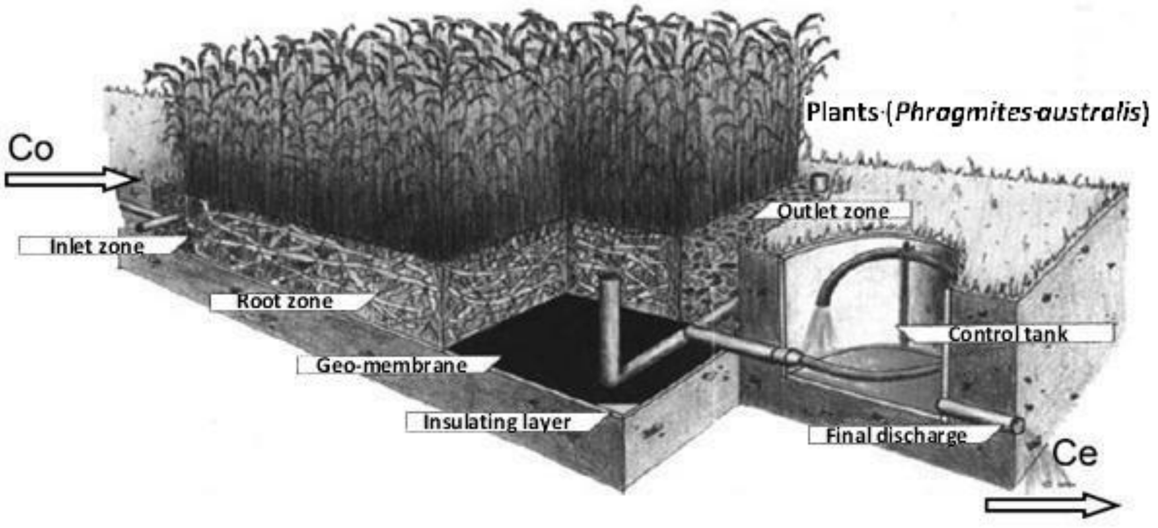


Figure 2 Scheme of a Horizontal Subsurface Flow Constructed Wetland (HSSFCW) (The figure was modified from Pastor et al., (2003) figure 3. Co and Ce denote the inflow and the outflow wastewater respectively).

In the root zone, temporal and spatial fluxes of material and energy take place inducing not uniformed gradients dependent on advective and diffusive processes (Kadlec and Wallace, 2009). Macro-gradients are less plant-mediated and more influenced by advective processes (e.g. water table fluctuations). In contrast, physico, chemical and biological processes at the micro zones in the near root zone are driven by diffusion (Imfeld et al., 2009). In addition, the differences in water temperature at different depths in the CWs (Dušek et al., 2008) favor the presence of anoxic/anaerobic interfaces enable areas within the wetland with different redox conditions (García et al., 2010).

In the upper zones of the wetland, close to the surface of the bed, oxygen concentrations are expected higher as a result of higher diffusion rates of oxygen from air into water phase and higher oxygen input from dense plant roots (Kadlec and Wallace, 2009). Therefore, high amount of aerobic bacteria is expected in the upper zones of CWs. Instead, at the bottom of the HSSFCW, reductive condition predominates and therefore anaerobic bacteria may become favorably established (Samsó and Garcia, 2013). From a mechanistic point of view, HSSFCW operate "theoretically" as an ideal plug flow, having higher water retention as compared to the idealization of complete stir tank mix reactors (Chazarenc et al., 2003). However, this is far away to occur in practice (Kadlec and Wallace, 2009). In HSSFCWs preferential water flow paths resulted from solute density effects and in-homogeneous distribution of the porous media occur in porous media (Suliman et al., 2005). The heterogeneity of the porous medium in HSSFCWs is the result of the dynamic growth of the plants and the in-homogeneous distribution of the support media (Brovelli et al., 2011). This heterogeneity enables longer wastewater retention time in the HSSFCW (Chazarenc et al., 2003; Brovelli et al., 2011). Thus, prolonging the contact between the contaminated water and microbes and potentially supporting bioremediation (Imfeld et al., 2009). The heterogeneous composition of the microbial communities (Samsó and Garcia, 2013) associated with the longer hydraulic retention times in CWs may be in benefit for the degradation/transformation of different type of contaminants, including OMPs.

1.4 Role of plants in CWs

Plants in CWs support the removal of several contaminants such as pathogens, carbonaceous material and nutrients (Kadlec and Wallace, 2009). Moreover, plant roots provide a large surface area for the settlement of bacteria communities as well as for adsorption of contaminants (García et al., 2010). In the root zone of CWs (Figure 2) the rhizosphere is stablished. The rhizosphere is the zone that surrounds a plant root and is influenced by compounds exuded by roots (Farrar et al., 2003; Narula et al., 2009). At the rhizosphere, microorganisms feed on these compounds resulting in complex biological and ecological processes (Bais et al., 2006). Root exudates also named “rhizodeposits” are a mix of several substances including, carbohydrates, organic acids and amino acids (Stottmeister et al., 2003). This rhizodeposits serve microorganisms as electron donors supporting the removal processes in CWs (Imfeld et al., 2009). At the saturated water porous media conditions of HSSFCWs, generally anoxic/anaerobic conditions predominate resulted in part from the additional supply of the carbon from the plant rhizodeposits (Farrar et al., 2003). Wetland plants like *Phragmites australis*, *Typha latifolia* and *Juncus effusus* are suited to survive under anoxic/anaerobic conditions. Moreover, they do release oxygen into the subsurface (Brix, 1997). The oxygen release by the wetland plants serves as electron acceptor for microorganisms and so influencing biodegradation processes. In laboratory conditions, the oxygen release by wetland plants (*Typha latifolia* and *Juncus effusus*) have been found to increase as the redox state of the rhizosphere is more reductive (Wießner et al., 2002).

In the rhizosphere, fluctuations of the redox conditions at micro-gradient scale occur from aerobic to strict anaerobic (Wießner et al., 2005). These micro-gradients influenced by changes of temperature and light, permit the establishment of different biological routes influencing the removal of the contaminants (Soda et al., 2007). The rhizosphere is considered the most active region of the wetland and plays a fundamental role in the contaminant removal (Imfeld et al., 2009).

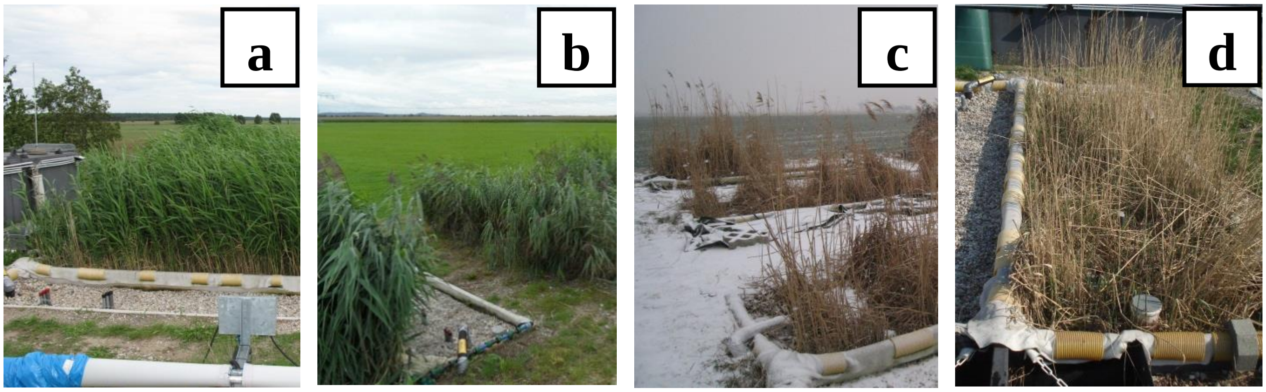
Rahman et al., (2014) observed a positive impact on the mobility As-species within the rhizosphere of helophytes in a model CWs as a result of the addition of electron donors (rhizodeposits). Factors influencing the exudation of the plants are (ii) type of plants, (iii) age of the plants, (iv) water composition, (v) redox environment, (vi) hydraulic conditions and (vii) temperature and light (Wießner et al., 2002, 2005; Lynch and Whipps, 1990; Münch et al., 2007). Many of these factors interact with each other in CWs, influence the physiological status and therefore the contaminant removal mechanisms (Kuschk et al., 2003; Hijosa-Valsero et al., 2010).

The most common plants used in HSSFCWs are *Phragmites australis*, *Typha latifolia* and *Juncus effusus* (Vymazal and Kröpfelová, 2008). They are used because of their high tolerance to contaminants, salinity and toxic compounds. The physiology of wetland plants is characterized by the *aerenchyma* which serves to exchange gases (i.e. oxygen) between the shoot and the root by either diffusion or convective flow (Brix, 1994). In addition, wetland plants “pump” water from the subsurface to the leaves through *stomata* (Kadlec and Wallace, 2009), inducing the uptake, translocation and metabolization of the contaminants from the water phase (Dordio and Carvalho, 2010). Moreover, CWs lose water to the atmosphere from the water and subsurface (evaporation), and from the plants (transpiration). The combination of the two processes is named *Evapotranspiration* (Kadlec and Wallace, 2009). Evapotranspiration is determined by climatic parameters and is partially compensated by precipitation (Kadlec and Wallace, 2009). Moreover, evapotranspiration is an important issue in CWs that causes increase of contaminant concentrations in the water phase as well as variations of the hydraulic retention time (Lim et al., 2001). The effect of evapotranspiration needs to be considered for computing removal efficiencies (Bojcevska and Tonderski, 2007).

In dependence on seasonal variations under different climatic conditions, the performance of CWs varies depending on the plants growth periods (Kuschk et al., 2003). An example of the variation of the physiological conditions of the plants along the year is shown in Figure 3. The pictures were taken at the eco-technology research facility Langenreichenbach (LRB) (Nivala et al., 2013).

With the decay of biomass from fall to winter period (Figure 3 b), transpiration and plant-microbial interactions are reduced (Kadlec and Wallace, 2009). In the winter time (Figure 3 c), plants show no green leaves as a result of the cold season. Moreover, lower water temperatures at the subsurface of the CW are expected as well as lower rates of root exudation (Farrar et al., 2003). In summer, the maximum growth of the plants and therefore the higher plant-microbial activity takes place (Figure 3 a). In spring, the sprouting period of the plants begins (Figure 3 d). Young plants emerge next to the old ones and gradually recover their biomass. The biological activity of the plants start to increase (e.g. the amount of rhizodeposits and oxygen), microbial communities get more active and evapotranspiration gradually increases, too (Münch et al., 2007). At sub- and tropical regions, planted HSSFCWs can work continuously during the whole year without having a large variation of the plant status.

Although, several investigations on wastewater treatment by CW have compared removal efficiencies winter/summer (e.g. Hijosa-Valsero et al., 2010; Reyes-Contreras et al., 2012), still poor attention has been given to the intermittent variation of the physiological status of the plants along the year and their role on the wastewater treatment efficiency. Instead, the changes in the seasons have served to explain the lower removals of contaminants during the winter and the higher removals in summer.



Summer **Fall** **Winter** **Spring**

Figure 3 Seasonal growth of the plants in pilot-scale HSSFCWs.

In HSSFCWs, the spatial and temporal growth of the plants is expected to influence the microbial activity and the redox condition in the saturated zone of the wetland at both spatial and temporal scale and therefore to influence the treatment efficiencies. The temporal redox condition dynamics as a result of the plant response to daylight has been observed in laboratory scale reactors (Wießner et al, 2005). In addition, in pilot scale CWs, it has been associated to be involved in the pollutant removal (Kuschk et al., 2003). In the context of OMPs removal, the role of the spatio-temporal redox condition dynamic influenced by the physiological status of the plants in HSSFCWs has been less investigated so far. Figure 4 shows two HSSFCWs, a planted and an unplanted variant. In the picture it can be seen the in-homogenous spatial distribution of the plants (*Phragmites australis*) in the HSSFCW probably as a result of the presence of toxic compounds or in-homogenous distribution of the nutrients within the wetland. The picture was taken during the summer time 2011 at eco-technology research facility LRB (Nivala et al., 2013).

Figure 4 Spatial preferential growth of the plants along the water flow path (from left to right) in a HSSFCW.

1.5 Removal processes of organic micropollutants in CWs

In municipal wastewater, OMPs occur at low concentration which is a limiting factor to be used for microorganisms as substrate (Onesios et al., 2009). Along the transit through the CWs, OMPs move between different redox areas where microbial-driven processes and chemical reactions can transform the OMPs (Pal et al., 1994; Dordio and Carvahlo, 2013) .

Imfeld et al., (2009) suggest that the removal mechanisms of organic chemicals in CWs (either biotic or abiotic) can be classified as destructive or non-destructive processes. Non-destructive processes indicate a transfer of the contaminants from one compartment to another within the wetland with no transformation of the compounds. In contrast, destructive processes refer to those where the contaminants are degraded or transformed. The possible routes of OMPs removal in HFCWs in terms of non-destructive and destructive processes are:

- Aerobic and anaerobic bacterial degradation/transformation
- Adsorption to the support medium, plants root and/or cell membranes of microorganisms
- Plant uptake and accumulation or further metabolism

1.5.1 Non-destructive processes of organic micropollutants

In CWs, non-destructive removal of OMPs includes adsorption, plant uptake and bioaccumulation and phytovolatilization (Li et al., 2014). Particularly, compounds with lipophilic properties tend to adsorb onto carbon rich surfaces such as roots, sludge, biofilms and on particulate matter suspended in the wastewater (Sikkema et al., 1995; Dordio and Carvahlo, 2013; Luo et al., 2014). Thus, lipophilic OMPs such as fragrance compounds are preferentially removed by adsorption in CWs (Li et al., 2014). However, some exception can be found. Accumulation of caffeine (Log K_{ow} of -0.07) was found in the gravel bed of HSSFCWs planted with *Phragmites australis*. Moreover, caffeine was also taken by plant roots of tomato and soybean in laboratory plant cultures (Dettenmaier et al., 2009), confirming the plant uptake of polar compounds. Adsorption of polar compounds via ionic interactions with minerals (e.g. clay) or matrix compounds (humic matter) can also occur in CWs, particularly at early stages of the operation. Prior reaching the stabilization or steady-state of the system, CWs are expected to serve as a sink of contaminants until a reversible adsorption and desorption equilibrium is reached (Imfeld et al., 2009).

The affinity of the compound to be adsorbed onto carbon rich surfaces also defines the facility to be transported along a subsurface porous media (Xu et al., 2007). In CWs, OMPs with lipophilic nature are slower transported than polar compounds with high solubility and low Log K_{ow} (Imfeld et al., 2009). For instance, Matamoros et al., (2005) observed no sorption of clofibric acid (Log K_{ow} of 2.84) to the gravel of an HSSFCW. The authors found that clofibric acid travels across an HSSFCW in a similar way than bromide which was used as a conservative tracer. The parameter characterizing the transport of OMPs in the porous medium is named *retardation factor* (Xu et al., 2007). The retardation factor of a specific compound depends on the adsorption coefficient K_d and the properties of the porous media (porosity, bulk density, solid density). Furthermore, the adsorption coefficient K_d, depends on the organic carbon/water partition coefficient (K_{oc}) which must be calculated for an specific site and soil type. Direct correlation between the organic carbon/water partition coefficient K_{oc} of a compound, the distribution coefficient K_d and the retardation factor of the compound have been observed (Xu et al., 2009b). High retardation factors of organic chemicals such as triclosan (Xu et al., 2009) implied higher contaminant residence time within the CWs (Imfeld et al., 2009) which could have relevant implications in CWs removal processes (e.g. a delay in transformation of triclosan).

As some OMPs are persistent in the wastewater and occur at low concentrations, the removal of these traces is expected to occur as a co-removal of the conventional wastewater parameters. For instance, filtration of OMPs is hardly possible. However, some OMPs adhered to suspended solids can be removed via filtration in the porous media of HSSFCWs. The associated problem of removing suspended solids in HSSFCWs is the clogging of the system. Ponding or water overflow in the HSSFCWs are indicators of a reduction of the effective volume of the system possibly caused by clogging (Ruiz et al., 2010). In addition, detriment of the water quality in HSSFCWs effluents may occur also as a result of clogging.

Other abiotic removal mechanism of OMPs in CWs is chemical precipitation. Chemical precipitation could be of importance in CWs for pH-sensitive compounds like diclofenac. Diclofenac becomes insoluble below pH 4 and precipitates (Pérez-Estrada et al., 2005). In addition, diclofenac possesses a carboxylic group as well as an amide functionality in its molecular structure which enable to dissociate at different pH conditions (pKa = 4.11) and to generate different reactive species. Variations of the redox potential in the pore water of CW (at the rhizosphere) as a result of changes in the intensity of daylight may result in variation of the pH in the pore water (Wießner et al., 2005; De Schamphelaire, et al., 2008). Such variations may cause different chemical processes (e.g. electron transfer or abstraction, radical stabilization) and therefore induce precipitation of some OMPs like diclofenac.

Plant uptake and bioaccumulation in wetland plants can reduce OMPs from the wastewater as demonstrated for triclosan by Zarate et al., (2012). In general, plant uptake of trace compounds is limited to the availability of the compounds in the water phase and the physicochemical properties of the compounds (Verlicchi and Zambello, 2014). For instance, Briggs et al., (1982) have correlated the plant uptake efficiency with the Log K_{ow} of selected organic chemicals. Previous studies indicated that compounds with Log K_{ow} between 0.5 and 3 are lipophilic enough to be taken up by plants (Pilon-Smits, 2005; Li et al., 2014). In addition, highly polar compounds (Log $K_{ow} < 1$) are less expected to be removed from the water by plant uptake. The transpiration of the plants is an important issue which influences the uptake of OMPs. For instance, high transpirations rates in summer may result in higher plant uptake rates (even for polar compounds) (Pilon-Smits, 2005; Dordio et al., 2010). Depending of the type of plant, some species transpire more than others; therefore the uptake rates of contaminants differ in accordance to the plant species selected for a wetland system.

The accumulation of selected OMPs (caffeine, triclosan, clofibric acid, diclofenac, carbamazepine and naproxen) in wetland plant roots and their translocation into shoots has been reported in literature (Zarate et al., 2012; Zhang et al., 2012b; 2013b, 2013c; 2013d; Bartha et al., 2014). Zarate et al., (2012) observed the accumulation of triclosan in roots and shoots of three different plants species (*Typha latifolia*, *Pontederia cordata*, and *Sagittaria graminea*). In their study, *Typha latifolia* showed the higher uptake rates of triclosan with respect to the other plants. These findings indicate the importance of wetland plants in the removal of OMPs as well as their function as storage compartment. Dordio et al., (2010) observed in their microcosm's experiments with LECA (light expanded clay aggregates) and planted with *Typha* spp. removal efficiencies in the water phase between 82-96% for ibuprofen and 88-97% for carbamazepine. The removal took place mainly due to adsorption of the OMPs on LECA. However, plants did contribute to the removal too. Although the contribution of microorganisms was not excluded in the study of Dordio et al., (2010) and no quantification of the compounds in the plants was assessed, plant uptake was proposed to account for 2-32% to the overall treatment performance. The fact that carbamazepine was removed almost equally like ibuprofen indicated that plants have the capacity to remove by uptake even recalcitrant compounds.

Phytovolatilization is a process where plants convert a contaminant into a volatile form (Susarla et al., 2002). Phytovolatilization in HSSFCWs of some organic chemicals like benzene and MTBE has been reported in literature (Seeger et al., 2011). However, in this study the contaminants occurred at concentrations in the range of mg L^{-1} in contrast to OMPs occurring at ng L^{-1} to µg L^{-1}. Moreover, benzene and MTBE are highly volatile compounds and can evaporate from the plant after absorption. For polar OMPs, volatilization or even phytovolatilization in CWs has not be deeply studied but is expected to not play a relevant role in the removal process because most of these compounds have low Henry constants (Verlicchi et al., 2012). However, phytovolatilization as a removal process of OMPs in CWs cannot be completely excluded particularly for volatile OMPs like certain fragrances (e.g. galaxolide® and tonalide®).

Another aspect to be considered is the toxicity that some organic contaminants may pose to the plants (Li et al., 2014). At the trace concentrations of single OMPs in wastewater, toxic effects to the plants are rather unlikely to occur (Dordio and Carvahlo, 2013). However, toxic impact of OMP mixtures in a complex matrix on plants is not well studied yet but it might occur, particularly, for antibiotic mixtures (Liu et al., 2013). Toxicity of contaminants to plants may be important for HSSFCWs treating industrial effluents.

Adsorption of OMPs in the cell membranes of microorganism is more possible as the pollutants are more lipophilic (Neumann, 2006). Although this phenomenon has not been deeply studied in CWs, it could be associated with the increase of pollutant concentrations at the outlet of WWTPs and CWs because bacteria may work as a carrier of OMPs. However, this phenomenon of concentration increase (Vieno et al., 2007; Verlicchi and Zambello, 2014) could be explained also by sorption-desorption equilibrium processes. Thus, OMPs could be “reversibly” retained and being released back into the water phase when the conditions in the CWs change (e.g. dilution by rain, changes of pH or bacterial communities) (Imfeld et al., 2009; Verlicchi et al., 2013).

1.5.2 Destructive processes of organic micropollutants

Aerobic and anaerobic degradation/transformation and phytodegradation are destructive processes that can be found in CWs (Imfeld et al., 2009). However, prior one of these destruction/transformation process takes place, the OMPs need to be adsorbed by a living organism for example the microbes, fungi or plants (Dordio and Carvahlo, 2013).

The adsorption of organic chemicals to cell membranes of microorganisms may cause toxic effects as confirmed by Newmann, (2006). While sorbed onto cells and other particulate matter, lipophilic OMPs are expected to be transported through HSSFCWs. In contrast, polar compounds will preferably be transported in the water phase (Ergüder and Demirer, 2010). As a result, high soluble compounds can be more accessible to microorganisms for biodegradation but simultaneously less expected to be sorbed onto cell walls. Certain types of organisms like fungi have the possibility to excrete enzymes for transformation of substances into the water phase (Asgher et al., 2008). By this, they are less exposed to toxic effects of chemicals. Although the contribution of fungi in the removal of OMPs in CWs cannot be excluded, very little research has been done in this field.

The structure of an OMP (e.g. number of aromatic rings and kind of functional groups) determines its biological degradability or recalcitrance (Imfeld et al., 2009). However, substance properties like the polarity seem not alone responsible for the fate of OMPs in CWs because correlations between the lipophilicity (Log K_{ow}) of the OMPs and their removal in CWs could not be proven (Verlicchi et al., 2013).

In HSSFCWs, microbial degradation/transformation of OMPs can follow aerobic or anaerobic routes (Li et al., 2014). Aerobic degradation of some pharmaceuticals like ibuprofen has been observed to occur in HSSFCWs (Matamoros et al., 2008). Contrary, other compounds like diclofenac or naproxen has been found to be removed under anaerobic conditions (Matamoros and Bayona, 2006; Ávila et al., 2010).

Microbial degradation/transformation of OMPs takes place favorably at the micro-zones located in the root zone of the HSSFCW (Imfeld et al., 2009). The degradation/transformations of the OMPs can occur as a result of the interaction with high diverse microbial communities, by the incorporation of the OMPs into the cells and further metabolism or as result of microbial co-metabolism (Sikkema et al., 1995; Jones et al., 2007b; Onesios et al., 2009).

Redox processes occurring in the rhizosphere allow establishing aerobic and anaerobic interfaces (Dušek et al., 2008) which promote high diversity of microorganisms capable to biodegrade the organic compounds (Imfeld et al., 2009). In addition, microbial processes are regulated by temperature gradients (Kadlec and Wallace, 2009) and in most cases higher temperatures increase the bioactivity of microorganisms (Verlicchi et al., 2013). The removal of selected OMPs (carbamazepine, naproxen, diclofenac and ibuprofen) was studied in tropical countries (Zhang et al., 2011). Although removals found ranging from 20 up to 90% are comparable to those reported in Mediterranean regions (e.g. Hijosa-Valsero et al., 2010), in tropical countries land requirement might be less as a result of high bioactivity of microorganisms.

Because the low concentration of the OMPs in CWs impedes the microbes to use them as substrate (Tran et al., 2013) biodegradation of OMPs might be driven by co-metabolic activities (Onesios et al., 2009). In this way, removal of the OMP by co-metabolism is expected to occur in HSSFCWs and may be limited by the content of organic carbon in the water phase (Zwiener and Frimmel, 2003). In the investigation of Matamoros et al., (2008), glucose was applied as carbon source but the removal of the OMPs was not enhanced when compared with starch. These findings indicated that other factors apart from the origin of the carbon source could be involved in the degradation/transformation of the OMPs in CWs. The complex wastewater matrix, the presence of compounds toxic to microbes can limiting the biodegradation as well as deficient operation conditions of the wetland system.

In this context, the influence of the plant exudates (additional carbon source) has been discussed assuming that they facilitate the co-metabolism of OMPs in CWs. Some findings have pointed that the influence of root exudates may be in benefit for the removal of some OMPs (Dordio and Carvahlo, 2013). Co-metabolism of dichloroethenes has been proposed to occur in a model HSSFCWs (Imfeld et al., 2008). However, the authors investigated dichloroethenes at concentrations of 1.5 and 6.5 and mg L^{-1} which are 1000 times higher than commonly found concentrations of OMPs in wastewater (Bell et al., 2011). To date, no publication has proven co-metabolism of OMPs in CWs.

As previously mentioned, microbial degradation/transformation of OMPs could also lead to concentration increases in the water phase at the outlet of CWs (Conkle et al., 2008). Conjugates of some pharmaceuticals are present in the wastewater resulted from human metabolism (Kovalova et al., 2012). These conjugates can include in their structures glucuronic acid, sulfate, glutathione, or acetyl coenzyme A. The real amount of pharmaceutical in the wastewater needs to account for both, the active pharmaceutical compound and its conjugate (Ternes and Joss, 2006). Enzymatic or chemical deconjugation processes implying a release of the parent compound into the water phase could be associated with the increase of carbamazepine and diclofenac concentrations in the effluent of WWTP (Vieno et al., 2007, 2014).

Little is known about the fate of the OMPs inside the wetland plants. Prior phytodegradation in CWs organic pollutants are taken up by plants despite the low concentrations in the wastewater (Dordio and Carvalho, 2013). The phytodegradation of selected OMPs in model plant systems has been reported by Huber et al., (2009). The authors studied the metabolism of acetaminophen in root cell cultures of *Armoracia rusticana* L. and found that the metabolites formed in the root cells revealed similarities to metabolites identified in mammalian metabolism. However, contrary to mammals, plants do not excrete contaminants but instead they either further metabolize them or store them in their tissues, vacuoles or cell walls (Bartha et al., 2014). The uptake and phytodegradation of triclosan was observed by Macherius et al., (2012). The authors found conjugates of triclosan in carrot cell cultures after exposition to 1 mg L^{-1}. The same metabolites were found in carrots cultivated on triclosan contaminated soils indicating the potential of triclosan to enter into the animal and human chain.

In general, destructive processes occur simultaneously with non-destructive processes. Thus, understanding removal mechanisms of OMPs in CWs remains to date a big challenge.

1.6 Operating conditions of the HSSFCWs

The operation conditions of HSSFCWs have to consider the designing basis. Although, several approaches for HSSFCWs design are available (e.g. Langergraber et al., 2009; Brovelli et al., 2011; Kadlec and Wallace, 2009), to date HSSFCWs design is mainly based on specific surface area requirements. For instance, the technical standards in Germany for HSSFCWs design (DWA-A 262, 2006) recommend a hydraulic loading: smaler or equal to 40 mm d^{-1} and a COD loading smaler or equal to 16 g m^{-2} d^{-1}. In addition, Kadlec and Wallace, (2009) recommend an areal-loading rate of less than 6 g BOD_5 m^{-2} d^{-1} to achieve an effluent concentration of less than 30 mg L^{-1}. In practice, several HSSFCWs operate out of these criteria (e.g. Ruiz et al., 2010). Thus, the systems are considered to operate at high organic load. This condition occurs when additional wastewater is fed into the system (e.g. clandestine discharges or increase in the number of houses connected to the HSSFCWs), by diffusive sources of wastewater (run off of fields or highways) or by extreme temporal variations of the inflow quality (e.g. winter/summer)

(Hijosa-Valsero et al., 2010). As a research objective, systems operating at high organic load have been poorly studied. However, CWs operating under these undesirable and sub-optimal conditions may pose a risk for environmental water quality. In addition, regarding the removal of OMPs in wetland systems running under high load conditions few is found in literature (Hijosa-Valsero et al., 2010, Reyes-Contreras et al., 2011).

When systems operate at high organic load, two important aspects need to be considered:

1. The potential of clogging and
2. The degradability of the wastewater.

In order to avoid clogging of HSSFCWs, the cross sectional load applied to the systems should not exceed 250 g BOD_5 m^{-2} d^{-1} (Nivala et al., 2012). In addition, an adequate pre-treatment of the inflow wastewater is required. Other factors to prevent clogging of the HSSFCW filter are the proper selection of the support media, adequate distribution of wastewater into the CW and the age of the system.

With respect to the biodegradability, a BOD_5/COD ratio of 0.5 indicates a suitable wastewater to be biodegraded (Huanosta-Gutiérrez, et al., 2012). It should be noted that use of the term “high organic load condition” in this work is in the context of municipal wastewater. Indeed, inflow concentrations in CWs treating industrial effluents and operating at high organic load could reach values of BOD_5 of 60,000 mg L^{-1} and COD of 135,000 mg L^{-1} (Olguín et al., 2008).

1.7 Objective of this study

The objective of this study was to investigate the removal of eleven selected OMPs in a planted and unplanted pilot-scale HSSFCWs operating at high organic load (> 6 g $CBOD_5$ m^{-2} d^{-1}). The planted HSSFCW was compared to the unplanted one in order to elucidate the role of the plants on the removal of the OMPs. Both HSSFCWs were running under the same organic loading conditions and were fed with the same municipal wastewater. The hypothesis behind this study was that plants support the removal of OMPs regardless the selected operation modus. The internal behavior of the selected OMPs was also investigated in four selected locations along the flow path and two different sampling depths. The removal mechanisms of the OMPs were investigated in relation with redox gradients and conventional wastewater parameters at the CW system scale (macro gradients in flow path and depth).

In this thesis, HSSFCWs were chosen to be investigated because it is the largest CWs technology implemented worldwide. Moreover, HSSFCWs are "passive" systems which require almost no special technical devices to operate. In comparison to other CW variants, HSSFCWs have the less energy requirement, are the most used decentralized wastewater treatment systems and have a huge potential to be implemented in developing countries. In addition, HSSFCWs deserves to be further studied because their efficiency to treat OMPs contained in municipal wastewater and and removal mechanisms are far away to be completely understood.

Chapter 2

Materials and methods

2.1 Description of the site

The investigations were carried out at the eco-technology research facility “Langenreichenbach” (LRB) described in detail by Nivala et al. (Nivala et al., 2013). The facility is located about 47 kilometers far from Leipzig (Germany) and gets part of the municipal wastewater of the nearby communities Langenreichenbach and others (12,000 PE). Due to absence of industries in this area, no input of industrial wastewater was realized.

The pilot-scale CWs investigated included one unplanted HSSFCW (H50) and one HSSFCW planted with *Phragmites australis* (H50p). Both systems (H50p and H50) had a depth of 0.5 m, length of 4.7 m and width of 1.2 m. After a pretreatment of the wastewater by a settling tank (HRT ≈ 1-2 days), the wastewater was dosed with the same loading regime to both beds (H50p and H50). This was realized by means of submergible pumps controlled from a lodge computer system at the site (Nivala et al., 2013).

The wastewater was intermittently fed at a rate of 5 L every 30 minutes resulting in hydraulic loading rate of 36 mm d^{-1} and a nominal hydraulic retention time of approximately of 5.5 days (Nivala, 2012). The surface area of each HSSFCWs was 5.64 m^2 and the size of the support medium was predominantly gravel (8-16 mm), but coarse gravel (16-32 mm) was used for the influent and effluent zones (Nivala et al., 2013).

At the LRB facility, outlet flow rates of both systems (H50p and H50) were measured by a “TIP” system. The TIP system consisted in a tube with a defined volume where the outflow water was measured. When the volume of the tube was filled up to 2 L, a magnetic sensor sent a signal to the lodge computer system in order to automatically release of the contained volume of the tube. This action was counted as “one” TIP. After that the tube is free of water again and prepared for the next volume of water. Per day between 30 and 40 TIPs were measured depending on the water loss (i.e. by evapotranspiration). The number of TIPs times and the volume of the tube (2 L) permited to measure the outlet flow rates of both systems (H50p and H50).

At the LRB facility, an onsite weather station is located (Nivala et al., 2013). Data of air temperature and precipitation were permanently recorded during the time of the investigation. In the Figure 5, data of precipitation (a) and air temperature (b) recorded during the year 2011 is shown. A maximum precipitation event occurred in July (38.7 mm) whereas the rest of the year, rain events did not exceed 15 mm d^{-1}. The minimum air temperature was measured of -9.9°C and maximum temperature was 32.8°C in June 2011. Data between 12th to the 27th of February were not recorded. The data were provided by Dr. Jaime Nivala (UFZ).

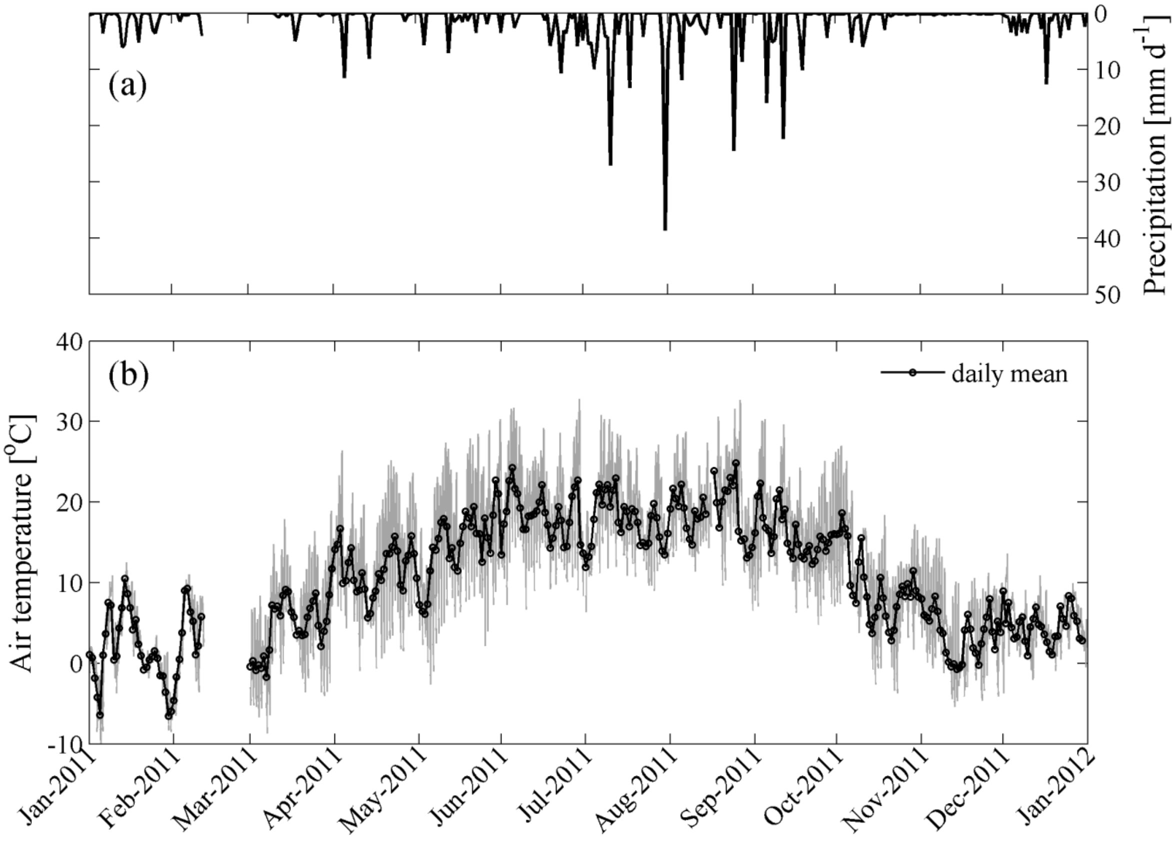


Figure 5 Precipitation and the air temperature during 2011 at the LRB facility (Daily means are shown in black, minimum and maximum values in gray). More data regarding minimum, maximum and daily mean air temperatures at the LRB facility can be found in figure 10 (a).

2.2 Selection of the target organic micropollutants

The eleven target OMPs and chemical information are presented in Table 1. The susbtances were selected based on general criteria such as persistency, production volume, occurrence in the environment and priority hazardous status (Table A1; EU, 2013). The physicochemical properties (Table 1) of the selected analytes included those being of lipophilic nature (polycyclic musks: galaxolide and tonalide) as well as being polar and good water soluble substances such as caffeine.

Table 1 Selected OMPs and their physicochemical properties (Chemical structures are shown in Table A2 of the Appendix).

Organic micropollutant (Acronym)	Use	CAS number	Log K_{ow}	Log K_{oc}	Water solubility (mg L^{-1})	Biological half-life in water (d)
Bisphenol A	Plasticizer	80-05-7	2.20-3.82[g]	2.17[f]	120-300[g]	2.5-4[g]
Caffeine	Stimulant	58-08-2	-0.07[c]	1[c]	21600[n]	0.2-0.9[h]
Carbamazepine	Antiepileptic	298-46-4	2.47[a]	3.59[c]	17.7[m]	*63[i]
Diclofenac	Anti-inflammatory/analgesic	15307-86-5	4.51[a]	2.92[c]	2.4[m]	*8[i]
Galaxolide	Fragrance	1222-05-5	5.9[k]	4.86[j]	1.75[k]	*4.5[j]
Ibuprofen	Anti-inflammatory/analgesic	15687-27-1	3.97[a]	2.59[c]	21-49[m]	*32[i]
Ketoprofen	Anti-inflammatory/analgesic	22071-15-4	3.12[a]	2.46[c]	51[m]	n.d.
Naproxen	Anti-inflammatory/analgesic	22204-53-1	3.18[a]	2.54[c]	15.9[m]	*14[i]
4-nonylphenols (Technical)**	Surfactant metabolite	84852-15-3	4.48[b]	3.97[e]	6[l]	150[b]
Tonalide	Fragrance	21145-77-7	5.7[k]	4.8[j]	1.25[k]	*0.2[j]
Triclosan	Antibacterial	3380-34-5	4.7[d]	4.26[d]	4.62[d]	n.d.

[a]Gross, et al., (2006); [b]Reinstorf et al., (2008); [c]Hijosa-Valsero, et al., (2010); [d]Ying et al., (2007); [e]Düring et al., (2002); [f]Clara et al., (2004); [g]Staples et al., (1998); [h]Bradley et al., (2007); [i]Tixier et al., (2003); [j]Buerge et al., (2003); [k]Chase et al., (2012); [l]Müller and Schlatter, (1998); [m]Imfeld et al., (2009); [n]National Library of Medicine Toxnet (http://toxnet.nlm.nih.gov).

n.d. = no detected.

*Obtained by model simulations on field measurements.

**Technical nonylphenol: isomer mixture of 4-nonylphenols (related from 4-nonylphenol polyethoxylates).

The selected OMPs included the neuroleptic pharmaceutical carbamazepine, known as very persistent and nearly ubiquitously occurring in aquatic systems (Tixier et al., 2003). Moreover, the analgesics ibuprofen, diclofenac, ketoprofen and naproxen were included based on their wide use in human therapy worldwide (Ternes and Joss, 2006). Frequently, these semi-polar compounds have been classified as potential environmental pollutants, as their reduction by conventional wastewater treatment has often been reported as inefficient (Cirja et al., 2008). In addition, the highly persistent antiseptic triclosan (Ying et al., 2007) was chosen. Four endocrine disruptor chemicals, the synthetic polycyclic musk fragrances Galaxolide® and Tonalide® (Chase et al., 2012), bisphenol-A (Clara et al., 2004) and 4-nonylphenols (Reinstorf et al., 2008) were also selected. The latter is considered priority hazardous substance according to the Water Framework Directive of the EU (Table A1) while diclofenac was recently added in the first watch list (EU, 2013). Caffeine was also included in order to verify the wetland performance by using an easily removable reference compound (Matamoros and Bayona, 2006).

2.3 Sampling

The sampling strategy was designed in order to achieve the objectives of this thesis (i) to determine the inflow-outflow mass removal of the selected organic OMPs and (ii) to understand the CWs internal behavior of the selected OMPs. The conventional wastewater parameters were measured by the Department of Environmental Biotechnology (UBT) of the UFZ. The water samples measured at the UBT included: the inflow and outflow of both studied CWs (H50p and H50) and the samping locations 0.13, 0.25, 0.5, 0.75 fractional distances within the beds for the whole period of investigation.

2.3.1 Inflow and outflow

Over a period of twelve months (from August 2011 to August 2012), 20 sampling events were performed. In each sampling campaign, grab samples were taken at the influent and the effluent of both CWs (H50 and H50p). This approach allowed studying the long term inflow-outflow performance in terms of mass removal in the CWs. The inflow samples were collected before dosing the wastewater into the wetland beds (Nivala et al., 2013). Due to the special outlet construction of the CWs where air contact could not be excluded, the outflow samples were taken within the wetland beds from the outflow collection pipe at the bottom of the wetlands.

2.3.2 Pore water samples

Pore water samples were taken within the beds during each sampling event. The samples samples were taken using steel lances and peristaltic pumps connected to a steel lance (Figure 6). A graphic description showing the internal sampling locations in the beds is shown in Figure 7.

Figure 6 Instruments used for the pore water sampling (still lance, sensor probes and peristaltic pump. The picture was taken by the LRB-Team).

The sampling locations were spatially defined as the fractional distance within the bed. The analysis to the internal behavior of the OMPs within the beds was performed based on concentrations because no internal flow rates were estimated. Moreover, the internal behavior of the selected OMPs in the H50 and the H50p beds was studied in three different time periods

(Table 2). Every period lasted two months and considered two different physiological conditions of the plants; the maximum growth (Period I and III) and the sprouting period (Period II) (see section 1.4).

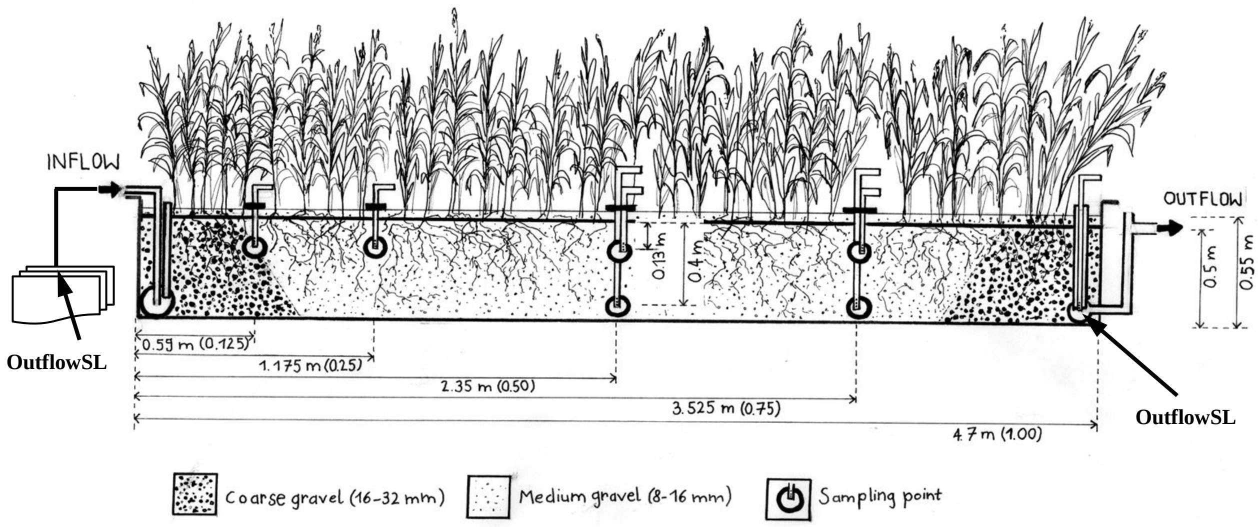


Figure 7 Diagram of the studied HSSFCW indicating the selected internal sampling locations (The steel lances were inserted into the beds and remained onsite during the 20 sampling events performed in this investigation. “InflowSL” indicates the inflow- and “OutflowSL” the outflow sampling locations. The Figure was drawn by Nancy Hachicho).

Table 2 Experimental design of sampling inside the wetland beds.

	Period I	**Period II**	**Period III**
Year/months	2011/8-9	2012/4-5	2012/7-8
Sampling points (fractional distance in% from inlet)	12.5, 25, 50 and 75	50 and 75	50 and 75
Sampling depths	0.13 m	0.13 and 0.4 m	0.13 and 0.4 m
Number of sampling events	5	4	4
Number of samples	55[a]	44[a]	44[a]
Duration of investigation (days)	61	61	62

In the period I, a one dimensional investigation of the behavior of the OMs was conducted at a single depth of 0.13 m from the upper layer of the wetland (Table 2). This depth was chosen because it can be assumed as the zone of highest root density (Imfeld et al., 2009). In addition, it is well documented that proceses that provide oxygen in HSSFCWs (surface reaeration and plant release) occur mainly in the upper layers of the wetland media (García et al., 2010). Thus, more oxidative reactions could be expected in the upper layers of the CW as compared to the bottom zones.

During Period I, internal concentration profiles were measured at four selected sampling locations along the flow path (Table 2). Period I was performed just after the maximum growth season of the plants. As mentioned in section 1.4, during the maximum growth of the plants, it can be assumed that plants develop a spatio-temporal redox condition dynamics inside the wetland which may influence the treatment efficiencies. Moreover, this would suggest that the removal of the OMPs could probably correlate with the growth status of the plants along the flow path of HSSFCWs.

During Periods II and III, the zones at 50% and 75% fractional distances within the bed were investigated (Table 2). Moreover, two internal sampling locations along the flow path at two different depths (0.13 m and 0.4 m) were studied. The sampling location of 0.4 m depth was chosen because more reductive conditions are expected in this zone and therefore more anoxic/anerobic processes could be studied (García et al., 2010). The period II was performed in the sprouting period of the plants whereas in the period III, the same conditions as applied in the period II were investigated but during the maximum growth season of the plants. The comparison between Period I and III at the sampling locations 0.50 and 0.75 fractional distance within the bed (depth of 0.13 m) was done in order to investigate the reproducibility of the results. However, it should be noted that in period III and period I the following differences applied: (i) the number of sampling events differ between Period I (n = 5) and Period III (n = 4) and (ii) temporal variations in the water quality content may occur as a result of the dynamic composition of the municipal wastewater (Metcalf and Eddy, 2003). For correlation analysis selected data from the twelve months period were evaluated.

2.4 Analytical methods

2.4.1 Chemicals

All reference compounds of the target analytes (Table 1) were delivered by Dr. Ehrenstorfer (Augsburg, Germany), Merck (Darmstadt, Germany) or Sigma Aldrich (Seelze, Germany) and were of ≥ 98% purity. The derivatization reagent BSTFA (bis-trimethylsilyl-trifluoroacetamide, Sigma Aldrich) was applied for trimethylsilylation of the H-acidic analytes prior to GC-MS analysis. Solvents (methanol, hexane) used for dissolution and dilution of reference compounds were supplied by Sigma Aldrich (Fluka). Stock solutions of the reference compounds were prepared in methanol at a concentration range of µg mL^{-1}. The respective amounts were spiked into 250 ml pure water for establishing calibration curves, determining the method characteristics, and for quantification by standard addition.

2.4.2 Sample preparation and derivatization

After sampling, the water samples were transported and stored under cold, dark and oxygen-free conditions. The water samples were prepared no longer than three days after sampling.

In the laboratory the water samples were filtered (45 µm glass fiber filter (Schleicher & Schüll, Dassel, Germany)) and adjusted to pH 2-3 (HCl, Merck) to inhibit biological activity and to enhance the extraction yield of the polar compounds. Moreover, the samples were spiked with internal standards (4n-nonylphenol, 9-chloromethylanthracene, beta-estradiol diacetate and bisphenol-d16, 200 ng each for 250 mL sample) prior to solid phase extraction. The filtered particles were not analyzed separately in this study. Solid phase extraction (SPE) was applied to extract 250 mL of the water sample using a sorbent mixture (100 mg LiChrolut EN/ 250 mg RP-C18, both from Merck) packed in a 6 mL glass cartridge. The sample holding time was less than 3 days. The SPE sorbents were conditioned with 6 mL methanol and 6 mL bidistilled water pH 3. After applying the sample at 3 mL min^{-1} and washing the sorbent with 6 mL water (10% methanol), the sorbent was dried using inert gas flow for 1 hour. The analytes were eluted with 12 mL methanol. The extract was evaporated to about 200 µL and purified by a silica gel clean-up with 1 g of silica gel filled in glass cartridge was conditioned with 6 mL aceton:hexane (35:65 v/v). After applying the sample extract, an elution was carried out with 12 mL aceton: hexane

(35:65 v/v). After evaporating the extract nearly to dryness, the analytes were derivatized with BSTFA (100 µL) at 80 °C for 30 min. After evaporating the excess reagent, the derivatized extract was reconstructed in 200 µL hexane. 1 µL of the extract was injected into GC-MS. The SPE recoveries ranged from 34% (caffeine) to 147% (galaxolide), respectively (Table A1). The precision of the measurements (see Table A3) allowed a correction of the recoveries if no internal standard was available.

2.4.3 GC-MS analysis

The analytes were determined with an Agilent 6890 series gas chromatograph coupled to an Agilent 5973 Mass Selective Detector (Agilent Technologies, San José, CA, USA). A 30 m long HP-5MS capillary with 0.25 mm internal diameter and 0.25 µm stationary phase (Agilent Technologies) was used for the separation. The oven temperature started at 70 °C, was hold for 2 min, increased at 5 K min^{-1} to 160 °C, further at 2 K min^{-1} to 180 °C and subsequently at 5 K min^{-1} to 280 °C, hold for 5 min. The transfer line temperature was set at 280 °C. The temperature of the ion source and the quadrupole analyzer were kept at 230 °C and 150 °C, respectively. 1 µL of each sample was injected splitless (2 min closed vent) into the hot injector at 280 °C. Helium was applied as carrier gas at constant flow conditions (1.0 mL min^{-1}). The mass spectrometer operated at electron ionization (70 eV) and selected ion monitoring (SIM) mode mass analysis (target ions and retention times see Table A3). Blank analyses were performed regularly to check carryover. Derivatized standard mixtures at concentrations of 0.25 ng mL^{-1} each analyte, at 1 ng mL^{-1}, 2.5 ng mL^{-1} and 10 ng mL^{-1} hexane were analyzed within a series of analysis to monitor the instrument performance and/or were used as external standard for quantification. Every sample was twice analyzed and the average value was used for overall investigations. The precision of two parallel measurements ranged from 4% (galaxolide and tonalide) to 15% (carbamazepine) with mean RSD (Relative Standard Deviation) for overall analytes inclusive the internal standard compounds at 7% (Table A3). In most cases external calibration was used for quantification or standard addition for samples with a very high matrix load (inflow wastewater). The preliminary substance screening of the inflow and the outflow wastewater of both CWs (H50 and H50p) showed that 11 selected OMPs occurred in the samples at concentrations above the Limits of Detection (LOD) of the applied analytical methods.

2.5 Analysis of the results

2.5.1. Quantification of mass removal

In order to account for the seasonal water loss by evapotranspiration and the variations in the inflow water quality, concentrations measured during the sampling events and monthly average flow rates were used for the calculation of contaminant loads (Kadlec and Wallace, 2009; Hijosa-Valsero et al., 2010). The monthly inflow and outflow rates were obtained from averaging the daily flow rates quantified at the site (Section 2.1). Then, the mass removal efficiencies (MREs) per sampling event per compound (%) were computed as follows:

$$\mathrm{MRE}(\%) = \frac{C_i \cdot Q_i - C_e \cdot Q_e}{C_i \cdot Q_i} \times 100 \qquad \text{equation (1)}$$

where C_i (µg L^{-1}) is the inflow concentration, Q_i (L day^{-1}) is the monthly average inflow rate, C_e (µg L^{-1}) is the outflow concentration and Q_e (L day^{-1}) is the monthly average outflow rate. Mean removal effiencies over the twelve month period were computed by the arithmetic mean of the 20 sampling events. MREs were also monthly computed in order to consider the different plant status influencing different rates of water loss during the year.

2.5.2 Statistical analysis

The inflow and outflow concentrations were statistically analyzed by using two mean comparison tests (Anderson et al., 2013). Firstly, the normality of the data was assessed by means of the Kolmogorov–Smirnov test (Dornelas et al., 2009). Then, comparisons were performed using the non-parametric Kruskal-Wallis test (Anderson et al., 2013). The inflow concentrations were compared against the internal concentrations of every sampling location for the Periods I, II and III. Thus, sampling locations showing significant concentration reduction inside the wetlands were identified when the significant differences were $p<0.05$. Correlation analyses were performed using the Spearman rank sum coefficient (ρ) (Braeckevelt et al., 2007). All computations were performed using the MATLAB® software (MathWorks, Natick, MA).

Chapter 3

Results and discussion

3.1 The system performance

The standard water quality parameters of the inflow and outflow water of both HSSFCWs are summarized in Table 3. The quality of the inflow wastewater discharged from 12,000 person equivalents corresponds to typical municipal wastewater as was reported for instance by Metcalf and Eddy (2003). The median COD influent concentration was 378 mg L^{-1} with a maximum value of 793 mg L^{-1}, and the ratio between $CBOD_5$/COD of about 0.59 indicated an easily biodegradable organic load (Metcalf and Eddy, 2003). In terms of $CBOD_5$ loading, the median organic loading rate applied to the systems was 7.1 g $CBOD_5$ m^{-2} d^{-1} which was higher than the design recommendation of 6 g BOD_5 m^{-2} d^{-1} (Kadlec and Wallace, 2009). However, in terms of COD loading, the median load rate applied to the systems was 12.7 g COD m^{-2} d^{-1} which accomplished the technical standards in Germany for HSSFCWs design (DWA-A 262, 2006). The recorded total sum of the parameters, namely the COD, $CBOD_5$ and TOC, reflected the high organic load conditions of both systems. The COD effluent concentrations in H50p and H50 varied only between 58% and 61%, $CBOD_5$ between 56% and 61% and TOC between 73% and 76%, respectively. The outflow concentration of less than 30 mg BOD_5 L^{-1} could neither be achieved in the H50p nor in the H50 (Table 3).

The operational regime is consistent with that used by García et al. (2004) who also observed small variations (60-65%) in the COD removal of a pilot-scale HSSFCW used for urban wastewater treatment. Moreover, in the study of Tanner, (2001) similar performances between a planted and unplanted HFCW were found. The median concentration of ammonium-N of 48.1 mg L^{-1} observed at the influent (Table 3) was found to be only slightly lower than those detected in the effluents of both planted and unplanted CWs at 53.6 mg L^{-1} (H50p) and 55.1 mg L^{-1}(H50), respectively which is a result of the conversion of organic nitrogen into ammonium. Total nitrogen decreased in H50p from 82.3 mg L^{-1} to 72.7 mg L^{-1} (with a removal of 11.6%) to a lesser extent in H50 from 82.3 mg L^{-1} to 78.9 mg L^{-1} (with a removal of 4.1%) (Table 3).

Table 3 Standard wastewater parameters of the in- and outflow of the H50p and H50 beds.

			Inflow			**Outflow**					
						H50p			**H50**		
Wastewater parameter	**Number of samples**	**Unit**	**median**	**min**	**max**	**median**	**min**	**max**	**median**	**min**	**max**
COD	19	mg L^{-1}	378	184	793	157	81.3	257	149	93.6	304
***CBOD$_5$**	a,b	mg L^{-1}	222[a]	84.4	352	97.1[b]	29.6	155	87.4[b]	25.3	129
TOC	19	mg L^{-1}	147	76.2	326	39.4	5.4	188	35.7	5.8	173
SO_4^{2-}-S	19	mg L^{-1}	40.3	9.1	63.5	9.1	4.1	50.7	7.3	2.5	23.4
S^{2-}	19	mg L^{-1}	12.4	4.1	22.2	23.7	0	32.0	23.1	16.9	30.0
NH_4^+-N	19	mg L^{-1}	48.1	12.8	84.6	53.6	19.3	78.0	55.1	17.6	113
NO_3^--N	19	mg L^{-1}	0.5	<LOD	1.0	0.2	0	1.9	0.3	0	1.0
NO_2^- -N	19	mg L^{-1}	0.01	<LOD	0.12	0.02	0	2.5	0.02	0.01	0.1
TN	19	mg L^{-1}	82.3	57.3	110	72.7	49.0	86.7	78.9	53.1	137
Dissolved oxygen	5	mg L^{-1}	0.1	0.1	0.4	0.1	0.04	0.31	0.2	0.10	0.8
Redox potential (E_h)	17	mV	-91	-158	-28	-123	-151	8	-117	-150	-84
pH	18	-	7.5	6.1	7.7	7.2	5.8	7.6	7.2	5.9	7.7

[a] Median of 11 samples

[b] Median of 10 samples

*CBOD$_5$ is the “Carbonaceous Biochemical Oxygen Demand”. The C refers to the oxygen consumed by microorganisms in the oxidation of organic matter only. The determination of the CBOD value was realized chemically using an inhibitor that suppresses the oxygen demand for nitrification (e.g. the inhibitor Allylthioharnstoff (ATH) used in this work). However, during the determination of the CBOD value, the wastewater samples did contain (i) sulfide (a reduced sulfur compound) and (ii) oxygen. Thus, certain amount of oxygen was used to oxidize sulfide. Such demand of oxygen was not accounted in the determination of the CBOD which leads to an overestimation of the carbon content in the wastewater. Hence, care has to be taken when interpreting the results of the CBOD value because the demand of oxygen for oxidation of sulfide during the determination of this parameter was not accounted for.

The comparatively better N-removal performance of the planted wetland might be attributed to the nitrogen uptake by the plants (Kadlec and Wallace, 2009) and their oxygen input into the rhizosphere stimulating aerobic processes such as microbial ammonium oxidation in this zone (Faulwetter et al., 2009). With the given high load of oxygen consuming compounds in the studied CWs, the small differences in the oxygen input between the H50p and the H50 caused by the plants were likely masked and are therefore not reflected by their redox related parameters such as redox potential and concentrations of oxygen, nitrite or nitrate.

Overall, both systems demonstrated rather reductive conditions. The median redox potentials of the effluent water for H50p (E_h of -123 mV) and H50 (E_h of -117 mV) were not significantly different. The median concentration of dissolved oxygen of the outflow was low in both CWs with 0.1 mg L^{-1} (H50p) and 0.2 mg L^{-1} (H50), respectively. In both wetland beds, microbial dissimilatory sulfate reduction (DSR) resulted in a decrease of the median sulfate concentration at the effluent by 77% (H50p) and 82% (H50) and an increase of the median sulfide concentrations to 23.7 mg L^{-1} (H50p) and 23.1 mg L^{-1} (H50). BOD and COD reduction are oxygen dependent processses. The high inflow concentrations of sulfide (12.4 mg L^{-1}) in both CWs also demanded oxygen for oxidation of sulfide. This competition for oxygen reduces the removal of $CBOD_5$ and COD. Moreover, the increase of sulfide in the effluents of the H50p and the H50 explains also the limited removals of COD and $CBOD_5$. During the twelve months period of investigation, no visual effect of clogging (ponding or overflowing) of the CWs were observed despite the high organic load of the system. This was attributed to three factors. First, the cross sectional load of the system of 66.7 g BOD_5 m^{-2} d^{-1} (calculated based on the median inflow $CBOD_5$) was much below the recommendation for avoiding clogging in HFCWs of 250 g BOD_5 m^{-2} d^{-1} (Nivala et al., 2012). Secondly, when the investigation was launched, the CWs had only 18 months of operation, which in terms of system longevity was not enough to see clogging effects in HSSFCWs (Nivala et al., 2012). Finally, the settling tank located prior the wetland treatment, worked as a primary step that allows settling of particles. Inside the studied CWs different redox conditions were recognized between the H50p and the H50 (Figure 8). Averages redox potentials measured in the internal sampling locations indicated prevalent reductive condition in both CWs ranging in average from -130 to -80 mV (Table 3). These conditions confirmed that anoxic/anaerobic process dominated in the both CWs. The slightly more reductive condition in the H50p could be attributed to the external amount of carbon coming from the plants roots as root exudates (Stottmeister et el., 2003). Instead in the H50, less reductive zones were observed probably attributed to higher diffusion rates of oxygen from the atmosphere to the subsurface due to the abscense of plants. These conditions potentially have influenced the microbial cenosis established in the systems differing between the H50p and the H50.

A homogeneous evolution of the redox potential (transversally homogenized) along the flow path in the H50p was observed. In the H50p, the redox potential gradually decreased from the inlet towards the outlet which negatively correlates with the observable plant growth in summer 2011 along the flow plants (Figure 4 of the introduction). This finding indicated closer ideal plug flow conditions in the H50p than in the H50. In contrast, in the H50, the redox potential was sligtly higher (-81 mV) towards the outlet in the sampling location 0.75 fractional distance within the bed (depth of 0.13 m) and was comparatively more oxic than in the H50p (-91.5 mV) at the same sampling location. General rules about the spatial growth of the plants in CWs are not found. However, it can be assumed that the growth of plants in CWs depends on two main factors (i) the water quality changing along the flow path (varying nutrient content “nitrate” or presence of toxic compounds) and (ii) the density of plants on the surface area (DeBusk et al., 1981; Reddy and DeBusk, 1984; Kadlec and Wallace, 2009). As observed in the Figure 4 of the introduction, the growth of the plants was less close the inlet zone and gradually increased towards the outlet. This trend could be due to (i) the inflow concententrations of sulfate of 40.3 mg L^{-1} (median basis) causing probably toxic effect to plants close to the inlet zone, (ii) the more negative redox potential close to the inlet zone and the gradual increase towards the outlet or (iii) the conversion of ammonia into nitrate as a result of nitrification along the flow path.

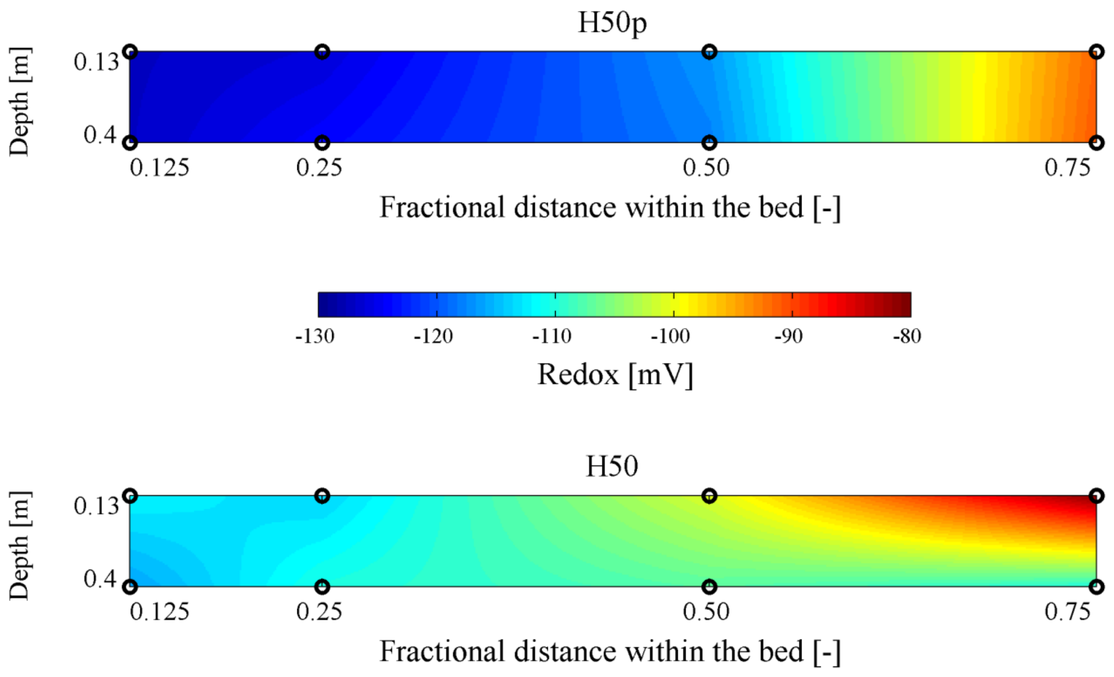


Figure 8 Two dimentional contour plot of the internal redox potential in the H50p and H50 beds (The black circles in the plots indicate the average of 17 sampling events measured during the whole period of investigation at the sampling locations 0.125, 0.25, 0.5 and 0.75 fractional distance and at the depths 0.13 and 0.4 m. The colors between the sampling locations are the linear numerical interpolations (n = 32,928 data) performed and rendered with Matlab®).

3.2 Inlet and outlet removal behavior of the organic micropollutants

3.2.1 Inflow and outflow concentrations

The inflow and outflow concentrations of the target OMPs measured over one year (from August 2011 to August 2012) are presented in Table 4. Caffeine and ibuprofen consistently showed the highest concentration in the raw municipal wastewater studied. Fluctuations in the median inflow concentrations of the selected OMPs ranged over one order of magnitude (1-100 µg L^{-1} concentration range).

Table 4 Median, minimum and maximum inflow and outflow concentrations of the investigated OMPs in µg L^{-1} (used data see in Tables A4, A5 and A6 of the appendix. The limits of detection are listed in Table A3. Compounds are alphabetically ordered).

		Inflow			**Outflow**					
					H50p			**H50**		
Organic micropollutant	**Number of samples**	**median**	**min**	**max**	**median**	**min**	**max**	**median**	**min**	**max**
bisphenol A	19	2.11[a]	0.50	19.99	3.47[a]	0.78	28.51	3.89[a]	0.77	62.09
caffeine	20	13.23[a]	1.91	63.92	2.16[a]	<LOD	63.13	2.04[a]	<LOD	18.92
carbamazepine	20	4.40	<LOD	29.65	4.07	<LOD	33.13	3.79	<LOD	37.78
diclofenac	19	7.97	1.14	36.86	14.80	1.56	73.11	9.34	1.40	73.40
galaxolide	20	2.08	0.13	9.01	2.14	0.41	10.42	2.33	0.79	6.73
ibuprofen	19	22.80	10.62	91.99	36.55	8.03	252.95	34.47	8.35	117.88
ketoprofen	19	1.32	<LOD	11.49	2.74	<LOD	21.36	2.38	<LOD	15.76
naproxen	19	2.97	0.71	8.09	3.58	1.10	15.35	3.22	0.92	15.65
4-nonylphenol (technical)	19	2.26[a]	0.57	8.98	4.34[a]	0.94	22.83	5.00[a]	0.28	59.04
tonalide	20	0.91	0.03	16.21	0.86	0.05	5.83	0.94	0.18	8.41
triclosan	19	0.97	0.01	2.86	1.05	0.40	5.12	1.06	0.32	3.25

[a] Significant differences ($p<0.05$) between the CW inflow and outflow.

<LOD = Data was below the limits of detection of the instrument.

The measured composition and concentration of some OMPs in the inflow were comparable to those given in the literature. For example, mean concentrations of caffeine of up to 7.37 µg L^{-1} have been reported for municipal wastewater from Spain (Santos et al., 2008). The same authors found mean concentrations of carbamazepine of 0.53 µg L^{-1}, ketoprofen of 1.58 µg L^{-1}, ibuprofen 69.7 µg L^{-1} and naproxen of 4.83 µg L^{-1}. Heberer (2002) reported concentrations of ketoprofen of 0.3 µg L^{-1}, naproxen 0.44 µg L^{-1}, diclofenac 3.02 µg L^{-1} and carbamazepine of 1.78 µg L^{-1}, which were obtained from municipal sewage in Berlin, Germany. In addition, Lindqvist et al. (2005) reported of having achieved concentrations of ketoprofen of 2.0 µg L^{-1}, naproxen of 4.9 µg L^{-1}, diclofenac 0.35 µg L^{-1} and ibuprofen of 13.11 µg L^{-1} in Finland. In the same country, mean concentrations of carbamazepine of 0.35 µg L^{-1} were found by Vieno et al. (2007).

In general, on the basis of the concentration values (Table 4) a rather limited elimination of the target substances was observed. Except for caffeine, bisphenol A and 4-nonylphenols, the concentrations of the other selected OMPs at the effluent of both beds hardly differed from those found in the inflow water. A significant reduction was only observed for caffeine, with 84% ($p=0.0004$) removed in H50p and 85% ($p=0.0001$) in H50. This is in agreement with results achieved in pilot-scale HSSFCWs of Matamoros and Bayona (2006), where caffeine was efficiently removed at 94%. Between the planted and unplanted beds, significant differences ($p < 0.05$) in the outlet concentrations of all studied compounds were not observed.

The outflow concentrations of bisphenol A for H50p ($p=0.0275$) and H50 ($p =0.0296$) were significantly higher than the inflow concentrations. In addition, the outflow concentrations of nonylphenols for H50p ($p=0.0148$) and the H50 ($p=0.0319$) were also significantly higher than the inflow concentrations. While the formation of 4-nonylphenols by the biodegradation of 4-nonylphenol polyethoxylate surfactants is well known in wastewater treatment (Klecka et al., 2010), the reason for the concentration increase of bisphenol A remains obscure. The release of relevant amounts from plastic parts of pipelines, valves and other technical devices along the sewage treatment path within the CWs seems unlikely. It can be speculated, that in the case that small polycarbonate and epoxy resin particles were entering with the wastewater; they could be hydrolyzed in the CWs to bisphenol A. However no evidence for this potential source could be found in the literature. On the other hand, it is known that bisphenol A is degraded under aerobic but not under anaerobic conditions (Ogawa et al., 2005). For instance, in activated sludge wastewater treatment plants, a removal of about 75% was found (Mohapatra et al., 2011).

Concentrations of OMPs in municipal wastewater are quite variable as a result of disposal and consumption which change depending on the period of the year and the time of the day (Ort et al., 2010; Ávila et al., 2014b). In order to demonstrate the variability of the inflow wastewater concentration at the eco-technology research facility LRB, the four selected anti-inflamatory drugs were investigated over a period of one week (Figure 9). The weekly sampling campaign was performed in august 2012. The inflow wastewater samples were taken daily at the settling tank of the reseach facility and were examined by means of LC-MS-MS analyses (methods not described). The results indicated highest variability for ibuprofen (σ^2 = 1.03 µg L^{-1}) followed by naproxen (σ^2 = 0.76 µg L^{-1}), diclofenac (σ^2 = 0.5 µg L^{-1}) and ketoprofen (σ^2 = 0.11 µg L^{-1}).

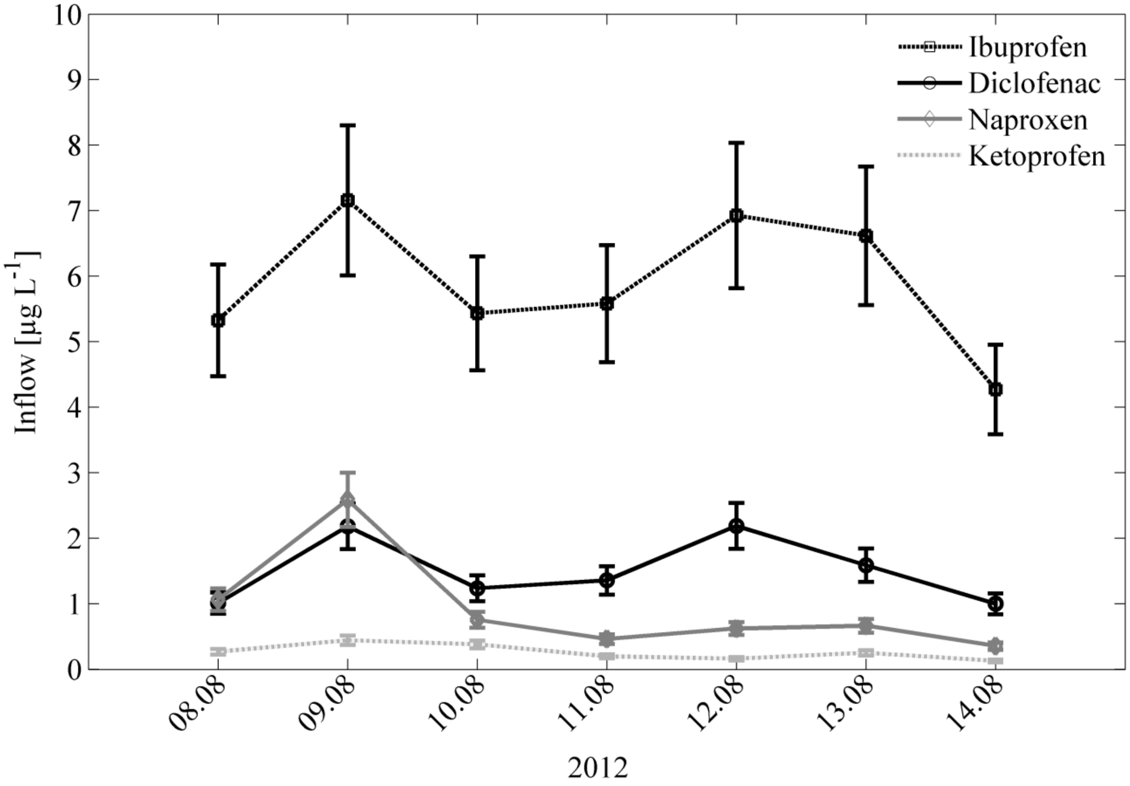


Figure 9 Weekly variation of the inflow concentration of ibuprofen, diclofenac, naproxen and ketoprofen (The wiskers indicate the mean RSD of the applied instrument after three injections).

3.2.2. Flow rates

Removal efficiencies based on concentration differences between inlet and outlet do not reflect the real wetland performance because water loss by evapotranspiration is not considered. Therefore, load based comparison is recommended in order to elucidate treatment efficiencies (Bojcevska and Tonderski, 2007). The net water loss in both CWs (H50p and H50p) by evapotranspiration was measured as the difference between the water inflow and outflow flow rates. The monthly average flow rates of the H50p and H50 as well as the mean air temperature during the entire year of investigation are shown in Figure 10 (raw data are shown in Table A7). Gray-shadow areas in (a) indicate minimum and maximum values of air temperatures.

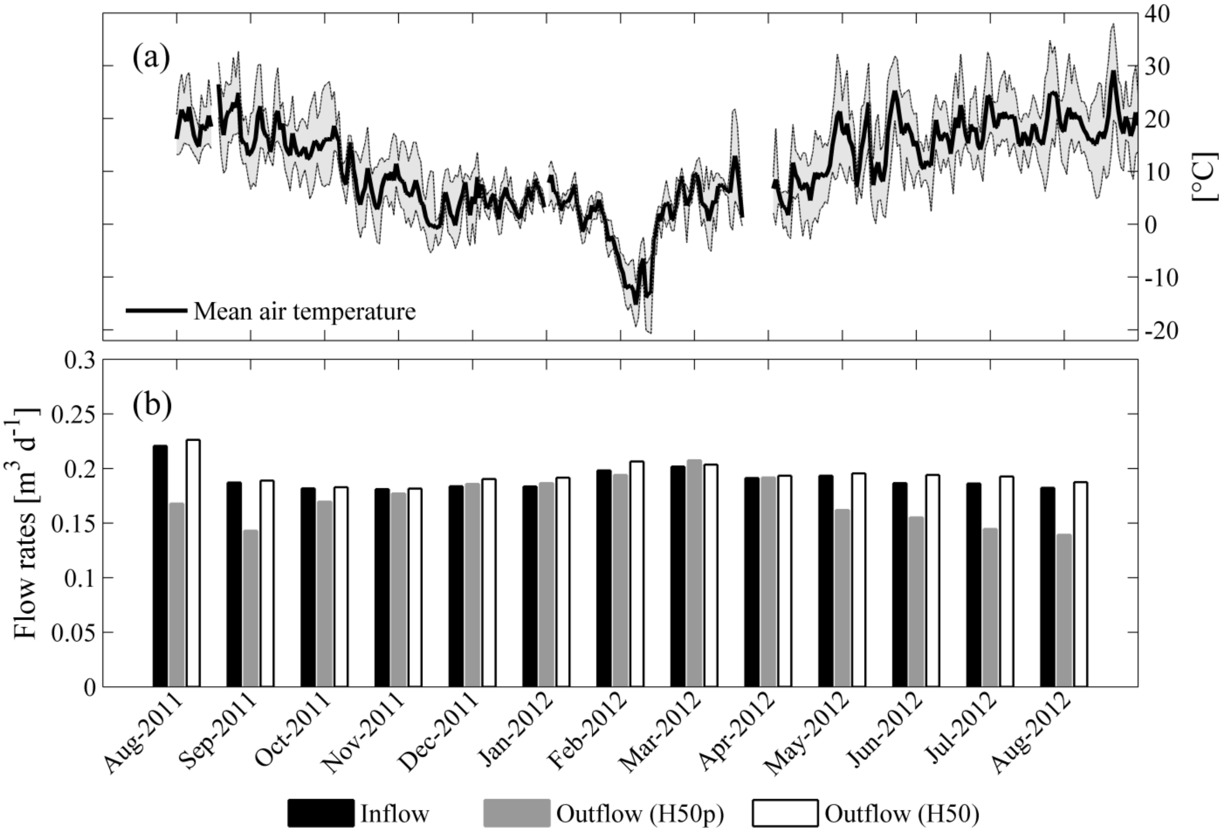


Figure 10 Daily mean air temperatures recorded at the LRB facility (a) and monthly average flow rates (b) over the one year period of investigation.

Although the H50p and H50 systems were set to operate at hydraulic loading rate of approximately 36 mm d^{-1} (Section 2.1) implying a daily flow rate of about 0.24 m^3 d^{-1} (Nivala, 2012), at the site the inflow rate ranged between 0.18 and 0.22 m^3 d^{-1} with a median value of 0.19 m^3 d^{-1} (Table A7). This inflow rate measured at the site lead to a hydraulic loading rate of 33.7 mm d^{-1} lower than the expected 36 mm d^{-1}. Furthermore, the outflow rate varied from 0.14 to 0.21 m^3 d^{-1} (median of 0.17 m^3 d^{-1}) for H50p and 0.18 to 0.23 m^3 d^{-1} (median of 0.19 m^3 d^{-1}) for H50. Over the entire period, a maximum water loss of up to 24% was observed in H50p during August 2011. The maximum air temperature of 38 °C was recorded in August 2012 and the minimum of -20.7 °C in February 2012. In the unplanted gravel bed, water loss is expected to be less relevant due to the absence of the plants.

3.2.3 Mass removals

The computation of the mass removal efficiencies (% of initial load) of the selected OMPs includes the water loss by evapotranspiration. Thus, from equation (1), the exemplarily calculation of the mass removal of galaxolide for the sampling date conducted on the 04.08.2011 is presented as follows:

$$\mathrm{MRE}_{galaxolide}(\%) = \frac{C_i \cdot Q_i - C_e \cdot Q_e}{C_i \cdot Q_i} \times 100$$

$$\mathrm{MRE}_{galaxolide}(\%) = \frac{(9.01)\cdot(0.22) - (10.42)\cdot(0.17)}{(9.01)\cdot(0.22)} \times 100$$

The value 9.01 was taken from Table A4 (brown cell).
The value 10.42 was taken from Table A5 (brown cell)
The values 0.22 and 0.17 were taken from Table A7 (brown cells)
Thus, the removal of galaxolide (%) = 10.63 ≈ 11 (see the green cell of the Table 5).

On the same basis, the mass removals efficiencies (average load-based) of the eleven OMPs in the H50p and H50 were computed for the whole period of investigation (Tables 5 and 6). Beside the removal of some OMPs, negative removals were found in both H50p and H50 as a result of the higher outflow concentration (Tables A5 and A6). Negative removals are marked as red cells in Tables 5 and 6. More number of negative removals was found in the H50 than in the H50p. The total number of analyzed samples was 213 (100%). The 64.78% (138/213) of the analized samples in the H50 showed negative removals (Table 6) whereas 56.8% (121/213) of the analized samples showed negative removals in the H50p (Table 5).

The total number of polar compounds analyzed in the samples was 133. The 75.18% (100/133) of the studied polar compounds showed negative removals in the H50 (Table 6) whereas 68.42% (91/133) of the studied polar compounds showed negative removals in the H50p (Table 5). Furthermore, the total number of neutral compounds analyzed in the samples was 80. The 47.5% (38/80) of the studied neutral compounds in the H50 showed negative removals (Table 6) whereas 37.5% (30/80) of the studied neutral compounds showed negative removals in the H50p (Table 5).

From these findings it can be concluded that the presence of plants reduce the number of negative removals for both, the polar and the neutral compounds (based on the number of analyzed samples). Thus, suggesting that planted CWs retain more OMPs (neutral and polar) than unplanted systems probably due to adsorption onto root surfaces or other transformation/degradation mechanisms. Further discussion about the negative removals is found in chapter 3.2.5.

Table 5 Outlet mass removals H50p (%) with negative removals.

	Organic micropollutants										
	Neutral compounds				Polar compounds						
Sampling date	**galaxolide**	**tonalide**	**caffeine**	**carbamazepine**	**ibuprofen**	**4-nonylphenols**	**naproxen**	**triclosan**	**bisphenol-A**	**ketoprofen**	**diclofenac**
04.08.2011	11	12	100*	14	9	**-95**	**-74**	**-34**	**-38**	**-68**	24
11.08.2011	33	53	98	40	64	12	73	43	6	81	62
01.09.2011	**-125**	**-13**	**-4**	25	10	**-17**	20	**-38**	**-5**	100*	20
08.09.2011	**-240**	**-140**	88	**-83**	**-327**	**-377**	**-204**	**-65**	**-196**	**-185**	**-831**
23.09.2011	39	30	93	69	**-44**	**-191**	23	24	**-28**	100*	33
20.10.2011	**-52**	**-40**	**-58**	14	**-10**	2	29	40	63	**-Inf**	17
17.11.2011	**-91**	64	52	**-Inf**	**-14**	**-72**	**-9**	**-2**	26	7	**-0.48**
01.12.2011	**-166**	82	**-170**	**-Inf**	**-248**	**-307**	**-108**	**-58**	**-140**	**-180**	**-237**
19.01.2012	22	19	89	100*	**-198**	**-293**	**-232**	**-6226**	**-202**	**-506**	**-182**
15.03.2012	**-289**	**-39**	**-167**	**-Inf**	**-42**	**-16**	**-36**	**-23**	**-21**	**-11**	**-69**
29.03.2012	14	**-2**	31	100*	21	**-73**	**-62**	**-26**	**-65**	**-77**	**-43**
12.04.2012	**-4**	17	48	**-Inf**	**-55**	**-91**	**-323**	**-31**	**-116**	**-110**	**-14**
26.04.2012	**-31**	**-36**	91	15	**-33**	**-17**	**-105**	**-178**	**-43**	**-126**	**-98**
10.05.2012	22	21	92	50	74	10	63	11	**-59**	45	57
24.05.2012	25	23	81	29	**-22**	**-5**	**-7**	2	**-11**	**-129**	**-126**
14.06.2012	29	24	57	55	No data	No data	No data	No data	No data	No data	No data
05.07.2012	**-73**	**-12**	100*	<LOD	**-265**	**-48**	**-102**	**-59**	**-566**	**-Inf**	**-288**
19.07.2012	80	55	100*	<LOD	75	25	35	38	4	25	62
02.08.2012	**-10**	**-32**	100*	<LOD	**-19**	**-97**	7	**-16**	**-459**	19	**-82**
16.08.2012	**-3**	**-15**	100*	<LOD	**-42**	**-169**	22	**-31**	**-109**	**-Inf**	**-193**

<LOD = Data of the inlet and the outlet were below the limits of detection of the instrument.

*100% Removal implies that the outlet concentration was below the LOD of the instrument.

-Inf = inflow concentration <LOD, but concentrations of OMP were detected along the flow path, implying therefore a negative removal.

Number of removals = 89 items

Number of negative removals = 121 items

Table 6 Outlet mass removals H50 (%) with negative removals.

	Organic micropollutants										
	Neutral compounds				Polar compounds						
Sampling date	**galaxolide**	**tonalide**	**caffeine**	**carbamazepine**	**ibuprofen**	**4-nonylphenols**	**naproxen**	**triclosan**	**bisphenol-A**	**ketoprofen**	**diclofenac**
04.08.2011	22	**-0.21**	100*	**-33**	**-5**	**-192**	**-25**	**-67**	**-94**	**-59**	39
11.08.2011	15	40	98	21	26	**-84**	41	**-15**	**-53**	42	21
01.09.2011	**-2977**	**-812**	**-3**	**-33**	**-11**	**-13**	14	**-40**	**-259**	100*	**-7**
08.09.2011	**-196**	**-123**	89	**-10**	**-115**	**-195**	2	**-43**	**-220**	**-14**	**-158**
23.09.2011	**-21**	**-8**	90	56	**-29**	**-322**	6	**-6**	**-101**	2	32
20.10.2011	**-39**	**-21**	93	37	14	1	42	45	37	**-Inf**	44
17.11.2011	**-105**	48	72	<LOD	**-107**	**-248**	**-37**	**-47**	**-50**	**-32**	**-40**
01.12.2011	**-52**	33	**-8**	<LOD	**-29**	**-181**	20	**-0.002**	**-411**	100*	**-21**
19.01.2012	21	15	70	100	**-186**	**-221**	**-255**	**-4942**	**-37**	**-313**	**-174**
15.03.2012	**-532**	**-57**	**-178**	**-Inf**	**-30**	**-32**	**-26**	**-29**	**-3**	**-5**	**-50**
29.03.2012	**-24**	**-8**	21	100*	22	51	**-30**	**-20**	**-55**	**-33**	**-23**
12.04.2012	**-7**	14	**-19**	**-Inf**	**-35**	**-55**	**-88**	**-24**	**-55**	**-28**	**-9**
26.04.2012	**-45**	**-31**	74	**-2**	**-30**	**-20**	**-109**	**-289**	**-78**	**-137**	**-99**
10.05.2012	**-27**	**-27**	87	6	61	0.01	47	**-11**	**-129**	28	24
24.05.2012	6	11	76	18	**-1**	11	**-0.01**	**-10**	**-34**	**-90**	**-64**
14.06.2012	**-17**	**-13**	49	15	No data	No data	No data	No data	No data	No data	No data
05.07.2012	**-88**	**-31**	100*	<LOD	**-85**	**-51**	**-25**	**-83**	**-489**	**-Inf**	**-103**
19.07.2012	41	22	100*	<LOD	52	**-46**	14	1	**-15**	1	44
02.08.2012	**-18**	**-60**	100*	<LOD	**-1**	**-103**	45	**-47**	**-641**	**-4**	**-23**
16.08.2012	**-13**	**-30**	100*	<LOD	**-105**	**-1887**	**-67**	**-68**	**-295**	**-Inf**	**-293**

<LOD = Data of the inlet and the outlet were below the limits of detection of the instrument.

*100% Removal implies that the outlet concentration was below the LOD of the instrument.

-Inf = inflow concentration <LOD, but concentrations of OMP were detected along the flow path, implying therefore a negative removal.

Number of removals = 71 items

Number of negative removals = 138 items

In order to investigate the removal capacity of both CWs, the negative removals in Tables 5 and 6 were considered as 0% removal (Tables A8 and A9 of the appendix). From these tables, mean values of the whole period of investigation were computed and Figure 11 was constructed to indicate the mean removal of the selected substances.

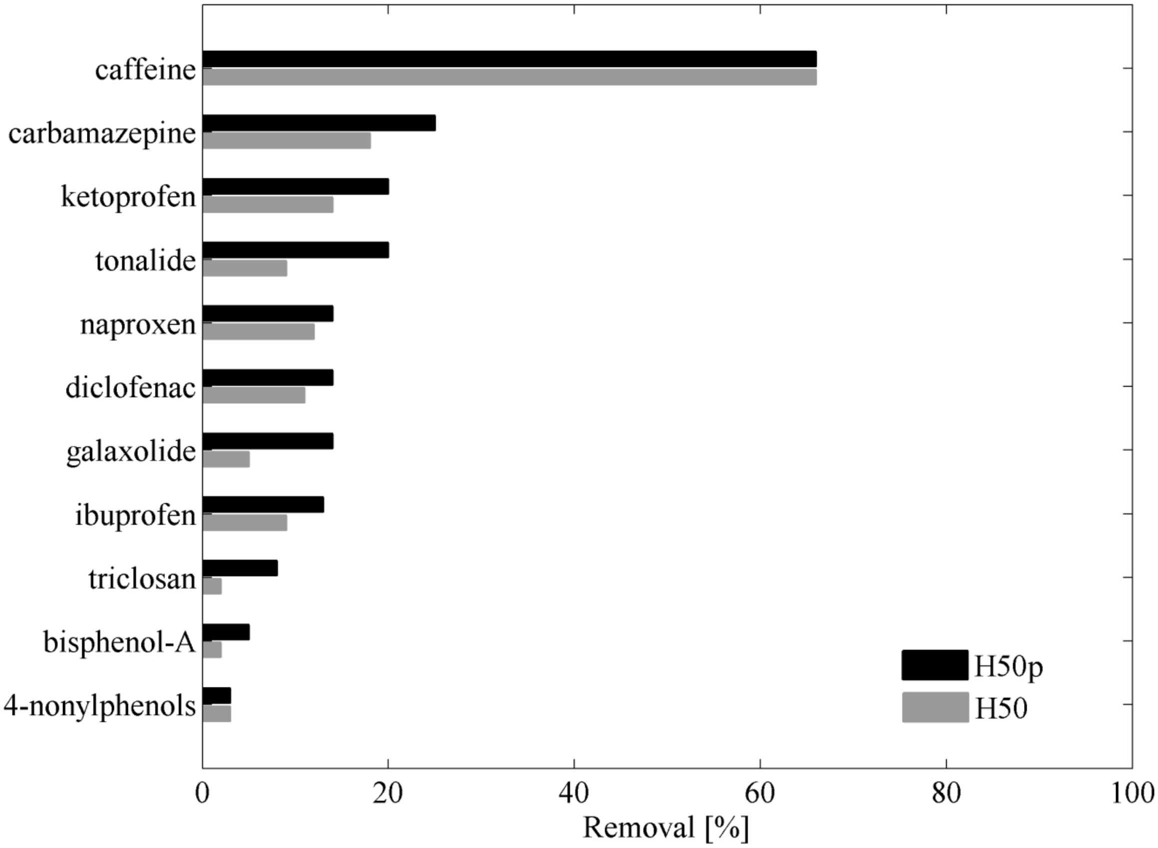


Figure 11 Comparison between the average removal of the H50p and the H50 for the whole period of investigation (Negative removals were considered as 0% removal. Data are averages of 20 sampling events for the neutral compounds and 19 sampling events for the polar ones).

The H50p bed performed better than the H50 except of caffeine which was similarly removed in both CWs. The other OMPs were removed with low efficiencies. With an average of 66±39% removal, caffeine was the most effectively eliminated target compound. The removal of caffeine is comparable to the overall removal of the $CBOD_5$ in the CWs (Table 3). Caffeine was efficiently eliminated also in the study of Matamoros and Bayona, (2006). The removal of this compound is related to anaerobic conditions (Hijosa-Valsero et al., 2010) although abiotic process such as adsorption onto carbon rich surfaces of the gravel bed of HSSFCWs or in plant roots cannot be completely excluded (Matamoros and Bayona, 2006; Dettenmaier et al., 2009).

In this study, the removal of caffeine was attributed to anaerobic processes since the increments in the organic surfaces (i.e. the plants roots in the H50p) did not influence the removal of the caffeine. The average removal of the other OMPs was below 30%. The high organic load applied to the CWs may explain the limited removal of the OMPs, since most of the oxygen was rapidly consumed for degrading the major organic matrix compounds and reductive conditions were prevalent. The recalcitrant compound carbamazepine was the second best removed OMP in both H50p and H50. Carbamazepine has been reported to be adsorbed in HSSFCWs by hydrophobic interactions with solid phases (Matamoros et al., 2005) and aerobic degradation (Hijosa-Valsero et al., 2010). Moreover, high values of carbon loading rates in CWs have been found to increase the removal of carbamazepine (Sharif et al., 2014). Because reductive conditions prevailed in both H50p and H50, abiotic processes seem to be responsible for the concentration reduction of carbamazepine from the wastewater. Furthermore, the higher specific surface in the H50p may have favored the sorption of carbamazepine onto organic surfaces when compared to the H50.

The lowest removals in this study were observed for triclosan and the two EDCs bisphenol A and the 4-nonylphenols. The low removal of triclosan could be attributed to its recalcitrance (Ying et al., 2007) and moderate affinity to sorb onto solids (Luo et al., 2014). The limited removal of bisphenol A could be also attributed to the reductive conditions prevailing in the beds. Bisphenol A is shown to be removed under aerobic conditions in wastewater treatment plants (Ogawa et al., 2005) and in HSSFCWs (Ávila et al., 2010). In this study, the high organic load applied to the system did not provide suitable aerobic conditions for the degradation of bisphenol A. Furthermore, the moderate adsorption of the 4-nonylphenols on solid matter (Luo et al., 2014) could have resulted in the low removal efficiencies.

The fragrances galaxolide and tonalide were better removed in the H50p than in the H50. Similar observations have been made in other studies (Hijosa-Valsero et al., 2010; Reyes-Contreras et al., 2012) where the removal was attributed to abiotic processes such as sorption onto organic surfaces (Matamoros and Bayona 2006) supported by the hydrophobic properties of the fragrance compounds (Table 1). The analgesics ibuprofen, naproxen, ketoprofen and diclofenac were removed between 13 and 20% in the H50p and between 9 and 14% in the H50. These analgesics have been found to be biologically removed in model experiments (Zwiener and Frimmel, 2003; Quintana et al., 2005) and in HSSFCWs (Matamoros and Bayona, 2006; Ávila et al., 2010, 2013). For instance, Ávila et al., (2010), showed that ibuprofen was removed by 50% due to the aerobic conditions prevailed in the studied CW. The continuous injection of ibuprofen at 75 µg L^{-1} into the HSSFCWs of Ávila et al., (2010) may have allowed the adaptation of the microbial consortia to this “substrate”. When compared to the investigations of this work, the inflow concentrations varied by a factor of 10 within the whole set of experimental data. Such variations in the inflow composition and the high organic carbon load seem no ideal conditions for the removal of organic trace pollutants. Few information is available about the removal of ketoprofen in CWs. Photodegradation (Hijosa-Valsero et al., 2010) and biodegradation (Tixier et al., 2003; Quintana et al., 2005) are proposed to drive the removal of ketoprofen. Because photodegradation can be excluded from this study due to the subsurface flow, the overall reductive condition of the H50p and H50 can explain the removal of ketoprofen from the water 20% (H50p) and 14% (H50). Furthermore, consistent with Ávila et al., (2010), the removal of naproxen and diclofenac was attributed to the prevalent anaerobic conditions in the H50p. However, the removal of naproxen and diclofenac can also occur under aerobic conditions (Quintana et al., 2005). Sorption of these analgesics on carbon rich surfaces is less expected due to their polarity (Luo et al., 2014).

The overall higher mass removals observed in the H50p over the H50 could be also caused by the different spatial-temporal redox conditions at micro-scale. Particularly in the vicinity of the plant root surface (Imfeld et al., 2009), the removal of OMPs can be affected by the additional release of electron donors (e.g. root exudates) and oxygen from the plant roots (Stottmeister et al., 2003). However, these processes occurring at the rhizosphere scale (Imfeld et al., 2009) were out of the scope of this study.

A correlation between the polarity of OMPs reflected by their log K_{ow} values and their removal, as previously found by Zhang et al. (2012a) and further discussed by Verlicchi and Zambello (2014), was not observed under the high organic load conditions of this study. In the Table 7, average removals of the selected OMPs and the standard deviations are compared to data reported in literature by Matamoros and Bayona, (2006) and Hijosa-Valsero et al. (2010).

Table 7 Average removal ± standard deviations (% of the initial load) of the investigated OMPs (Load increase was considered as 0% removal. Comparisons with literature data for similar systems are included [a,b]).

	Pilot-scale CW		Pilot-scale CW[a]	Mesocosm CW[b]			
	HLR = 36 SA = 5.64 OLR = 7.1		HLR = 36 SA = 54-56 OLR = 4.6	HLR = 50 SA = 1			
				OLR (Summer) = 3		OLR (Winter) = 10	
Organic micropollutant	H50p	H50	Planted	Planted	Unplanted	Planted	Unplanted
Bisphenol A	5±15	2±8	-	-	-	-	-
Caffeine	66±39	66±39	94±1	98±1	98±1	57±18	65±27
Carbamazepine	25±33	18±32	-	39±49	0±120	24±47	19±30
Diclofenac	14±23	11±17	11±42	36±27	0±7	17±22	11±26
Galaxolide	14±20	5± 11	31±11	64±16	29±15	13±25	0±7
Ibuprofen	13±26	9±18	52±1	54±19	5±21	39±24	28±26
Ketoprofen	20±35	14±32	0±14	-	-	20±27	11±39
Naproxen	14±22	12±18	0±10	83±7	57±11	43±16	27±23
4-nonylphenols	3± 6	3±12	-	-	-	-	-
Tonalide	20±25	9±15	32±10	65±16	35±13	21±30	0±24
Triclosan	8±15	2±10	-	-	-	-	-

OLR = Organic loading rate (g BOD$_5$ m^{-2} d^{-1}).

HLR = Hydraulic loading rate (mm d^{-1}).

SA = Surface area (m^2).

[a] Matamoros and Bayona, (2006). Values from the sampling campaign in May 2004. The removal rates were calculated based on the concentrations.

[b] Hijosa-Valsero et al. (2010).

The pilot-scale HSSFCW investigated by Matamoros and Bayona (2006), received a lower organic loading rate (OLR) than the systems investigated in the current study. Both systems are, however, comparable with regard to the hydraulic loading rate despite the fact that the system footprint of the Matamoros and Bayona (2006) study was ten times larger than the systems in this study. In contrast to previous studies, in this invstigation micropollutants were not continuously injected into the inflow. Thus, this study was focused on the behavior of micropollutants at real wastewater concentrations without any fortification. Caffeine, galaxolide, ibuprofen and tonalide were better removed at low OLRs (Matamoros and Bayona, 2006) than in this study. Ketoprofen and naproxen were removed better at higher OLRs. The removal of diclofenac was comparatively similar at both OLRs, but slightly better at high OLR (H50p).

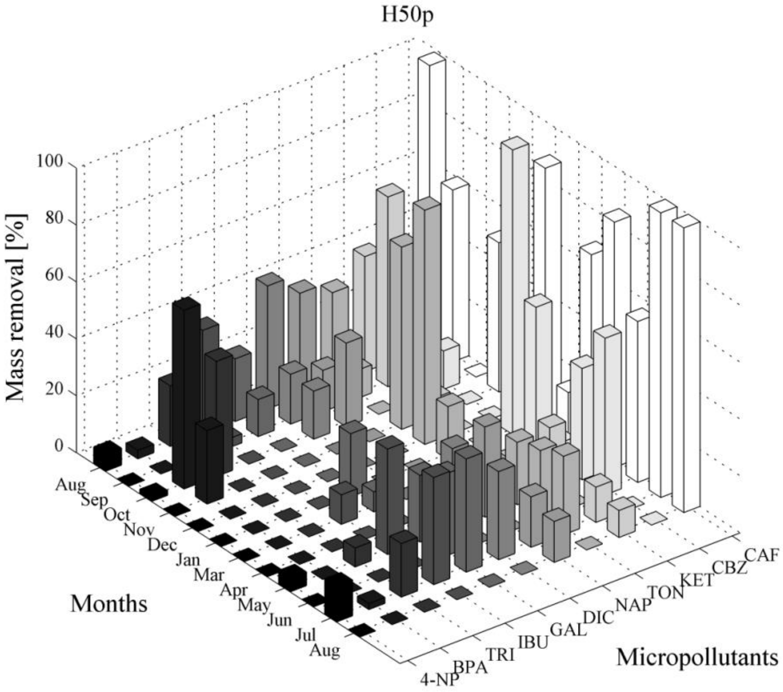
Hijosa-Valsero et al. (2010) investigated two different OLR conditions in their mesocosm HSSFCWs. The surface area of their mesocosm was smaller than the investigated pilot-scale HSSFCWs of this work, but the applied hydraulic loading rate of 50 mm d^{-1} was higher than in this study 36 mm d^{-1}. Micropollutants were not continuously injected into the inflow of the Hijosa-Valsero study either. When compared to results of this study, the planted mesocosm showed better removal efficiencies at low OLRs (in summer) than the H50p system. In the H50 system, only for carbamazepine, diclofenac and ibuprofen better removal efficiencies were obtained than in the unplanted mesocosm. At high OLRs (in winter), mass removals comparable to the planted mesocosm and H50p were found. H50p was more suitable for the removal of caffeine, carbamazepine and galaxolide than the planted mesocosm. On the contrary, the unplanted mesocosm proved to be remove carbamazepine, ibuprofen and naproxen more efficiently than H50. This could be caused by the fewer inflow variations of the OMPs in the study of Hijosa-Valsero et al. (2010) compared to the one-year investigation of this study covered real wastewater feeding from an active community.

3.2.4 Temporal variability of the organic micropollutant removal

The temporal variability removal of some OMP has been reported in literature (Hijosa-Valsero et al., 2010; Reyes-Contreras et al., 2012). However, no publication has studied the removal of OMPs in CWs under condition of high organic load of wastewater. The removal performance of the H50p and H50 along the year of investigation is presented in Figure 12 (raw data are presented in Table A10). The mass removals on the figures were in some cases calculated based on average values of two or three sampling events and in other cases presented as single values (Table A10). The data in both plots was ordered from the maximum removal (caffeine) to the minimum removal (4-nonylphenols). Consistent with the average twelve-month removal (Figure 9), the removal of the OMPs along the year was higher in the H50p than in the H50. Furthermore, the mass removal of the OMPs exhibited large temporal variability along the year. Higher mass removals were observed in the summer seasons of the year compared to the winter time. This relation could be associated with the plant status which changes according to the season of the year. Instead in the H50, the absence of plants can explain the lower observed removals. Caffeine was best removed along the year with respect to the other studied OMPs. The anti-inflammatory pharmaceuticals, ibuprofen, naproxen, ketoprofen and diclofenac were removed between 20 and 22% in months with higher ambient temperature in 2011 (Aug.-Sep.) and 2012 (Jun.-Jul.) and less than 20% in the cold months of 2012 (Dec.-Mar.). Limited removals of the endocrine disrupting chemicals were observed in both the H50p and the H50 over the whole year. These observations are consistent with the average twelve-month removal of the EDCs which indicated also low removals (Figure 11). Hijosa-Valsero et al., (2010) investigated the removal of some OMPs during winter time. The corresponding organic load was 10 g BOD_5 m^{-2} d^{-1}. Indeed, lower removals were found during the winter time compared to the summer (3 g BOD_5 m^{-2} d^{-1}). However, it could not be elucidated if the low removals were attributed to the high organic load or to variations on the plant status. Due to the Mediterranean conditions where the investigation of Hijosa-Valsero et al., (2010) was conducted, the variations of the plant status are expected low along the year. Thus, it can be supposed that the organic load may have played a relevant role over the plant status regarding the low observed removals in winter. In this investigation, with the high organic load applied over the whole year of investigation, lower removals during the cold months were observed (Figure 12) contradicting the forgoing hypothesis. Thus, the data support the fact that even under conditions of high organic load, the plant status may play a relevant role in the removal of OMPs in CWs.

Zhang et al., (2012a) demonstrated the removal of selected OMPs when systems operated in tropical climates. The high removal efficiencies between 60 to 90% of caffeine, ibuprofen, naproxen and ketoprofen were attributed to the high temperatures associated to tropical climates (23 to 32 °C). However, no relation with the plant status could be also stablished because the experiments were performed at fairly constant temperature, constant degree of solar radiation and no seasonal variations take place in tropical climates as compare to temperate countries.

In summary, the results of this study show that the mass removal of the investigated OMPs is improved by the presence of the plants and is dependent on their growth status.



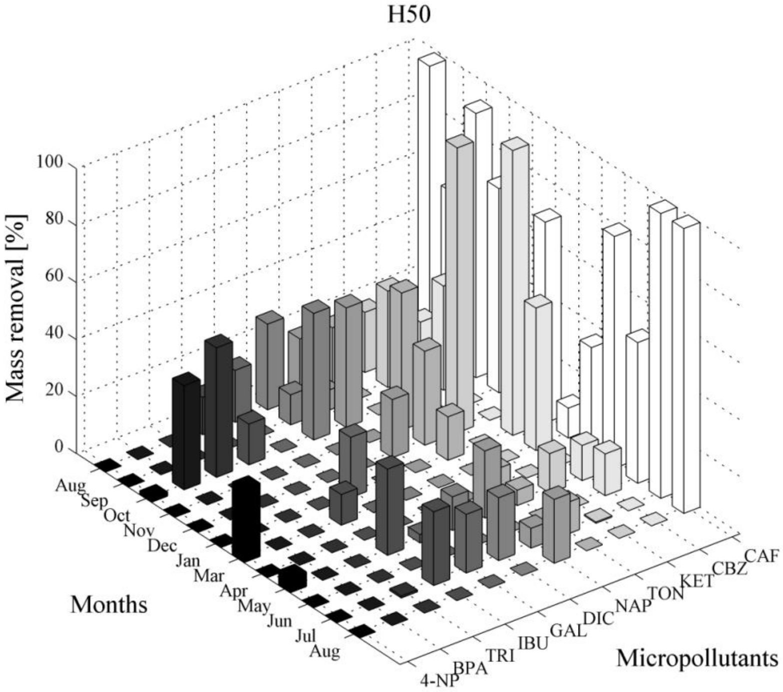


Figure 12 Removals of the selected OMPs in the H50p and H50 bed in dependence of the season (Definition of acronyms can be found in the list of abbreviations).

3.2.5 Negative removals

Negative removals were found in both, the H50 and H50p. Average negative removals were computed on the same basis as in Figure 11 (considering negative removals as 0%) but in this case considering removals as 0%. In the Figure 13 removals and the negative removals of the selected OMPs are compared. Over the 12 month period, the maximum negative removal was found for triclosan of -357% (H50p) and -302% (H50) whereas carbamazepine showed the lowest negative removals of -4% in both the H50p and in the H50. Plants increased negative removals for caffeine, ketoprofen, naproxen, diclofenac, ibuprofen and triclosan, all of them polar compounds. The unplanted bed (H50) indicated higher negative removals for the lipophilic compounds tonalide, galaxolide and the two EDCs, bisphenol A and 4-nonylphenols than those observed in the planted bed. The comparative low Log K_{oc} of the polar compounds with respect to the neutral ones (Table 1) could explain the higher negative removals in the H50p of the polar compounds whereas the organic surfaces associated to the plant roots could have retained the more lipophilic compounds. Contrarily, the higher negative removals of the more lipophilic compounds in the H50 could be attributed to the absence of plants. In other words, plants increase the amount of organic surfaces at the subsurface where low polar compounds can be sorbed. Instead, the absence of plants may favor the higher negative removals of the same compounds.

Negative removals of some OMPs in CWs have been reported in literature (Conkle et al., 2008; Reyes-Contreras et al., 2012; Li et al., 2014). Verlicchi and Zambello, (2014) proposed in their review four main reasons which lead to pharmaceutical release phenomena during treatment in CWs. They are, (i) biotic degradation of some conjugated compounds, (ii) the release of compounds previously sorbed onto the particulate dissolving after the biological treatment, (iii) instrumental errors and (iv) sampling variation. This latter aspect is related with the proper collection of effluent samples. If water samples are not time-adjusted to account for long HRTs in CWs, apparent negative removal may occur. As discussed by Ort et al., (2010) appropriate selection of the sampling regime in WWTPs is needed in order to have reliable results.

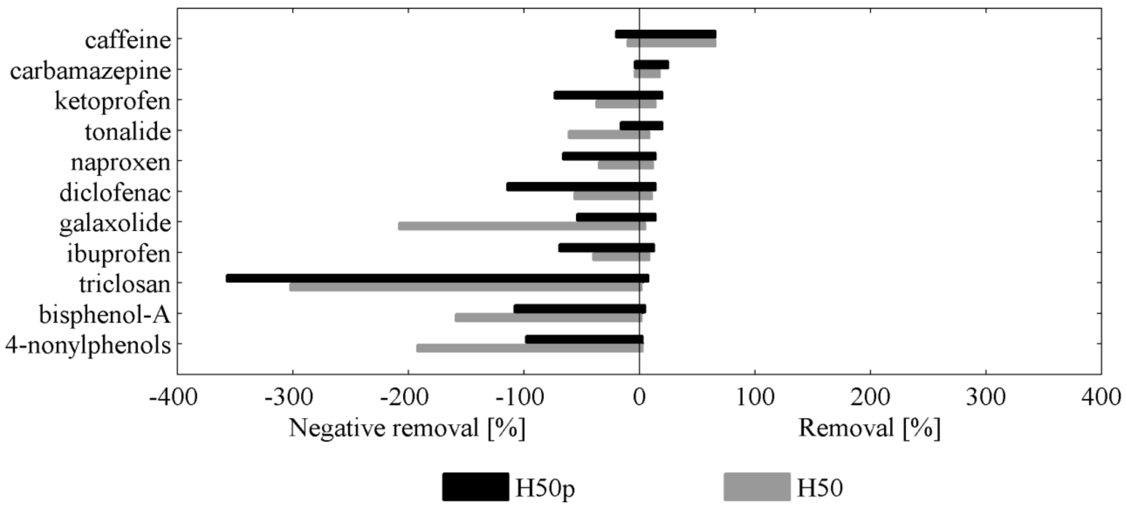


Figure 13 Average removals and negative removals of the selected OMPs (Mean values of the 20 sampling events for neutral compounds and 19 sampling events for polar ones).

Concentration increments of some OMPs like carbamazepine and diclofenac at the outlet of WWTP has been associated with hydrolysis into parent compounds and/or microbial de-conjugation of conjugate compounds (Clara et al., 2005b; Ternes and Joss, 2006; Vieno et al., 2007). In order to investigate these different hypotheses concerning possible negative removals as a result of bacterial de-conjugation of conjugates, the occurrence of the conjugates, naproxen acyl-glucuronide, diclofenac-acyl-beta-D-glucuronide and ibuprofen acyl glucuronide was examined in the inlet wastewater by means of LC-MS-MS analyses (methods not described). These conjugates were selected because the analgesics naproxen, diclofenac and ibuprofen were found among the highest concentrations in the analyzed wastewater samples (Table 4). Moreover, negative removals of these substances in the range of -35 to -114% were found (Figure 13). The mentioned conjugates of naproxen, diclofenac and ibuprofen were available commercially and they are known as phase-II metabolites excreted by humans after these substances were administered as painkillers. The stability of these conjugates in tap and influent wastewater was checked at laboratory scale in batch experiments. In both water types, the conjugates spiked at 100 ng L^{-1} were hydrolyzed within 3-5 hours to form the free drugs. The conjugates of naproxen, diclofenac and ibuprofen could not be detected in the influent water of the pilot HSSFCWs assuming that their hydrolysis was completed during the transport from the sources to the CWs (about 2 km long pipeline). Thus, the previous stated assumption, the increased concentration of e.g. diclofenac in the effluent of the CWs could be related from the respective de-conjugation reactions in the wetland beds, was not proved. However, for other target analytes such as bispehenol A, 4-nonylphenols and triclosan, the hypothesis that microbial de-conjugation can contribute to the effect of their negative removals can not be excluded yet. Among the technical origin of BPA and 4-NP, a release from former conjugates is possible, too. In human urine, BPA is excreted mostly conjugated as glucuronide and sulfate (less free BPA) and it was found at concentrations from 1 µg L^{-1} to 30 µg L^{-1} (Matsumoto et al., 2003, Califat et al., 2005). Moreover, NPs and triclosan could be detected in human urine at concentrations of about 16 µg L^{-1} and 4 µg L^{-1}, respectively (Li et al., 2013).

Plant uptake of organic chemicals is expected to take place in CWs simultaneously with microbial processes (Imfeld et al., 2009). After uptake of the OMPs, different routes for assimilation and incorporation within the plants take place. For instance, OMPs can be accumulated in the plant tissues and/or further metabolized within the plant to form also conjugates (Macherius et al., 2012; Li et al., 2014). Among the influence of microbial activity, also abiotic processes can affect the partition of the OMPs such as by adsorption-desorption on sludge, gravel, cell membranes of bacteria (Sikkema et al., 1995) and plant roots. These processes vary in dependence of temperature, pH or matrix ingredients (e.g. surfactants or humic matter for soptive interactions) (Loffredo and Senesi, 2006). In this investigation, the variation of the inflow concentration (Table 4) and the organic loading regime applied to the systems was playing an important role and probably caused the picture of negative removals of some OMPs.

Although, the loading rate of 33.7 mm d^{-1} and the organic loading rate of 12.7 g COD m^{-2} d^{-1} were lower than 40 mm d^{-1} and 16 g COD m^{-2} d^{-1} recommended by the technical standards in Germany for HSSFCWs design (DWA-A 262, 2006), the system performance (Section 3.1) as well as the low removal efficiencies found in this work compared to literature (Table 7), confirms (i) that the system were operating at high organic load and (ii) let suggest that the areal-loading rates based on g BOD_5 m^{-2} d^{-1} recommended by Kadlec and Wallace, (2009) may provide a better insigth of the operating conditions of HSSFCWs than the one based on g COD m^{-2} d^{-1} (DWA-A 262, 2006).

No evidence of clogging was observed (Section 3.1). However, it can not be excluded that a fraction of the pore water volume of the beds was reduced due to suspendid solids entering in the beds. Thus, reducing the HRT and consequently affecting the treatment efficiencies. In addition, in case of grap sampling, effluent concentrations are not corrected by the HRT being reflected as negative removal of a compound. Furthermore, the removal of the OMPs cannot be considered without the wastewater matrix composition (in this case municipal wastewater). Matrix effects associated to the type of wastewater need also to be considered for a proper interpretation of the treatment efficiencies. A complex matrix or an improper sample preparation could bias the true concentration value. Thus, depending on the matrix ingredients, the complexity to assess accurate concentration values may importantly vary. In this study, only the soluble fraction of the wastewater was investigated. However, by analyzing also the particulated fraction of the water samples another picture regarding the negative removal phenomena could be found.

The effect of water loss due to the evapotranspiration by plants was found independent of the occurrence of negative removals. Water loss was not observed in the H50 (Figure 10). However, negative removals were found in both the H50p and the H50. As shown in Figure 10, most of the water loss occurred during the warm months in the H50p. In addition, higher mass removals of some OMPs were found during the warm months (Figure 12). Based on these observations, it can be assumed that the concentration effect caused by water loss was over-compensated by efficient degradation processes of some of the investigated OMPs.

In this work, also the high amount of oxygen consuming compounds may have limited the aerobic removal of the OMPs. The high organic load applied to the CWs and the HRT (about 5.5 days) was not suited to allow efficient anaerobic degradation of the OMPs since those processes are often characterized by slow kinetics. Therefore, it can be concluded that the high organic load, the short HRT of the CWs and the inflow variations made the OMPs pass through the wetland with very limited removal and probably inducing negative removals. The finding of the negative removals within the open CW systems needs further investigation to find out the reasons and finally to overcome this undesired behavior.

3.2.6 Conclusions part I

The results of the inlet-outlet investigation of the behavior of the selected OMPs are shown in the following bullets:

- The two pilot-scale HSSFCWs (H50p and H50) operated during this investigation above the recommended areal-loading rate of 6 g $CBOD_5$ m^{-2} d^{-1}.
- Removals of all studied OMPs were observed in both the H50p and H50 beds.
- Except of caffeine that was similarly removed in the H50p and the H50, for the rest of the studied OMPs, the H50p performed better than the H50, confirming that plants improve the removal of OMPs, even when CWs operated at high organic loading.
- Temporal variability on the removal of the OMPs was observed.
- Higher removals were found in the warm months than in cold ones for both, the H50p and the H50.
- Negative removals were observed in both systems as a result of outflow concentration increase.
- Higher number of negative removals was found in the unplanted (H50) than in the planted (H50p) system.
- Inflow variation of the concentration of the OMPs, the short HRT and the operating conditions (high organic load) were identified as the relevant factors inducing low efficient removals of the OMPs.

3.3 The internal behavior of the organic micropollutants

In order to get more detailed insight on the removal processes of the OMPs in the studied CWs, the internal behavior of the OMPs was studied at defined locations and depths within the CWs (see Section 2.3.2). Because internal flow rates were not determined in this work (only for inflow and outflow) the analysis was done on the basis of concentration data. Outliers were considered for the purpose of this analysis for all the three studied periods. The findings obtained per separate period are presented in the following subsections.

3.3.1 Periods I (2011/August-September)

The internal behavior of the studied OMPs in the CWs showed different trends for the compounds Thus, the results are discussed according to the different groups of target substances.

3.3.1.1 Pharmaceutical and personal care products (PPCPs)

In accordance with Matamoros and Bayona (2006), an exponential concentration decrease of caffeine was observed along the flow path of the H50p and H50 (Figure 14 a and b). Moreover, significant reductions ($p<0.05$) in the concentration between the influent and the sampling locations at a 0.5, 0.75 fractional distance as well as the outflow were found.

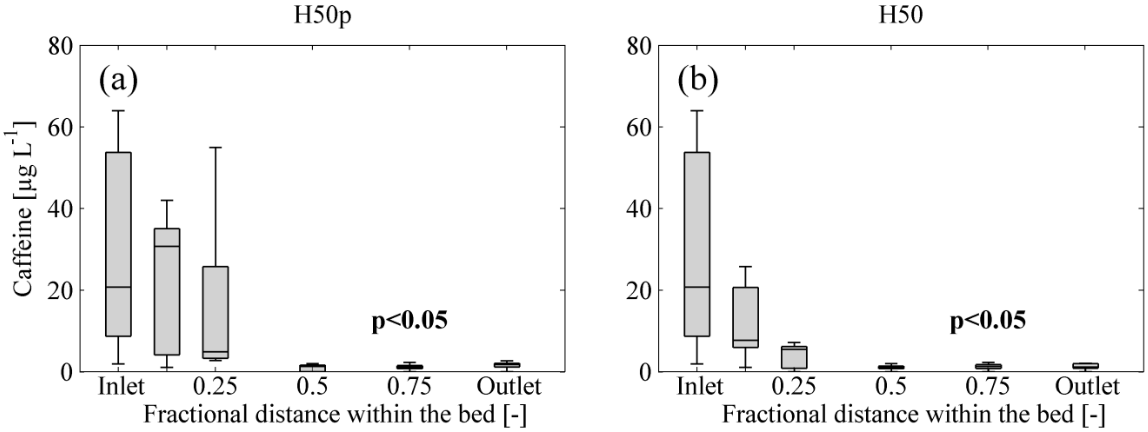


Figure 14 Distribution of caffeine concentration along the flow path during period I.

The evolution of the concentrations of the anti-inflammatory drugs ibuprofen, ketoprofen, naproxen and diclofenac in the H50p and H50 during the Period I is presented in Figures 15, 16, 17 and 18. The internal concentrations of the four anti-inflammatory drugs in H50p fluctuated considerably along the flow path, oscillating from the inlet towards the outlet. The same effect but to a lower extent was observed in H50. The outliers in the H50p indicated higher variability of the concentrations than the H50. In addition, the higher whiskers observed in H50p (Figures 15a, 16a, 17a and 18a) with respect to H50 (Figures 15b, 16b, 17b and 18b) were attributed to concentration increase as a result of plant evapotranspiration. From the statistical investigation, significant concentration reductions of ketoprofen, naproxen and diclofenac were found between the inflow and the sampling locations at a 0.5 and 0.75 fractional distance in H50 (Figures 16b, 17b and 18b). Ibuprofen was only significantly reduced in 0.50 fractional distance from the inlet of the H50 (Figure 15b). Raw data of the concentrations used for the statistical investigation in Period I are shown in Tables A11 to A18 of the appendix.

The results suggest that inside the wetlands preferential zones of removal may occured which might be associated with (i) the plant status, (ii) the establishment of preferential zones of micro and macro gradients of redox parameters and (iii) the associated internal CW microbial cenosis (Imfeld et al., 2009; García et al., 2010). In addition, the anti-inflammatory drugs are characterized by semipolar properties and molecular functionalities such as carboxylic and amino groups facilitate a microbial biodegradation and higher mobility than expected by their Log K_{ow} and low sorption properties (Gröning et al. 2007). Considering that the physicochemical properties of the compounds determine the fate of organic chemicals in the environment (Schwarzenbach et al., 2006), compounds of higher polarity (lower K_{ow}) like ibuprofen (Log K_{ow} of 3.97), ketoprofen (Log K_{ow} of 3.12), naproxen (Log K_{ow} of 3.18) and diclofenac (Log K_{ow} of 4.51) can be expected to be easier transferred into cells (e.g. microorganism or plant cells) allowing bioaccumulation (Jjemba, 2006), or microbial degradation/transformation.

The fluctuations of the internal concentrations of the anti-inflammatory drugs could be attributed to several factors. While the internal pore water samples represent only a fraction of the whole cross section of the wetland, the outflow grab samples covered the entire profile load and could explain the higher outflow concentrations. Moreover, the variability of the inflow concentrations of the studied pharmaceuticals can also explain the high variability of the OMPs concentration within the CWs (Figure 19). The HRT also needs to be considered for understanding the behavior of the OMPs within the CWs. The nominal HRT of 5.5 days used in this investigation let suggest that the internal fluctuation behavior may be also the result of the transit of the changed inflow concentration through the CWs.

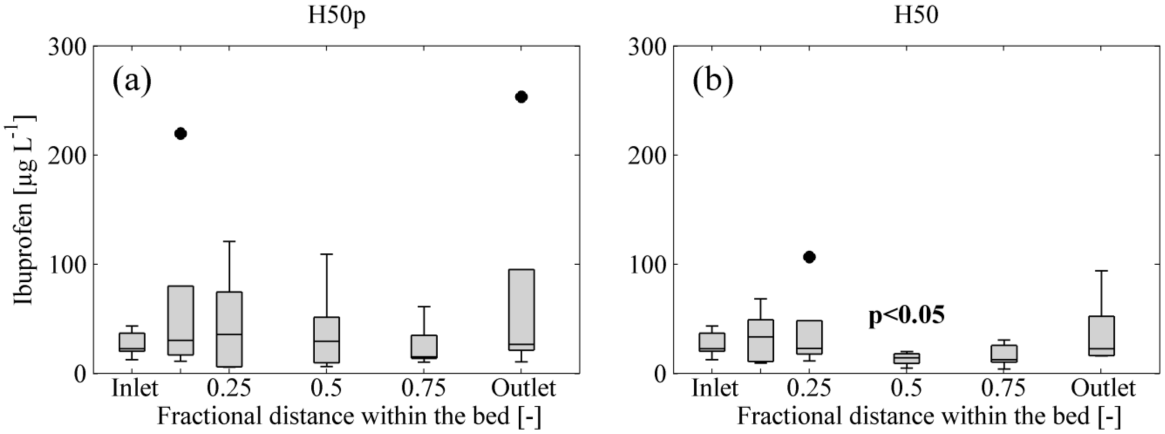


Figure 15 Distribution of the ibuprofen concentration along the flow path during period I (black dots indicate outliers).

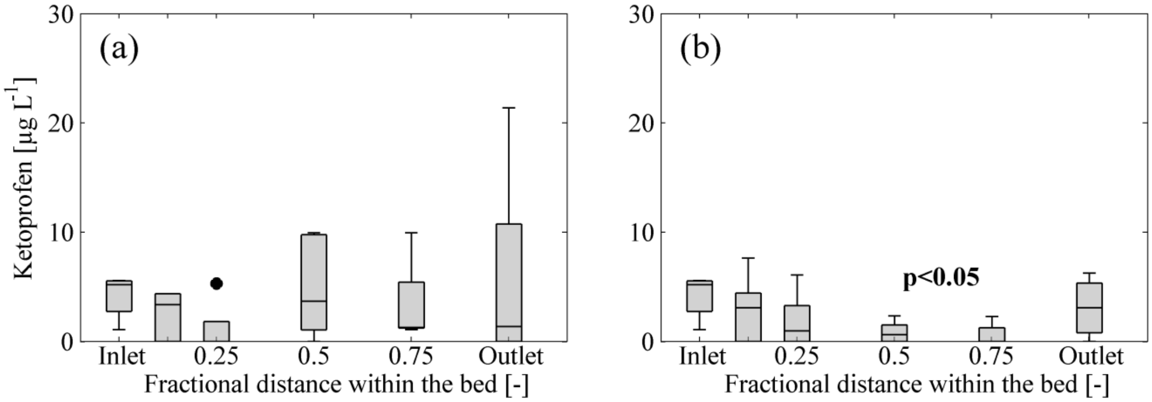


Figure 16 Distribution of the ketoprofen concentration along the flow path during period I (black dot indicates outlier).

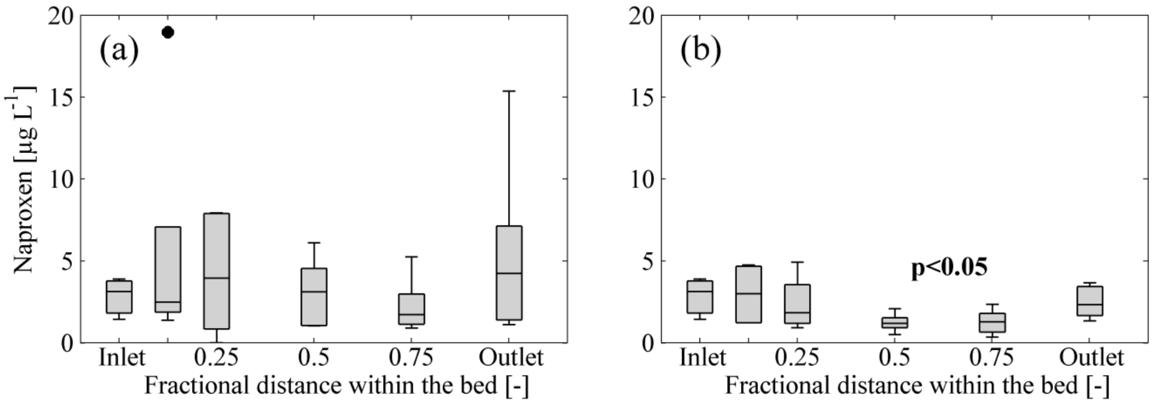


Figure 17 Distribution of the naproxen concentration along the flow path during period I (black dot indicates outlier).

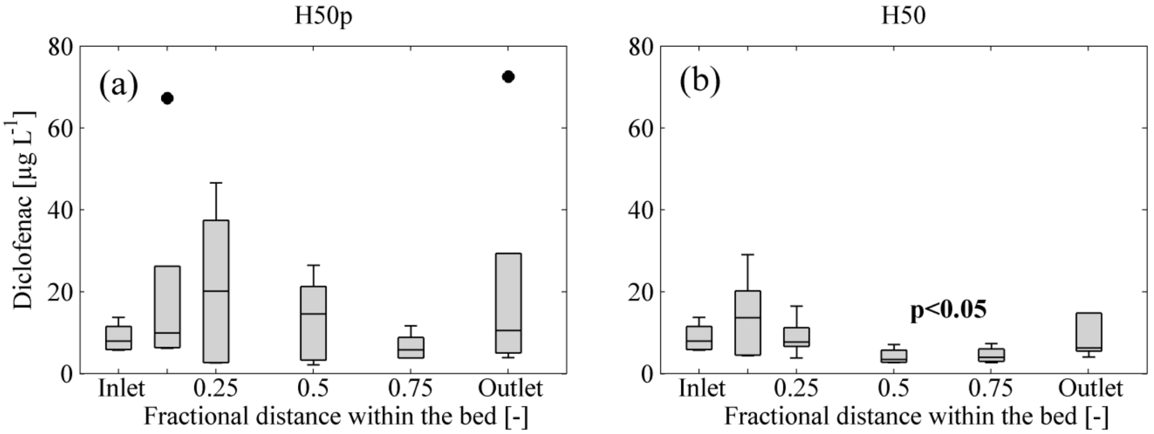


Figure 18 Distribution of the diclofenac concentration along the flow path during period I (black dots indicates outliers).

Figure 19 shows the inflow variability of the four investigated analgesics ibuprofen, ketoprofen, naproxen, diclofenac during the twelve months period of investigation. These variations can be attributed to the varying human consumption of pharmaceuticals in dependence of seasonal and health issues (Ternes and Joss, 2006). The gray shadow areas in Figure 19 indicate the three studied periods. In agreement with Figure 9, Ibuprofen presented the higher absolute inflow variability among the rest of the anti-inflammatory drugs (ketoprofen, naproxen and diclofenac) within the time Period II (σ^2 = 34.32 µg L^{-1}), followed by Period III (σ^2 = 19.41 µg L^{-1}) and Period I (σ^2 = 11.98 µg L^{-1}). Such variability of the inflow concentrations of some of the studied OMPs (e.g. the anti-inflammatory drugs) could also explain the fluctuation of these compounds along the flow path as well as the concentrations increases at the outlet of both CWs. Thus, the clear significant reduction of the anti-inflamatory drugs in period I is well recognized due to less inflow variations.

To account for the seasonal variability of the compound concentration in the wastewater, the time resolution of sampling is a critical aspect in order to get valid conclusions (Ort et al., 2010). In this investigation, grab samples were taken as practiced in previous investigations (Matamoros and Bayona, 2006; Hijosa-Valsero et al., 2010; Ávila et al., 2014b) and the monthly average data were used for the interpretation of removals (Kadlec and Wallace, 2009). In other studies composite samples have been prefered to compute removals (Matamoros et al., 2005; Ávila et al., 2010) which may show different results as compared to the grab sampling approach.

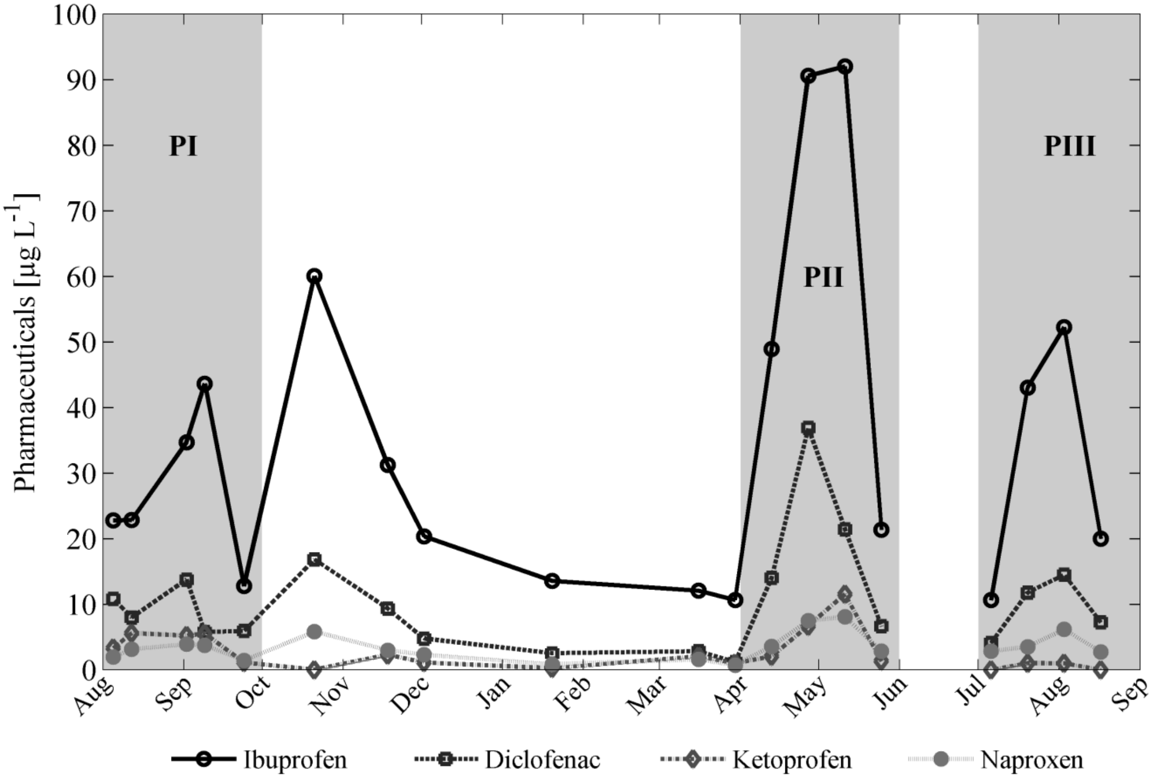


Figure 19 Seasonal variations of the pharmaceutical concentrations in the inflow wastewater.

The pharmaceutical carbamazepine known as widely persistent (Zhang et al., 2008) was removed neither in the H50p nor in the H50 (Figure 20). Carbamazepine transited the CWs without remarkable reduction along the flow path. The variations of the concentrations inside of the CWs could have been associated to the temporal variability of the inflow concentrations caused by human consumption (Ternes and Joss, 2006).

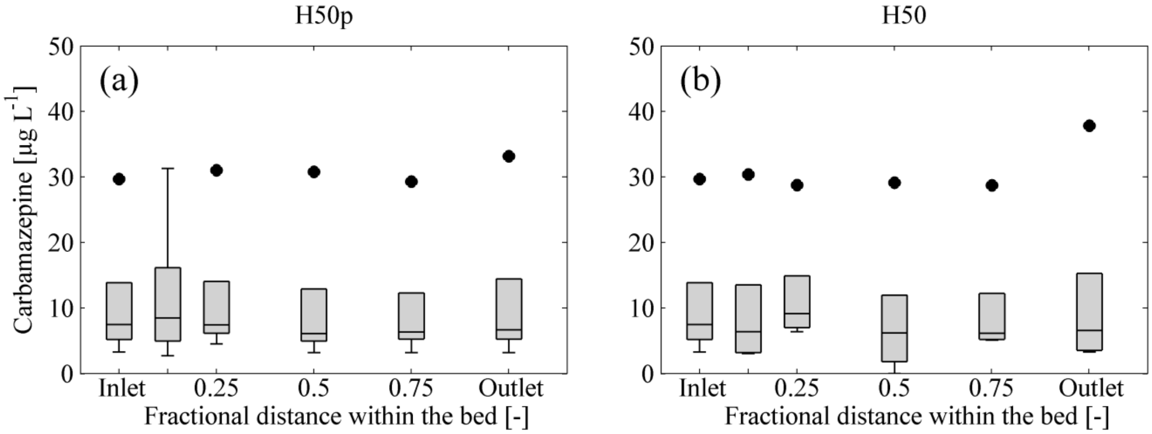


Figure 20 Distribution of the studied neuroleptic drug carbamazepine concentration along the flow path during period I (black dots indicate outliers).

Although carbamazepine was slightly removed with 25% (H50p) and 18% (H50) in terms of loads (Table 7), the limited concentration decrease along the flow path seems to be attributed to the high organic load and the short HRT of the CWs. In other wastewater treatment facilities, carbamazepine has also shown low removals. For instance, Matamoros et al., (2005) observed a removal of carbamazepine of 26% in a conventional HSSFCW. In addition, low removals of carbamazepine has been observed in conventional WWTPs. Joss et al., (2005) reported a removal of carbamazepine < 10% whereas Vieno et al., (2007) reported increases of carbamazepine at the effluent of a WWTPs in Finland.

Triclosan also did not show a significant change in its concentration along the flow path (Figure 21 a and b) most likely attributed to its persistent behavior (Ying et al., 2007). From the Figure 21 only the effect of over-concentration of the compound due to water loss by evapotranspiration can be observed.

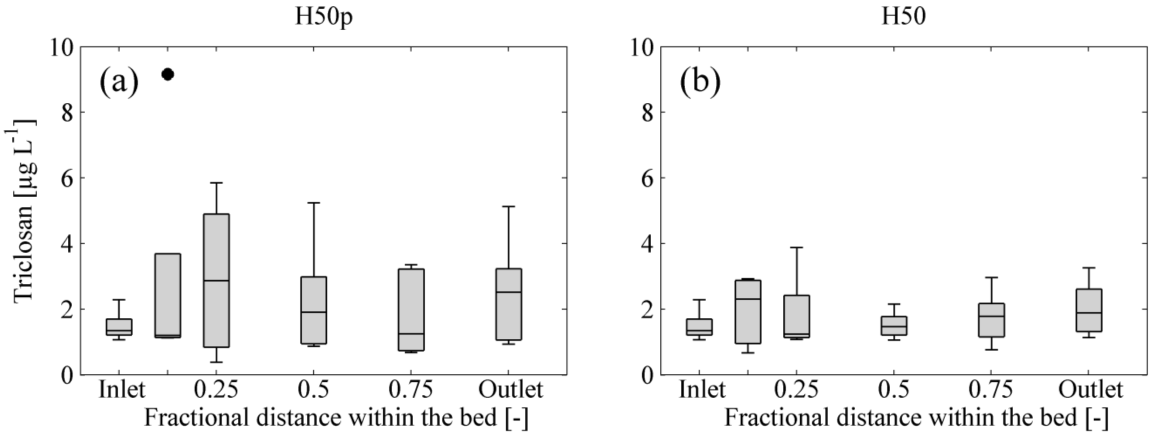


Figure 21 Distribution of the antibacterial compound triclosan concentration along the flow path during period I (black dot indicates outlier).

3.3.1.2 Endocrine disrupting chemicals (EDCs)

Galaxolide and tonalide, polycyclic musk fragrances applied in household and personal care products are suspected to pose endocrine disrupting effects (Chase et al., 2012). Their internal behavior in the wetland systems was quite similar (Figure 22 a b c and d). However, the concentrations of tonalide were in average 10 times less than those for galaxolide caused by their different amounts applied initially in the different household goods. The fluctuations of these compounds within the CWs can be attributed to the variation of the inflow during the time of investigation (Period I). Both compounds are favored transformed by oxidative reactions. For instance, the degradation product of galaxolide “galaxolidone” has been observed to increase in the effluents of WWTPs from to be not detected in the inflow up to 280 ng L^{-1} in the outflow (Vallecillos et al., 2014). In this investigation, the low availability of oxygen in the CWs limited the removal of both fragrance compounds as was observed in Figure 22 and Table 7.

The concentrations of the EDCs bisphenol A and 4-nonylphenols showed limited removal along the flow path, too (Figure 23 a b c and d). Actually, the concentrations of these compounds increased at the outflow with respect to the inflow concentrations, leading to significant increments ($p<0.05$) of 4-nonylphenols at the outflow of the H50 (Figure 23 d). This particular behavior of significant concentration increase could be probably caused by a slow degradation and the additional formation of 4-nonylphenols from the respective nonylphenol polyethoxylate surfactants in the course of biodegradation (Klecka et al., 2010).

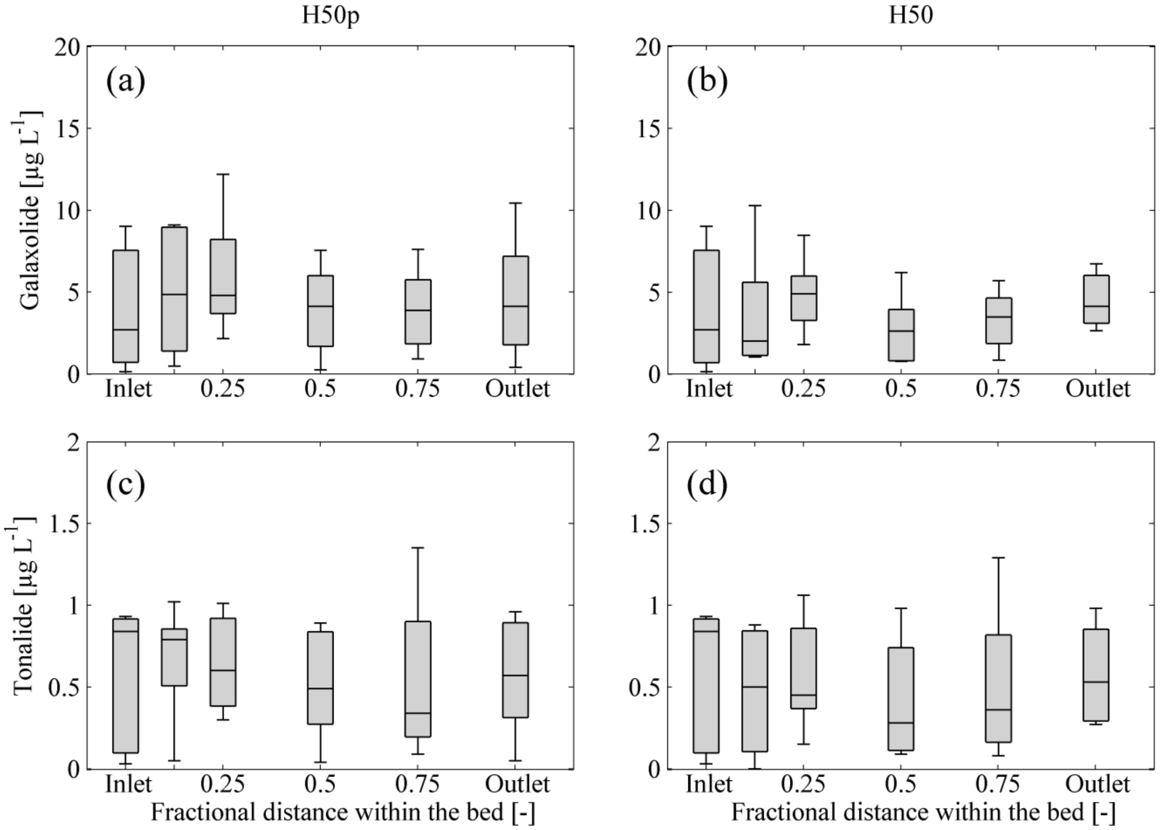


Figure 22 Distribution of galaxolide and tonalide concentrations along the flow path during period I (black dots: outliers).

Bisphenol A (Log K_{oc} of 2.17) and 4-nonylphenols (Log K_{oc} of 3.97) are characterized by high organic carbon partition coefficients (Log K_{oc}) (Table 1), that point to their affinity to be sorbed on carbon rich surfaces. Thus, these two compounds are expected to stay longer in the wetland as compared to other much more polar one like caffeine (Log K_{oc} of 1) which can be easily transported with water through the porous media of the HSSFCW. The slow transport of Bisphenol A and 4-nonylphenols within the CWs as oppose to caffeine reflects the heterogeneity of retardation factors within the CWs and the complexity of degradation processes (Imfeld et al., 2009).

The Table 8 shows the results of the whole statistical investigation corresponding to the period I. Either reduction or increase of the OMPs concentrations as a result of the transit through the H50p and the H50 can be elucidated by looking at the p-values marked with asterisks (Table 8). Over the all investigated OMPs, the behavior of the selected anti-inflamatory drugs and caffeine in the selected sampling locations at 0.50 and 0.75 fractional distances and at the outflow showed relevant decrease of concentrations. For the rest of the OMPs, the results indicated less removal efficiencies.

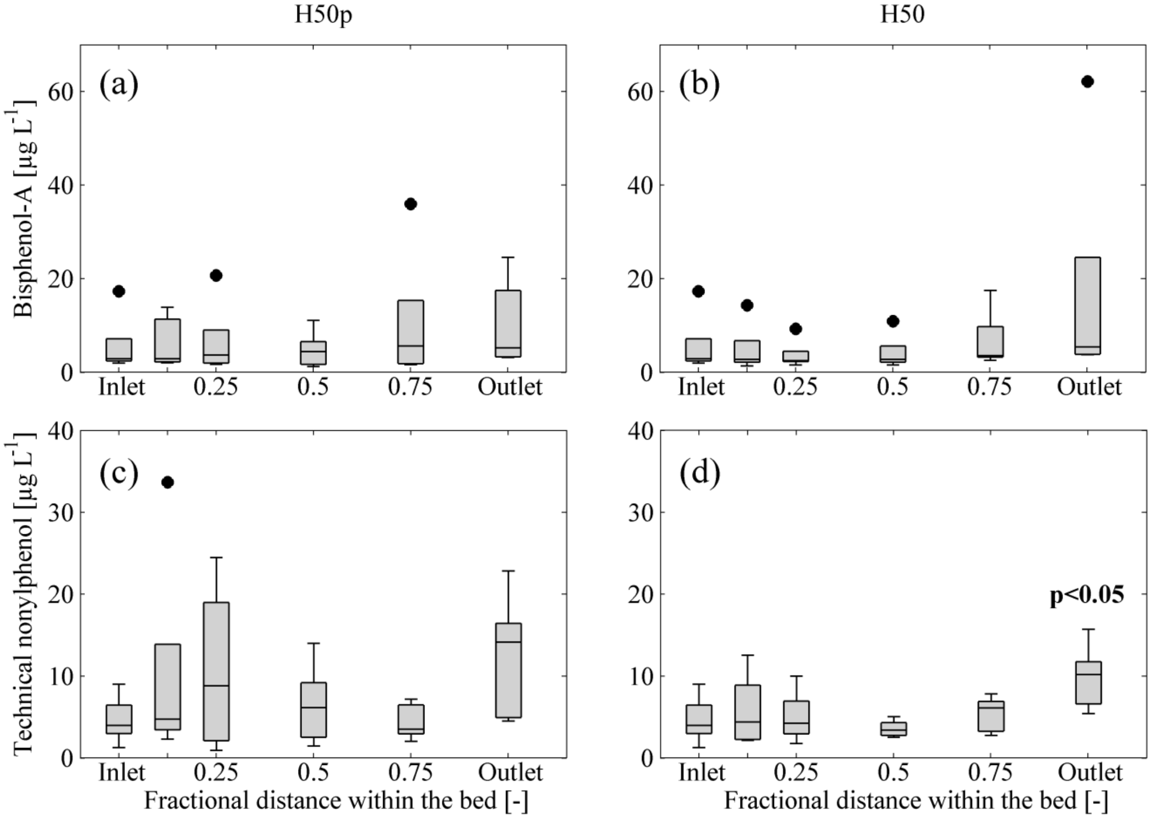


Figure 23 Distribution of bisphenol A and (technical) 4-nonylphenol concentration along the flow path during period I (black dots: outliers).

Based on the results achieved during period I, the mentioned sampling locations were further investigated in Periods II and III. Thus, the influence of different redox conditions (in dependence on the depth) and the same conditions under a different plant status (maximum growth) was evaluated. Results of periods II and III are discussed based on the statistical analysis.

Table 8 Kruskal-Wallis test of OMPs concentrations between the inflow and the samplings locations 0.13, 0.25, 0.50, 0.75 (fractional distance within the bed) and the outflow in the period I at the sampling depth of 0.13 m.

	Organic micropollutants										
	Neutral compounds				Polar compounds						
H50p	**Galaxolide**	**Tonalide**	**Caffeine**	**Carbamazepine**	**Ibuprofen**	**4-nonylphenols**	**Naproxen**	**Triclosan**	**Bisphenol-A**	**Ketoprofen**	**Diclofenac**
0.125	0.6015**	0.9168*	0.6015**	0.9168**	0.9168**	0.6015**	0.7540*	0.9168*	0.9168	0.1732*	0.4647**
0.25	0.4647**	0.7540*	0.4647*	0.9168*	0.7540**	0.6015**	0.6015**	0.6015**	0.9168**	0.0445*	0.6015**
0.50	0.9168**	0.6015*	**0.0160***	0.6015*	0.7540**	0.6015**	0.9168*	0.9168*	0.9168**	0.9168*	0.6015**
0.75	0.7540**	0.9168*	**0.0163***	0.6015*	0.6015*	0.7540*	0.3472*	0.7540*	0.8340**	0.5296*	0.2506*
Outflow	0.7540**	0.9168*	**0.0163***	0.9168*	0.6015**	0.0758**	0.4647**	0.4647**	0.1745**	0.7533*	0.9168**
H50											
0.125	0.7540*	0.4647*	0.2506*	0.6015*	0.9168**	0.7540**	0.9168*	0.6015**	0.7540*	0.3457*	0.7540**
0.25	0.6015**	0.9168*	0.0472*	0.4647**	0.9168**	0.7540**	0.3472*	0.9168*	0.4020*	0.1732*	0.9168*
0.50	0.6015*	0.8340*	**0.0163***	0.3472*	**0.0472***	0.4647*	**0.0283***	0.9168**	0.7540*	**0.0278***	**0.0283***
0.75	0.9168**	0.9168*	**0.0163***	0.6015*	0.2506*	0.7540**	**0.0472***	0.6015**	0.4647**	**0.0150***	**0.0283***
Outflow	0.7540**	0.9168*	**0.0283***	0.8340*	0.9168*	**0.0472****	0.4647*	0.2506**	0.0758**	0.3472*	0.7540*

n = 5

*marks removal: The median of the inlet was bigger than the median of the treatments (sampling points 0.125, 0.25. 0.50, 0.75, outlet).

**marks increase: The median of the treatment (sampling points 0.125, 0.25. 0.50, 0.75, outlet) was higher than the median of the inlet.

No asterisk indicates that the medians between the inlet and the treatment were the same.

Red values indicate significant differences.

3.3.2 Period II (2012/April-May)

The results of the statistical investigation during the Period II are presented in the Table 9 (for the sampling points at 0.5 and 0.75 fractional distance from inlet at 0.13 m depth) and Table 10 (for the same sampling points at 0.4 m depth). The results of showed that only caffeine and galaxolide showed significant concentration changes. A significant concentration decrease was found for caffeine only at the outflow of the H50p during the period II. In comparison to the inlet, increased concentrations of Galaxolide were observed at the sampling location 0.75 fractional distance from the inlet in the H50 (water depth of 0.13 m) and at the same sampling point in the H50p (water depth of 0.4 m)

Apart of that, none other significant removal was observed at the selected sampling points, neither at 0.13 m depth nor at 0.4 m depth. Raw data of the concentrations used for the statistical investigation are shown in Table A19 to A26 of the appendix. A short description of the relevant findings is presented below the table with a description of whether the compounds showed concentration increase/decrease.

Table 9 Kruskal-Wallis test of OMPs concentrations between the inflow and the samplings locations 0.50, 0.75 (fractional distance within the bed) and the outflow in the period II at the sampling depth of 0.13 m.

	Organic micropollutants										
	Neutral compounds				Polar compounds						
H50p	**Galaxolide**	**Tonalide**	**Caffeine**	**Carbamazepine**	**Ibuprofen**	**4-nonylphenols**	**Naproxen**	**Triclosan**	**Bisphenol-A**	**Ketoprofen**	**Diclofenac**
0.5	0.0433**	0.5590**	0.0833*	0.3865**	0.3865**	0.7728**	0.1489**	0.3836*	1.0000*	0.7728**	0.1489**
0.75	0.5637**	0.5614**	0.0833*	0.8845*	0.3865*	0.3865*	0.7728*	0.7728*	0.7728*	0.7728**	1.0000**
Outflow	0.7728**	0.5614*	**0.0209***	1.0000**	1.0000*	0.3865**	0.3865**	0.3865*	0.2482*	0.3865**	0.7728*
H50											
0.5	0.0833**	0.2454**	0.0833*	0.2482**	0.3865**	0.3865**	0.3865**	0.3865*	0.3865**	0.5637**	0.2482**
0.75	**0.0209****	0.1465**	0.2482*	0.1489**	0.5637*	0.3865**	0.5637**	0.3865*	0.5637**	0.7728**	0.2482**
Outflow	0.5637**	1.0000**	0.0833*	0.7728**	0.7728*	1.0000*	1.0000*	0.3865*	0.3865**	0.3865**	0.7728*

n = 4

Table 10 Kruskal-Wallis test of OMPs concentrations between the inflow and the samplings locations 0.50, 0.75 (fractional distance within the bed) and the outflow in the period II at the sampling depth of 0.4 m.

	Organic micropollutants										
	Neutral compounds				Polar compounds						
H50p	**Galaxolide**	**Tonalide**	**Caffeine**	**Carbamazepine**	**Ibuprofen**	**4-nonylphenols**	**Naproxen**	**Triclosan**	**Bisphenol-A**	**Ketoprofen**	**Diclofenac**
0.5	0.0833**	0.3836**	0.1489*	0.0833**	0.3865**	0.1489**	0.1489**	0.3865**	0.1489**	0.5637**	0.0833**
0.75	**0.0433****	0.5590**	0.0833*	0.5637**	0.5637*	0.7728**	0.5637**	0.3865**	0.5637**	0.3865**	0.2482**
Outflow	0.7728**	0.5614*	**0.0209***	1.0000**	1.0000*	0.3865**	0.3865**	0.3865**	0.2482**	0.3865**	0.7728*
H50											
0.5	0.1489**	0.3836**	0.2482*	0.2482**	1.0000*	0.7728**	0.5637*	0.3865**	1.0000**	0.5637**	0.5637**
0.75	0.2482**	0.2454*	0.2482*	0.8845**	1.0000*	1.0000**	0.7728*	0.3865**	1.0000**	0.3865**	0.5637**
Outflow	0.5637**	1.0000**	0.0833*	0.7728**	0.7728*	1.0000**	1.0000*	0.3865**	0.3865**	0.3865**	0.7728*

n = 4

*marks removal: The median of the inlet was higher than the median of the treatment (sampling points 0.50, 0.75, outlet).

**marks increase: The median of the treatment (sampling points 0.50, 0.75, outlet) was higher than the median of the inlet.

Red values indicate significant differences.

3.3.3 Period III (2012/July-August)

The results of Period III are presented in the Table 11 (for the sampling points at 0.5 and 0.75 fractional distances to inlet at 0.13 m depth) and Table 12 (for the same sampling points at 0.4 m depth). Raw data of the concentrations used for the statistical investigation are shown in Table A27 to A34 of the appendix. The internal behavior on the OMPs can be evaluated from the p-value of the Tables. Bisphenol A, caffeine, naproxen, ketoprofen, 4-nonylphenols and triclosan displayed significant concentrations reductions or increments within the CWs.

The concentration of caffeine was significantly reduced at the sampling locations at 0.5, 0.75 and the outflow of the H50p and H50 for both sampling depths (0.13 and 0.4 m) which indicates a sufficient environment for removal of the easy degradable caffeine. For a significant reduction of naproxen**,** proper conditions were found in the unplanted bed (H50) at sampling location 0.5 fractional distances to the inlet and at a water depth of 0.13 m. An increase of the concentrations of bisphenol A, ketoprofen, 4-nonylphenols and triclosan were observed mainly near the outflow and in the upper zone (0.13 m) of both wetland beds.

With regard to the reproducibility of the results between Period I and III, only for caffeine and partially for naproxen could be demonstrated. The higher number of significant concentration increases found in Period III (Tables 11 and 12) compared to Period I (Table 8) suggest once more that the temporal composition of the inflow municipal wastewater was involved in the significant concentration reductions found in Period I.

Table 11 Kruskal-Wallis test of OMPs concentrations between the inflow and the samplings locations 0.50, 0.75 (fractional distance within the bed) and the outflow in the period III at the sampling depth of 0.13 m.

Organic micropollutants											
	Neutral compounds				Polar compounds						
H50p	**Galaxolide**	**Tonalide**	**Caffeine**	**Carbamazeine**	**Ibuprofen**	**4-nonylphenols**	**Naproxen**	**Triclosan**	**Bisphenol-A**	**Ketoprofen**	**Diclofenac**
0.5	0.3865**	0.3865**	**0.0139***	0.3173**	0.2482**	0.0833**	0.2482**	**0.0209****	0.7728*	0.1886**	0.0833**
0.75	0.5637**	0.4678**	**0.0139***	<LOD	0.5637*	**0.0209****	0.2482*	0.0833**	1.0000**	0.2454**	0.7728*
Outflow	1.0000*	0.7728**	**0.0139***	<LOD	0.3865**	**0.0209****	0.3865**	0.1489**	**0.0433****	**0.0194****	0.1489**
H50											
0.5	0.5637**	0.3865**	**0.0139***	<LOD	0.0833*	0.2482**	**0.0209***	**0.0209****	0.7728**	0.3211*	0.5637*
0.75	0.5637*	0.4678**	**0.0139***	<LOD	0.3865*	0.3865**	0.5637*	0.1102**	0.2482**	0.5385**	0.3865**
Outflow	0.7728**	0.7728**	**0.0139***	<LOD	1.0000*	0.0833**	0.5637**	0.2482**	**0.0209****	0.1465**	0.3865**

n = 4

Table 12 Kruskal-Wallis test of OMPs concentrations between the inflow and the samplings locations 0.50, 0.75 (fractional distance within the bed) and the outflow in the period III at the sampling depth of 0.4 m.

Organic micropollutants											
	Neutral compounds				Polar compounds						
H50p	**Galaxolide**	**Tonalide**	**Caffeine**	**Carbamazepine**	**Ibuprofen**	**4-nonylphenols**	**Naproxen**	**Triclosan**	**Bisphenol-A**	**Ketoprofen**	**Diclofenac**
0.5	0.7728	0.6631**	**0.0139***	<LOD	1.0000**	0.1913**	1.0000**	0.2454**	0.7728**	0.1391**	0.2482**
0.75	0.7728**	0.3865**	**0.0139***	<LOD	0.2482**	0.2482**	0.5637**	0.1489**	0.7728**	0.2454**	0.1489**
Outflow	1.0000*	0.7728**	**0.0139***	<LOD	0.3865**	**0.0209****	0.3865**	0.1489**	**0.0433****	**0.0194****	0.1489**
H50											
0.5	0.7728**	0.3865**	**0.0180***	<LOD	0.5637**	0.7728**	0.7728**	0.0833**	0.3865*	0.3749**	0.3865**
0.75	0.3865**	0.3865**	**0.0139***	<LOD	0.5637**	0.2482**	1.0000**	0.0814**	0.7728*	0.3749**	0.3865**
Outflow	0.7728**	0.7728**	**0.0139***	<LOD	1.0000*	0.0833**	0.5637**	0.2482**	**0.0209****	0.1465**	0.3865**

n = 4

*marks removal: The median of the inlet was higher than the median of the treatment (sampling points 0.50, 0.75, outlet).

**marks increase: The median of the treatment (sampling points 0.50, 0.75, outlet) was higher than the median of the inlet.

No asterisk indicates that the medians between the inlet and the treatment were the same.

Red values indicate significant differences.

<LOD means below the limit of detection of the instrument.

As a summary of the investigation during periods I, II and III, Table 13 shows the sampling zones where selected OMPs were significantly reduced. In period I, most of the significant removals were observed with respect to period II and III. Except of caffeine in the Period III, significant concentration reductions of the OMPs in dependence on the depth in the CW could not be demonstrated. Even though no significant concentration reductions were found in the H50p, in terms of load, the H50p performed better than the H50 (Figure 11, Table 7). Thus, the absence of significant concentration reductions in the H50p could be associated to over-concentration of the OMPs in the internal pore water samples resulted from water loss by evapotranspiration. This particularity of planted wetlands highlights once more the complexity and the challenge to understand the internal removal behavior of the OMPs.

Table 13 Internal sampling locations showing preferential removal of the OMPs in the HFCWs.

	Sampling location	
Organic micropollutant	**H50p**	**H50**
Caffeine	0.50, 0.75	0.50, 0.75
Ibuprofen	-	0.50
Diclofenac	-	0.50, 0.75
Naproxen	-	0.50, 0.75
Ketoprofen	-	0.50, 0.75

Remark: The sampling locations correspond to the depth 0.13 m.

Another aspect to consider for understanding the internal behavior of the selected OMPs in the wetlands is the transport of the compounds through the porous media of the CWs. The increase of the organic carbon/water partition coefficient (Log K_{oc}) of the liphophilic compounds such as galaxolide (Log K_{oc} of 4.86) and tonalide (Log K_{oc} of 4.8) may have increased the retardation factor of those compounds in the CWs (Xu et al., 2009b; Imfeld et al., 2009). In addition, other compounds like 4-nonylphenols, bisphenol A and triclosan are also expected to stay longer in the wetland as a result of their physicochemical property (Log K_{oc}) when compared to polar and good water soluble substances like caffeine (Log K_{oc} of 1) which are instead easily transported through the porous media of the CWs.

Ibuprofen (Log K_{oc} of 2.59) was found to be easily transported in agricultural soils whereas triclosan (Log K_{oc} of 4.26) and bisphenol A (Log K_{oc} of 2.17) were readily retarded in the tested soils (Xu et al., 2009b). The unknown residence times of most of the target substances makes a relation to the initial concentration difficult as well as the evaluation of their removal efficiency since along the flow path certain removal occurs simultaneously with the transport process.

Along the flow path of the investigated CWs, the nutrient content in the water changes as a result of the treatment process. Such effect may have induced prefential growth of the plants along the flow path, as was observed during summer time 2011 (Figure 4). Because, plant density and growth influence (i) the establishment of different microbial communities, (ii) the surface area for adsorption of OMPs, (iii) the redox condition in the rizhosphere and (iii) the root exudation and oxygen transfer rates. It can be concluded that the physiological status of the plants was involved on the degradation/transformation of OMPs and may have resulted in the establishment of preferential zones of removal.

3.3.4 Relations between redox processes at the CW system scale and the behavior of the selected organic micropollutants

When the inflow wastewater and the outflow of the H50p and H50 were analyzed in terms of the temporal evolution of the redox potential, fluctuations in dependence of the season were observed (Figure 24 a and b). In fact, a variability of the inflow redox potential of σ^2 = 35.51 mV, (n = 17) was found (Figure 24 a). In addition, higher variability of the redox potentials was found at the outflow of the H50p (σ^2 = 41.25 mV, n = 17) compared to the outflow of the H50 (σ^2 = 20.15 mV, n = 17) (Figure 24 b). While the higher variability in the H50p could be attributed to the presence of the plants which provided an extra amount of carbon into the system, the lower variability observed in the H50 could be the result of the lower water temperatures connected to increased solubility of oxygen in the water. Indeed, the average water temperature in the outflow of the H50p was 16 °C (σ^2 = 7.5 °C, n = 18) which was higher than the average value in the H50 of 14.2 °C (σ^2 = 6.8 °C, n = 18). The reason of the higher temperatures in the H50p compared to the H50 could be the result of the plant covering that insulates the subsurface of planted CW whereas the unplanted bed lacks of this feature (Brix, 1994).

Furthermore, temporal pulses of the redox potential in the inflow and outflow of both systems were observed (Figure 24 a and b). These pulses appeared in the months of December, April-

May and July-August and were more pronounced in the H50p than in the H50 (Figure 24 b). The observed pulsing behavior of the redox potential also reflects the temporal changing composition of the inflow wastewater which is expected to influence the microbial communities within the CWs and therefore the seasonal removal efficiency of the investigated OMPs.

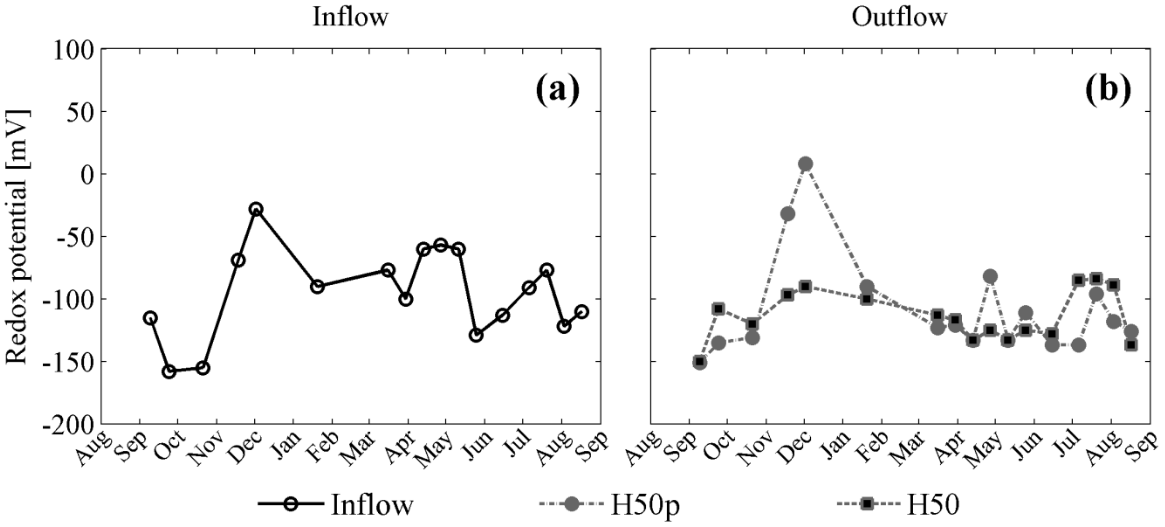


Figure 24 Inflow and outflow redox potentials in the H50p and H50 beds in dependence of the season.

Indeed, the high variability of the redox potential observed in the outflow samples of both H50p and H50 went along with a high variability of the OMP outflow concentration as exemplarily demonstrated for ibuprofen, diclofenac, ketoprofen and naproxen (Figure 25 a and b). The outflow concentrations of the pharmaceuticals ibuprofen, naproxen, ketoprofen and diclofenac displayed higher variation (1-250 µg L^{-1} concentration range) during the three periods I, II and III in the planted wetland (H50p) than in the unplanted one (H50) (Figure 25 a and b). For instance, in the H50p the variation of ibuprofen was σ^2 = 102.02 µg L^{-1} (Period I), followed by σ^2 = 43.61 µg L^{-1} (Period II) and finally σ^2 = 27.50 µg L^{-1} (period III). In the H50 the variability of ibuprofen was observed in Period II (σ^2 = 43.19 µg L^{-1}) followed by Period I (σ^2 = 32.79 µg L^{-1}) and finally period III (σ^2 = 14.63 µg L^{-1}).

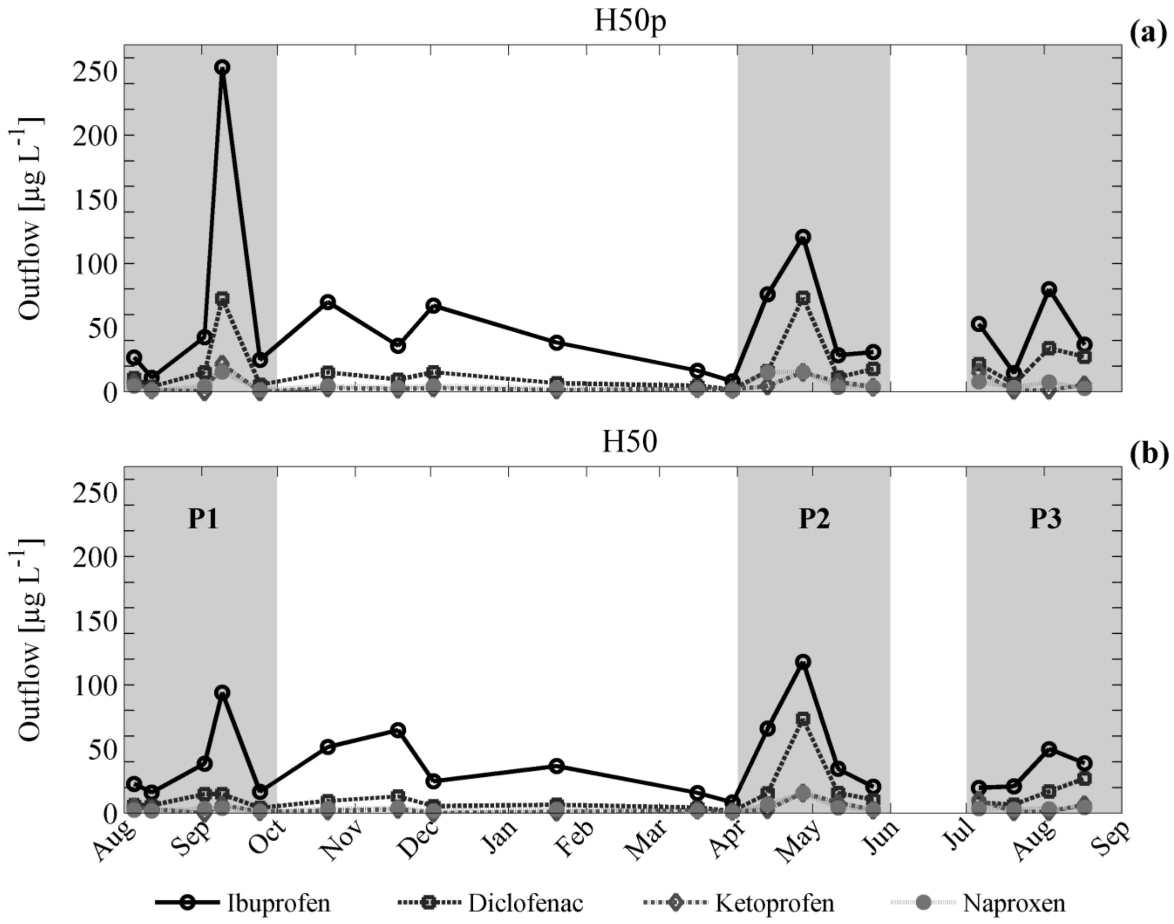


Figure 25 Outflow concentrations of the anti-inflamatory pharmaceuticals in the planted (H50p) and unplanted (H50) HSSFCWs.

The inflow concentrations of the four anti-inflamatory drugs showed a seasonal variability during the twelve-month of study (Figure 19) surely predetermined by varying consumption within the seasons (Ternes and Joss, 2006). As the variation of the inflow concentration was the same for both systems (1-100 µg L^{-1} concentration range), these results support the previous hypothesis implying that inflow variability of the wastewater composition interfered with the concentration reduction of the target substances at internal sampling locations. In addition, as the sampling strategy of both outflows (H50p and H50) was the same, the higher variability of the outflow concentrations (Figure 25 a and b) when compared to the inflow (Figure 19) could be the result of (i) water loss by evapotranspiration and/or (ii) possible proceeses like de-conjugation and desorption processes.

Imfeld et al., (2009) described that redox processes occurring at the wetland system scale (macro gradients in flow path and depth) influence biochemical reactions that affect the removal of organic contaminants. In order to further understand the internal removal behavior of naproxen, ketoprofen and diclofenac, the total nitrogen (TN) and sulfide (S^{2-}) were investigated at the 0.75 sampling location in the H50p and the H50 to elucidate potential reasons why these group anti-inflamatory drugs was removed in that selected zone. In this work, some relations between the behavior of the OMPs and selected wastewater parameters could be stablished. They are presented and dicussed in the following section in regard to the individual wetland beds H50p and H50.

3.3.4.1 The planted HSSFCW (H50p)

The dynamics of the redox indicator sulfide (S^{2-}) and the wastewater parameter total nitrogen (TN) were investigated at the 0.75 sampling location in both H50p and H50. TN data include not only the inorganic nitrogen compounds (ammonia, nitrate and nitrite) but also organic nitrogen arising from proteins, plant and microorganism which are transformed during wastewater passage through the CWs. The TN and sulfide concentrations in the H50p are shown in Figure 26 a and c (sampling location 0.75). Over the twelve months period, the dynamics of TN and sulfide were similar except between the months January and May which covers a part of the studied Period II (April-May 2012). During January and May, the concentrations of TN and sulfide decreased more in the upper zone (0.13 m depth) than at the bottom at 0.4 m depth. With the plant sprouting in May, the TN and sulfide concentrations increased in the upper zone more clearly than in the lower layer probably caused by a small, but significant C-flux from the plant roots e.g., of root exudates (Farrar et al., 2003) and the increasing bacterial growth. As known from former investigations, the intermittent release of rhizodeposition products in CWs is varying throughout the year (Stottmeister et al., 2003) and occurs favoured in the near-root zone (e.g. the sampling depth of 0.13m). The stronger bioactivity in this upper zone by plants and bacteria may stimulate the removals of OMs. In contrast, close to the bottom of H50p (at 0.4 m depth), the influence of plant roots concerning oxygen supply is less marked, and reductive conditions were favored.

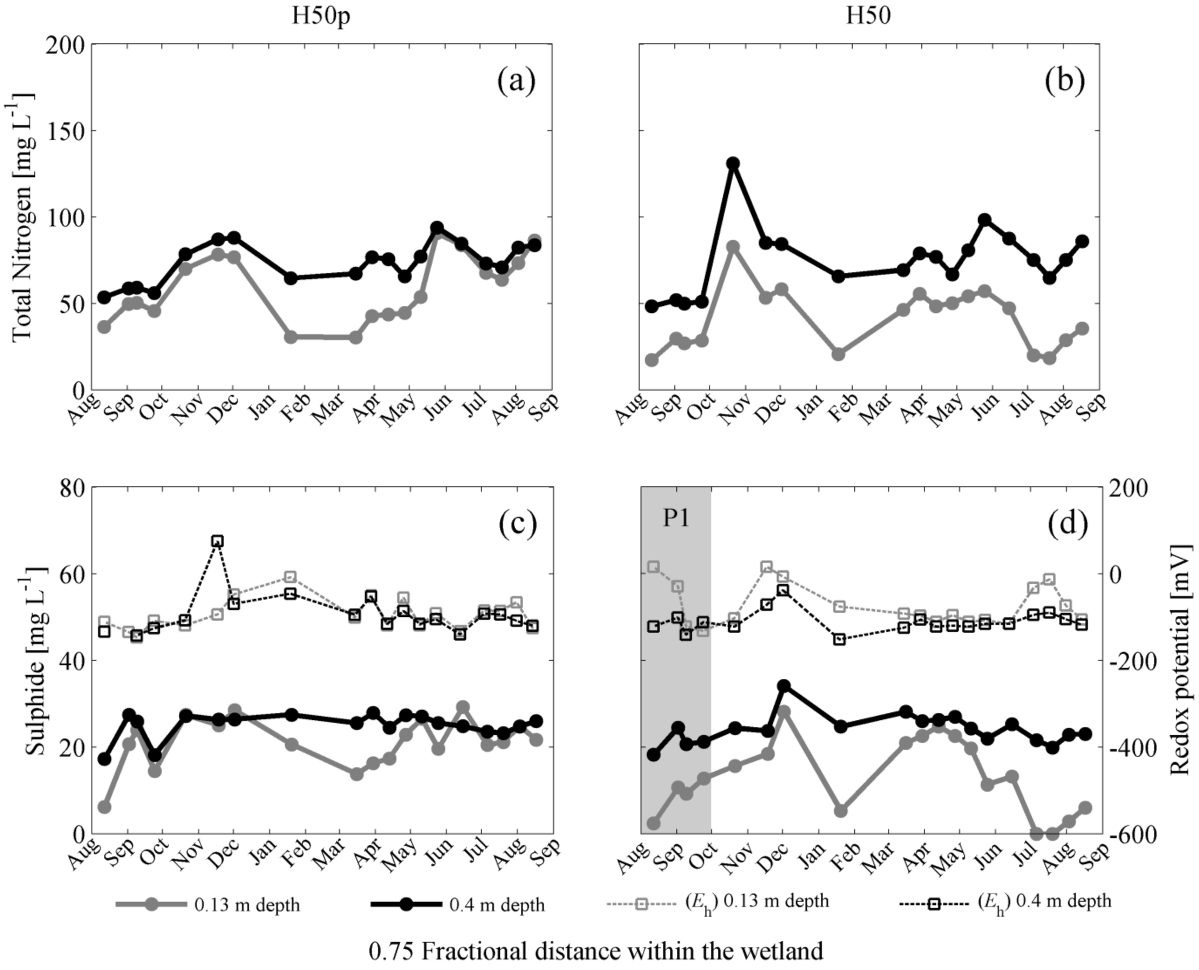


Figure 26 Temporal behavior of TN and S^{2-} in the planted and unplanted HSSFCWs at two different depths and at 0.75 fractional flow distance (The gray area denotes Period I. Panel (a) and (b) show the temporal behavior of TN in the H50p and the H50, respectively whereas panels (c) and (d) show the temporal behavior of S^{2-} in the H50p and the H50).

From the prevalent reductive conditions in H50p (Figure 24) in the range of -123 mV (Table 3) the mass removal of the three analgesics seem to be triggered by anaerobic processes. Moreover, two positive correlations were found. Correlation analysis between the OMPs (naproxen, ketoprofen and diclofenac) and the wastewater parameters (TN and sulfide) revealed a similar trend between naproxen and sulfide concentrations at the sampling depth 0.13 m ($\rho = 0.6594$; $p = 0.0037$; $n = 18$) and between diclofenac and sulfide ($\rho = 0.6512$; $p = 0.0043$; $n = 18$) (Figure 27). Furthermore, at the sampling depth 0.4 m a positive correlation between diclofenac and sulfide

($\rho = 0.8810$; $p = 0.0072$; $n = 8$) was found. Correlations between the concentrations of naproxen, ketoprofen, diclofenac and the TN content were not observed.

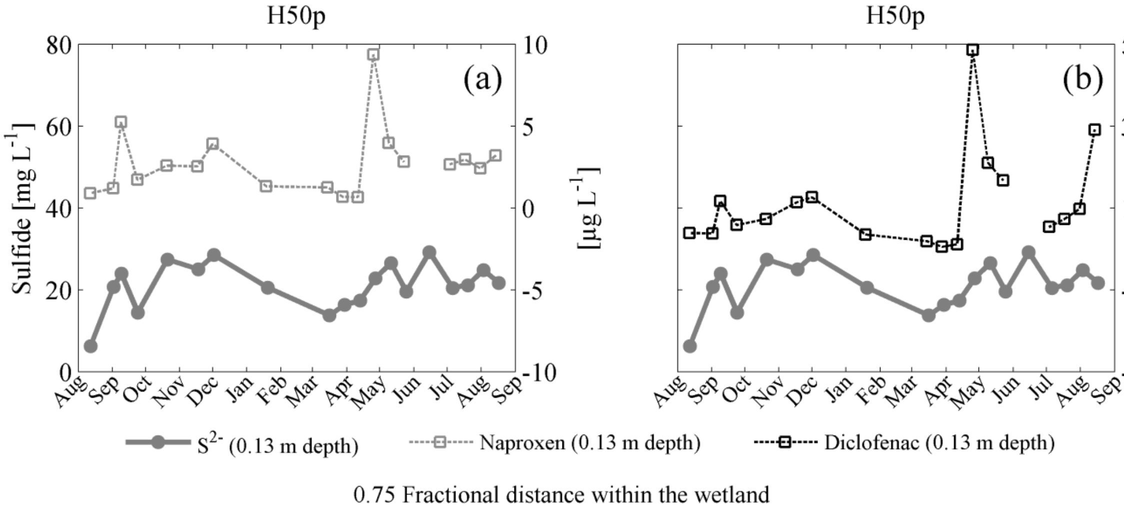


Figure 27 Correlations between the concentrations of naproxen and sulfide (a) and diclofenac and sulfide (b) in the H50p in dependence on the season.

Based on (i) the previous investigations by Avila et al. (2010) who indicated that also under anaerobic conditions naproxen and diclofenac could be removed in HSSFCWs, (ii) the fact that microbial activity of sulfate-reducing bacteria can be monitored by measuring an increase/decrease in the sulfide concentration (Annweiler et al., 2001), (iii) the low concentrations of naproxen and diclofenac around 1000 times lower (µg L^{-1}) than the sulfide concentrations (mg L^{-1}) that do not support biomass growth and the induction of the relevant degradative enzymes (Tran et al., 2013) and (iv) considering the high organic load of the wetlands (growth substrate was not a limited factor), it can be concluded that the dynamics of sulfate-reducing bacteria, predominating in the H50p may be involved in the removal of naproxen and diclofenac and that co-metabolism could be a potential removal pathway.

Bacterial co-metabolism of organic compounds is well decribed in literature (Onesios et al., 2009; Tran et al., 2013). Particularily, co-metabolic transformation/degradation of naproxen, ketoprofen and diclofenac has been reported in the model batch experiments running under aerobic conditions and municipal wastewater (Quintana et al., 2005). The authors found that ketoprofen followed an aerobic metabolic transformation route; naproxen followed an aerobic co-metabolic complete biodegradation route whereas diclofenac was proposed to follow an anaerobic pathway driven by reductive dehalogenation (Schwarzenbach et al., 2003). No publication has reported so far about possible aerobic or anaerobic co-metabolism of OMPs in CWs.

Although under the operational conditions of this study the removal rates of OMPs in the H50p and H50 due to the aerobic biodegradation can be expected to be low, the plant roots do release oxygen (Brix, 1997), and the contribution of aerobic bacteria to the removal of the OMPs is probable to occur as occurred for ibuprofen in the microcosms HSSFCW experiments of Matamoros et al., (2008). Suárez et al. (2010) reported that diclofenac is highly biodegradable under aerobic (nitrifying) conditions but relatively persistent under anoxic (denitrifying) conditions suggesting that diclofenac is positively affected by the development of nitrifying biomass. However, long term experiments under oxic conditions resulted in a limited removal of diclofenac (Zwiener and Frimmel, 2003). The findings of Zwiener and Frimmel, (2003) are in agreement with Quintana et al., (2005) who also noticed a limited removal of diclofenac. From these results, it can be concluded that the removal of some OMPs such as diclofenac seems to be fast under aerobic conditions but can also proceed under anaerobic conditions. The observed low removal efficiency of 14% for diclofenac in H50p under high organic load conditions (Table 7) and the correlations with sulfide amounts, strengthens the assumption of a potential anaerobic degradation of diclofenac in CWs, also indicated in literature (Verlicchi and Zambello, 2014).

3.3.4.2 The unplanted HSSFCW (H50)

Internal sampling in H50 showed that the concentrations of naproxen, ketoprofen and diclofenac were significantly reduced in the upper zone (0.13 m depth), at a fractional distance of 0.75 from the inflow (Section 3.3.1.1). A correlation between the concentrations of naproxen, ketoprofen and diclofenac and the TN and sulfide contents was not found. However, some relationships between the dynamics of TN and sulfide and the concentration reduction of naproxen, ketoprofen, and diclofenac became obvious. Stratification of both TN and sulfide occurrence depending on the sampling depths was observed over the twelve months of investigation (Figure

26 b and d). During the studied Period I (indicated in Figure 26 as the gray area) at 0.13 m depth, the concentration of sulfide was lower at a depth of 0.13 m than in the deeper part of the bed (Figure 26 d). Additionally, in the upper zone less reductive conditions were measured (Figure 26 d), and consequently a lower concentration of TN was observed (Figure 26 b). At the deeper internal sampling location (0.4 m depth), the sulfide concentration was considerably higher (by a factor of 3) compared to the upper zone (Figure 26 d) and the concentrations of TN were higher too (Figure 26 b). These findings revealing less reductive conditions in the upper zone (0.13 m depth) associated with the lower concentrations of TN and sulfide suggest that aerobic degradation/transformation processes might be involved in the significant concentration reductions of naproxen, diclofenac and ketoprofen. Furthermore, the water temperature tends to differ between depths (Dušek et al., 2008) as well as the diffusion rate of oxygen. Thus, implying that the amount of aerobic bacteria in the upper zones should be higher compared to the deeper zone. Moreover, compared to the H50p at the same sampling location less reductive conditions were found in the H50 (Figure 8, Section 3.1) supporting once more (i) the stablishment of different microbial communities between systems and (ii) the probability that more aerobic bacteria were located in the H50 than in the H50p.

As sulfide was detected at the outflow of the H50 (Table 3) and has been reported to be toxic to aerobic bacteria at relatively low concentrations (Wu et al., 2013), it can be assumed that the activity of aerobic bacteria located in the upper zone (0.13 m) was influenced by the amount of sulfide in the H50, therefore suggesting that the presence of sulfide reduces the removal of naproxen, ketoprofen and diclofenac. Contrary, in the deeper zone (0.4 m), the higher concentration of sulfide suggests that anaerobic bacteria dominate and the removal of the trace compounds is associated to anaerobic removal pathways. The significant removal of naproxen, ketoprofen, diclofenac and ibuprofen observed in the H50 suggests that different degradation/transformation routes occur simultaneously within the unplanted bed. These mixed mode mechanisms allow the removal of OMPs even if they have different physicochemical properties (e.g. solubility, chemical structure and Log K_{ow}) and follow different biological removal pathways.

3.3.5 Conclusions part II

The conclusions of this section are summarized as follows:

- Internal zones showing preferential removal of some OMPs (caffeine, ibuprofen, ketoprofen, naproxen and diclofenac) were recognized by means of the statistical analysis (sampling locations 0.5 and 0.75 fractional distance from the inlet and 0.13 m sampling depth).
- Positive correlations between naproxen (sampling depth of 0.13 m), diclofenac (sampling depth of 0.13 m and 0.4 m) and sulfide concentrations were found in the H50p whereas no correlations were found in the H50.
- On the basis of the observed correlations, sulfide is proposed as an indicator of the internal removal of naproxen and diclofenac in CWs, even when operating at high organic load.
- The dynamics of TN and sulfide revealed that redox processes at the CW system scale are involved in the removal of naproxen and diclofenac at the 0.75 fractional distance sampling location.
- Concentration increase of the some OMPs including bisphenol A, 4-nonylphenols and triclosan were found in H50p and in the H50.
- Significant concentration reductions of the OMPs in dependence on the depth in the CW could not be demonstrated.
- The removal of the OMPs in the CWs is attributed to aerobic (in H50) and anaerobic processes (in H50p). In both cases reflected by the different amounts of sulfide.

Chapter 4

Summary and outlook

Many HSSFCWs are in operation worldwide under conditions of high organic load. However, studies on the removal of OMPs under such conditions are rare. Here, the removal of eleven OMPs contained in real municipal wastewater at concentrations range from 1 to 100 µg L^{-1} was investigated in two (planted and unplanted) pilot-scale HSSFCWs. The systems were running under conditions of high organic load. A removal of all studied OMPs was observed in both, the planted and unplanted HSSFCWs. When the average removal of the OMPs over the twelve-month study period was compared using the inflow and outflow mass loads, even under the high organic load conditions, the planted system demonstrated better performance than the unplanted one (load increase was considered as 0% removal). This was the case for all selected OMPs except caffeine which was similarly removed in both HSSFCWs. Higher removals were found during June-October with mean air temperatures of 20 °C than in the months at lower air temperature (November-April) indicating a seasonal dependence of the removals of the OMPs.

Different negative removals of all OMPs were observed. Carbamazepine showed the same percentage of negative removal in the planted and in the unplanted HSSFCW. Higher number of negative removals was found for polar compounds than for the neutral ones in the planted HSSFCW. Moreover, the unplanted bed indicated higher negative removals for the lipophilic compounds and the two EDCs than those observed in the planted one.

The organic mass load showed to be not the only important factor in the performance of HSSFCWs for OMP removal, but also the variation of the inflow concentration. High organic loads applied to CWs may decrease the removal efficiency of OMPs and therefore, potentially increase the number of negative removals. Although a complete explanation of this phenomenon is not available yet, the grab sampling strategy and the not time-adjusted sampling regime could be involved in the ocurrece of this phenomenon. It would be interesting to compare the results of this investigation with one employing composite sampling approach.

The evaluation of the removal behavior of OMPs inside the bed of the CWs has to consider complex interactions of the several parts of the CWs. Particularly, within the planted bed, plant-related factors such as:

1. non-ideal hydraulic flow conditions caused by plant transpiration and the depth-dependent root density
2. potential uptake of certain compounds by the plants and
3. inhomogeneous excretion of oxygen and root exudates caused by depth-dependent root density

seem to superimpose the different effects on the removal of the OMPs, which are difficult to differentiate by means of standard analytical approaches. However, by investigating the redox conditions at the CW system scale and the internal removal behavior of naproxen, ketoprofen and diclofenac some relations were found. In the planted HSSFCW, a positive correlation between concentrations of naproxen, diclofenac and sulfide was found at sampling location 0.75 fractional distance from the inlet (sampling depth of 0.13 m), Moreover, a positive correlation was found between the concentrations of diclofenac and sulfide at the sampling depth of 0.4 m. These results confirmed the findings of Ávila et al., (2010) and suggest that sulfate-reducing bacteria could be involved in the removal of naproxen and diclofenac. Thus, sulfide was proposed as an indicator of the removal behavior of naproxen and diclofenac in CWs, even when operating at high organic load. The results of this thesis open a new question regarding the role of sulfur compounds in CWs on the removal of OMPs. Further attention should be given at the sampling locations 0.50 and 0.75 fractional distance from the inlet (0.13 m depth) to confirm the hypothesis that sulfate-reducing or other anaerobic bacteria were involved in the removal of naproxen and diclofenac.

Contrarily, in the unplanted HSSFCW, partially aerobic conditions were measured which may influence the removal of naproxen, ketoprofen and diclofenac. This was statistically confirmed when the period I was analyzed and the corresponding dynamics of total nitrogen in the unplanted bed. Except of caffeine in the Period III, significant concentration reductions of the OMPs in dependence on the depth in the CW could not be demonstrated (Period II and III) which was attributed to the inflow variations of the OMPs concentrations influencing the treatment efficiency. In order to further understand the internal removal behavior of OMPs in

HSSFCWs, the internal flow rates are needed. Geophysical approaches such as electrical resistivity tomography, internal pore water velocities computed by tracer experiments can help to cope with this problem.

Future research should also focus on the aspect of the formation of OMP metabolites which could also have a biological activity (e.g. toxic effects) to certain microbial communities or plants. Thus, treated CWs effluents need to be more deeply considered through combining traditional chemical analysis with biological effect-directed analysis. Apart from the chemical structure which influences the biodegradation of the OMPs, the aspect of limited bioavailability due to the low OMP concentration (low µg L^{-1} range) should be also considered.

Increasing the amount of wastewater to CWs operating at the design criteria may lead to overloading of the system (organic load > 6 g BOD_5 m^{-2} d^{-1}). From this investigation, it was concluded that despite this undesirable situation, the system will remove OMPs and the plants will support the removal. However, the removals may be low as was found in this investigation. Moreover, care has to be taken regarding the negative removal phenomenon. For further understanding of the removal mechanisms of OMPs in CWs and for enhancing their operation, the origin of the negative removals needs to be further investigated. It would be also interesting to compare the results of this investigation with those findings obtained in CWs operated under low load conditions.

CWs are promising technology for the removal or OMPs contained in wastewater. However, benefits and constrains of this technology need to be discussed before practical implementations. CWs demand low cost of construction and maintenance with respect to advanced physiochemical treatments. But the need of area can be an enormous disadvantage in countries where space is limited or the cost per square meter is high. In this work, the most common type of CW (HSSFCW) was investigated. HSSFCWs can be further optimized to increase their performance by ameliorating their operation conditions or by developing other engineered variants of CWs. More efficient CW techniques are important for the future because the issue of OMPs in the environment and their removal from the wastewater will become compulsory as environmental regulations will become stricter.

Abbreviations

ANA	Department of analytical chemistry
Apr	April
ARSs	Activated Sludge Reactors
Aug	August
BOD_5	Biological oxygen demand (incubation period of 5 days)
BPA	Bisphenol-A
BSTFA	bis-trimethylsilyltrifluoroacetamide
CAS	Chemical Abstracts Service
CAF	Caffeine
CBZ	Carbamazepine
$CBOD_5$	Carbonaceous biological oxygen demand (incubation period of 5 days)
COD	Chemical oxygen demand
CWs	Constructed Wetlands
Dec	December
DIC	Diclofenac
DSR	Dissimilatory sulfate reduction
DT_{50}	Half-life
EC_{50}	Half maximal effective concentration
EDCs	Endocrine disrupting chemicals
EPA	US Environmental Protection Agency
EU	European union
4-NP	4-nonylphenols (technical)
FDB	Fractional Distance within the Bed.
GAL	Galaxolide
GC-MS	Gas Chromatography-Mass Spectrometry
HSSFCWs	Horizontal Subsurface-Flow Constructed Wetlands
H50p	Planted horizontal subsurface-flow constructed wetland
H50	Unplanted horizontal subsurface-flow constructed wetland
HLR	Hydraulic loading rate
HRT	Hydraulic Retention Time
IBU	Ibuprofen
Jan	January
Jul	July

Jun	June
KET	Ketoprofen
K_d	Sorption distribution coefficient
K_{ow}	Octanol/water partition coefficient
K_{oc}	Organic carbon/water partition coefficient
LC-MS-MS	Liquid chromatography–mass spectrometry Tandem mass spectrometry
LOD	Limit of detection
LRB	Langenreichenbach eco-technology research facility
LRB-Team	L. Schultze-Nobre, D. Wang, S. Müller, C. Bartsch, A.-S. Al-Dahoodi, F. Schulz and V. Castillo-Taleno.
NAP	Naproxen
n.d.	no detectable
Nov	November
NPs	Nonylphenols
Mar	March
min	Minutes
MREs	Mass removal efficiencies
MTBE	methyl-*tert*-butyl-ether
Oct	October
OLRs	Organic loading rates
OMPs	Organic Micropollutants
PEC	Predicted environmental concentration
PNEC	Predicted no-effect concentration
PPCPs	Pharmaceutical and Personal Care Products
REACH	European Regulation for Registration, Evaluation, Authorisation and Restriction of chemicals
RSD	Relative standard deviation
SA	Surface area
Sep	September
Sd	Sampling depth
SIM	Selected ion monitoring
SPE	Solid Phase Extraction
SS	Suspended solids
TN	Total nitrogen
TOC	Total Organic Carbon

TON	Tonalide
TRI	Triclosan
UBT	Department of Environmental Biotechnology
UBZ	Center of Environmental Biotechnology
UFZ	Helmholtz Centre for Environmental Research-UFZ
WWTP	Wastewater treatment plant

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Appendix

Table A1 List of priority pollutants included in the European Water Framework Directive.

Number	**CAS Number**	**Substance**
1	15972-60-8	Alachlor
2	120-12-7	Anthracene*
3	1912-24-9	Atrazine
4	71-43-2	Benzene
5	not applicable 32534-81-9	Brominated diphenyletheriv* Pentabromodiphenylether (congener numbers 28, 47, 99, 100, 153 and 154)
6	7440-43-9	Cadmium and its compounds*
7	85535-84-8	Chloroalkanes, C10-13 iv*
8	470-90-6	Chlorfenvinphos
9	2921-88-2	Chlorpyrifos (Chlorpyrifos‐ethyl)
10	107-06-2	1,2-Dichloroethane
11	75-09-2	Dichloromethane
12	117-81-7	Di(2-ethylhexyl)phthalate (DEHP)
13	330-54-1	Diuron
14	115-29-7	Endosulfan*
15	206-44-0	Fluoranthene
16	118-74-1	Hexachlorobenzene*
17	87-68-3	Hexachlorobutadiene*
18	608-73-1	Hexachlorocyclohexane*
19	34123-59-6	Isoproturon
20	7439-92-1	Lead and its compounds
21	7439-97-6	Mercury and its compounds*
22	91-20-3	Naphthalene
23	7440-02-0	Nickel and its compounds
24	**25154-52-3** **104-40-5**	**Nonylphenols*** **(4-nonylphenol)***
25	1806-26-4 140-66-9	Octylphenols (4-(1,1',3,3'-tetramethylbutyl)-phenol)
26	608-93-5	Pentachlorobenzene*
27	87-86-5	Pentachlorophenol
28	not applicable 50-32-8 205-99-2 191-24-2 207-08-9 193-39-5	Polyaromatic hydrocarbons* (Benzo(a)pyrene)* (Benzo(b)fluoranthene)* (Benzo(g,h,i)perylene)* (Benzo(k)fluoranthene)* (Indeno(1,2,3-cd)pyrene)*
29	122-34-9	Simazine
30	not applicable 36643-28-4	Tributyltin compounds* (Tributyltin-cation)*
31	12002-48-1	Trichlorobenzenes
32	67-66-3	Trichloromethane (chloroform)
33	1582-09-8	Trifluralin

*****Identified as priority hazardous substance. Further details can be found in the Annex II of Directive 2008/105/EC (http://ec.europa.eu/environment/water/water-framework/priority_substances.htm) and EU, (2013).

Table A2 Chemical structures of the selected OMPs.

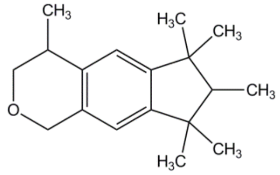
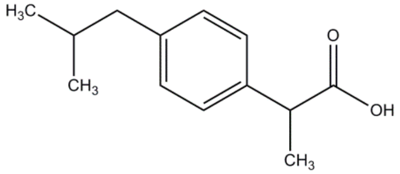
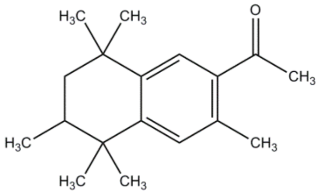
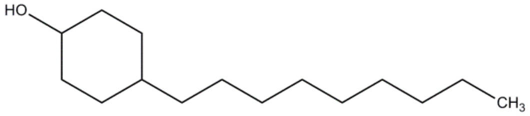
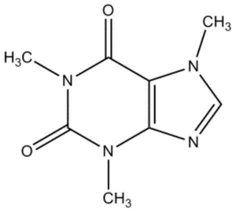
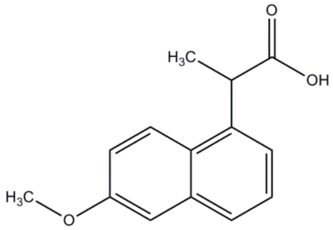
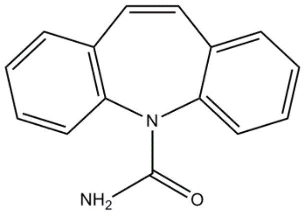
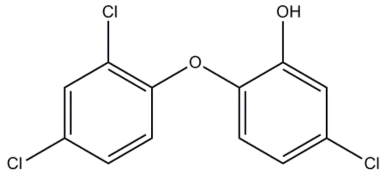
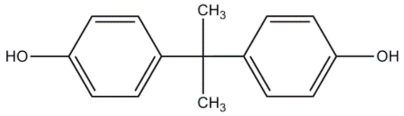
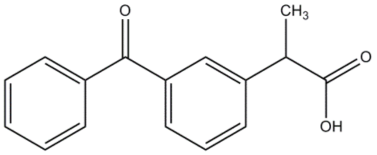
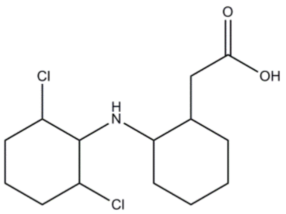
Neutral compounds		Polar compounds	
OMPs	**Chemical stucture**	**OMPs**	**Chemical structure**
Galaxolide		Ibuprofen	
Tonalide		4-nonylphenols	
Caffeine		Naproxen	
Carbamazepine		Triclosan	
		Bisphenol A	
		Ketoprofen	
		Diclofenac	

Table A3 Method parameters of the target GC-MS analysis (TMS-trimethylsilyl derivative). Compounds are ordered based on their retention time.

Micropollutant	**Instrument-LOD (ng/µL)**	**Method precision**		**SPE-recovery (%)**	**Target ions (m/z)** Quantifier	**Retention time (min)**
		Intraday mean RSD (%)	**Interday mean RSD (%)**			
Ibuprofen (TMS)	0.1	4	1	38	160, 263, 278	21.04
Technical nonylphenol (TMS)	0.1	4	5	114	207, 221	23.8-25.8
Caffeine	0.16	15	14	34	194, 109	27.27
Galaxolide	0.05	6	5	147	243, 258	27.66
Tonalide	0.08	6	5	109	243, 258	28.07
4-n-Nonylphenol*	0.5	4	7	87	107, 220	28.13
as a TMS derivative	0.4	6	10	80	179, 292	30.08
Naproxen (TMS)	0.1	4	1	117	185, 243	34.71
9-Chloromethyl-anthracene*	0.4	3	4	99	191, 222	35.27
Triclosan (TMS)	0.1	4	2	61	347, 362	35.89
Ketoprofen	2.5	2	4	127	282, 311	37.59
Bisphenol A-d16* (TMS)	0.5	6	8	98	368, 386	37.82
Bisphenol A (TMS)	0.3	4	3	101	357, 372	37.97
Diclofenac (TMS)	0.2	4	5	109	214, 242	39.44
Carbamazepine	0.2	15	13	135	193, 236, 165	40.39
Beta-Estradiol diacetate*	0.5	3	5	79	314, 172, 356	48.56

*Internal standard

Table A4 Concentrations of the selected OMPs (µg L^{-1}) in the inflow municipal wastewater of the LRB facility per sampling date.

	Organic micropollutants										
	Neutral compounds				Polar compounds						
Sampling date	**galaxolide**	**tonalide**	**caffeine**	**carbamazepine**	**ibuprofen**	**4-nonylphenols**	**naproxen**	**triclosan**	**bisphenol A**	**ketoprofen**	**diclofenac**
04.08.2011	9.01	0.84	63.92	29.65	22.74	5.61	1.95	1.50	2.92	3.32	10.79
11.08.2011	7.06	0.93	50.34	8.63	22.80	3.96	3.12	1.25	2.58	5.57	7.97
01.09.2011	0.13	0.03	1.91	5.83	34.69	8.98	3.89	1.34	17.29	5.20	13.74
08.09.2011	0.89	0.12	10.94	3.31	43.62	3.53	3.72	2.28	3.76	5.52	5.73
23.09.2011	2.69	0.91	20.72	7.49	12.75	1.28	1.42	1.06	1.93	1.08	5.90
20.10.2011	2.66	0.18	37.77	17.71	60.02	6.13	5.79	2.86	8.80	<LOD	16.81
17.11.2011	0.47	16.21	16.69	<LOD	31.20	2.15	2.97	0.70	5.54	2.26	9.33
01.12.2011	0.85	5.19	8.28	<LOD	20.31	1.13	2.27	0.61	1.33	1.09	4.74
19.01.2012	1.79	0.22	38.56	10.67	13.52	0.86	0.82	0.01	0.78	0.22	2.50
15.03.2012	0.31	0.79	5.16	<LOD	12.05	1.12	1.55	0.37	0.91	2.03	2.82
29.03.2012	0.76	0.91	23.93	4.33	10.63	0.57	0.71	0.97	0.50	0.96	1.14
12.04.2012	2.36	1.02	7.78	0.00	48.90	1.73	3.57	0.98	1.42	2.04	14.02
26.04.2012	2.65	1.34	27.38	4.47	90.55	5.39	7.47	0.15	19.99	6.66	36.86
10.05.2012	3.07	1.34	15.51	11.28	91.99	3.43	8.09	1.78	3.02	11.49	21.40
24.05.2012	2.39	1.06	8.10	5.11	21.34	1.71	2.81	1.02	2.11	1.32	6.61
14.06.2012	2.70	0.90	9.46	12.87	No data	No data	No data	No data	No data	No data	No data
05.07.2012	0.42	0.88	3.78	<LOD	10.62	1.72	2.82	0.34	0.54	<LOD	4.06
19.07.2012	2.70	1.36	19.12	<LOD	43.03	3.43	3.49	0.76	3.04	0.99	11.76
02.08.2012	1.49	0.28	7.54	<LOD	52.22	2.26	6.15	0.70	1.38	0.97	14.48
16.08.2012	0.96	0.21	5.10	<LOD	20.00	3.14	2.70	0.46	1.04	<LOD	7.24
Median	2.08	0.91	13.23	4.40	22.80	2.26	2.97	0.97	2.11	1.32	7.97
Min	0.13	0.03	1.91	<LOD	10.62	0.57	0.71	0.01	0.50	<LOD	1.14
Max	9.01	16.21	63.92	29.65	91.99	8.98	8.09	2.86	19.99	11.49	36.86

<LOD = Data was below the limits of detection of the instrument.

No data was acquired in the week 16 for the polar compounds due to tecnical problems with the instrument (GC-MS).

Table A5 Concentrations of the selected OMPs (µg L^{-1}) in the outflow water samples of the planted HSSFCW (H50p) per sampling date.

	Organic micropollutants										
	Neutral compounds				Polar compounds						
Sampling date	**galaxolide**	**tonalide**	**caffeine**	**carbamazepine**	**ibuprofen**	**4-nonylphenols**	**naproxen**	**triclosan**	**bisphenol A**	**ketoprofen**	**diclofenac**
04.08.2011	10.42	0.96	<LOD	33.13	26.75	14.14	4.37	2.59	5.23	7.21	10.56
11.08.2011	6.10	0.57	1.59	6.66	10.72	4.51	1.11	0.93	3.14	1.38	3.91
01.09.2011	0.41	0.05	2.69	5.93	42.56	14.29	4.22	2.51	24.54	<LOD	14.95
08.09.2011	4.12	0.40	1.82	8.23	252.95	22.83	15.35	5.12	15.08	21.36	72.46
23.09.2011	2.23	0.87	1.87	3.19	24.86	5.06	1.48	1.10	3.35	0.00	5.39
20.10.2011	4.29	0.27	63.13	16.22	69.84	6.38	4.33	1.80	3.47	2.74	14.80
17.11.2011	0.89	5.83	7.95	1.07	35.67	3.70	3.23	0.71	4.11	2.11	9.37
01.12.2011	2.15	0.87	21.15	24.70	66.95	4.34	4.48	0.90	3.02	2.88	15.15
19.01.2012	1.32	0.17	3.95	0.00	38.19	3.21	2.57	0.40	2.23	1.26	6.67
15.03.2012	1.16	1.04	13.15	3.12	16.28	1.24	2.01	0.43	1.05	2.15	4.54
29.03.2012	0.62	0.88	15.64	<LOD	8.03	0.94	1.10	1.17	0.78	1.61	1.56
12.04.2012	2.45	0.85	4.07	7.99	75.82	3.30	15.10	1.28	3.07	4.29	15.96
26.04.2012	3.47	1.82	2.44	3.80	120.50	6.30	15.29	0.41	28.51	15.06	73.11
10.05.2012	2.84	1.27	1.53	6.74	28.33	3.68	3.52	1.88	5.71	7.47	10.95
24.05.2012	2.13	0.97	1.87	4.34	30.82	2.12	3.58	1.19	2.77	3.60	17.73
14.06.2012	2.28	0.81	4.88	6.87	No data	No data	No data	No data	No data	No data	No data
05.07.2012	0.99	1.34	<LOD	<LOD	52.67	3.45	7.74	0.74	4.88	15.46	21.37
19.07.2012	0.75	0.83	<LOD	<LOD	14.44	3.48	3.05	0.65	3.96	1.01	6.08
02.08.2012	2.11	0.48	<LOD	<LOD	79.75	5.75	7.36	1.05	9.94	1.01	33.81
16.08.2012	1.27	0.31	<LOD	<LOD	36.55	10.86	2.71	0.77	2.80	5.44	27.27
Median	2.14	0.86	2.16	4.07	36.55	4.34	3.58	1.05	3.47	2.74	14.80
Min	0.41	0.05	<LOD	<LOD	8.03	0.94	1.10	0.40	0.78	<LOD	1.56
Max	10.42	5.83	63.13	33.13	252.95	22.83	15.35	5.12	28.51	21.36	73.11

<LOD = Data was below the limits of detection of the instrument.

No data was acquired in the week 16 for the polar compounds due to tecnical problems with the instrument (GC-MS).

Table A6 Concentrations of the selected OMPs (μg L^{-1}) in the outflow water samples of the unplanted HSSFCW (H50) per sampling date.

	Organic micropollutants										
	Neutral compounds				Polar compounds						
Sampling date	**galaxolide**	**tonalide**	**caffeine**	**carbamazepine**	**ibuprofen**	**4-nonylphenols**	**naproxen**	**triclosan**	**bisphenol A**	**ketoprofen**	**diclofenac**
04.08.2011	6.73	0.81	<LOD	37.78	22.77	15.69	2.33	2.39	5.42	5.05	6.30
11.08.2011	5.77	0.53	1.20	6.56	16.06	6.98	1.76	1.37	3.77	3.08	6.01
01.09.2011	4.12	0.30	1.96	7.77	38.52	10.17	3.35	1.88	62.09	<LOD	14.76
08.09.2011	2.64	0.27	1.21	3.63	93.93	10.42	3.66	3.25	12.01	6.27	14.79
23.09.2011	3.25	0.98	2.11	3.31	16.51	5.41	1.34	1.13	3.88	1.06	4.03
20.10.2011	3.71	0.22	2.73	11.23	51.43	6.04	3.36	1.56	5.58	1.75	9.34
17.11.2011	0.96	8.41	4.73	<LOD	64.54	7.48	4.07	1.02	8.29	2.98	13.02
01.12.2011	1.23	3.30	8.50	<LOD	24.77	3.00	1.72	0.57	6.44	<LOD	5.42
19.01.2012	1.34	0.18	10.78	<LOD	36.58	2.61	2.75	0.32	1.01	0.86	6.47
15.03.2012	1.98	1.24	14.36	3.98	15.67	1.47	1.95	0.48	0.94	2.13	4.22
29.03.2012	0.94	0.98	18.92	<LOD	8.35	0.28	0.92	1.16	0.77	1.27	1.40
12.04.2012	2.52	0.88	9.29	8.09	65.79	2.68	6.69	1.21	2.21	2.61	15.32
26.04.2012	3.85	1.76	7.11	4.57	117.88	6.47	15.65	0.57	35.63	15.76	73.40
10.05.2012	3.71	1.62	1.89	10.03	34.47	3.26	4.07	1.89	6.56	7.89	15.53
24.05.2012	2.14	0.90	1.88	3.96	20.48	1.46	2.67	1.06	2.68	2.38	10.29
14.06.2012	3.16	1.01	4.79	10.98	No data	No data	No data	No data	No data	No data	No data
05.07.2012	0.79	1.15	<LOD	<LOD	19.71	2.58	3.52	0.62	3.18	10.07	8.23
19.07.2012	1.59	1.06	<LOD	<LOD	20.76	5.00	3.00	0.75	3.49	0.98	6.57
02.08.2012	1.66	0.43	<LOD	<LOD	49.85	4.36	3.22	0.98	9.71	0.95	16.84
16.08.2012	1.02	0.26	<LOD	<LOD	38.82	59.04	4.26	0.73	3.89	6.56	26.93
Median	2.33	0.94	2.04	3.80	34.47	5.00	3.22	1.06	3.89	2.38	9.34
Min	0.79	0.18	<LOD	<LOD	8.35	0.28	0.92	0.32	0.77	<LOD	1.40
Max	6.73	8.41	18.92	37.78	117.88	59.04	15.65	3.25	62.09	15.76	73.40

<LOD = Data was below the limits of detection of the instrument.

No data was acquired in the week 16 for the polar compounds due to tecnical problems with the instrument (GC-MS).

Table A7 Monthly average flow rates over the period of investigation (m^3 d^{-1}). Data was provided by Dr. Jaime Nivala from the Centre of Environmental Biotechnology of the UFZ.

	H50p		H50	
Month	Q_i	Q_p	Q_i	Q_u
Aug-2011	0.22	0.17	0.22	0.23
Sep-2011	0.19	0.14	0.19	0.19
Oct-2011	0.18	0.17	0.18	0.18
Nov-2011	0.18	0.18	0.18	0.18
Dec-2011	0.18	0.19	0.18	0.19
Jan-2012	0.18	0.19	0.18	0.19
Feb-2012	0.20	0.19	0.20	0.21
Mar-2012	0.20	0.21	0.20	0.20
Apr-2012	0.19	0.19	0.19	0.19
May-2012	0.19	0.16	0.19	0.20
Jun-2012	0.19	0.16	0.19	0.19
Jul-2012	0.19	0.14	0.19	0.19
Aug-2012	0.18	0.14	0.18	0.19
Median	0.19	0.17	0.19	0.19
Min	0.18	0.14	0.18	0.18
Max	0.22	0.21	0.22	0.23

H50p = planted pilot-scale CW;

H50 = unplanted pilot-scale CW;

Q_i = Flow rates at the influent

Q_p = Flow rates (for planted CWs) at the effluent;

Q_u = Flow rates (for unplanted CWs) at the effluent.

Table A8 Mass removal efficiencies (%) of the selected OMPs in the planted HSSFCW (H50p) per sampling date. Negative removals are considered as 0% removal.

	Organic micropollutants										
	Neutral compounds				Polar compounds						
Sampling date	**galaxolide**	**tonalide**	**caffeine**	**carbamazepine**	**ibuprofen**	**4-nonylphenols**	**naproxen**	**triclosan**	**bisphenol-A**	**ketoprofen**	**diclofenac**
04.08.2011	11	12	100*	14	9	0	0	0	0	0	24
11.08.2011	33	53	98	40	64	12	73	43	6	81	62
01.09.2011	0	0	0	25	10	0	20	0	0	100*	20
08.09.2011	0	0	88	0	0	0	0	0	0	0	0
23.09.2011	39	30	93	69	0	0	23	24	0	100*	33
20.10.2011	0	0	0	14	0	2	29	40	63	0	17
17.11.2011	0	64	52	0	0	0	0	0	26	7	0
01.12.2011	0	82	0	0	0	0	0	0	0	0	0
19.01.2012	22	19	89	100*	0	0	0	0	0	0	0
15.03.2012	0	0	0	0	0	0	0	0	0	0	0
29.03.2012	14	0	31	100*	21	0	0	0	0	0	0
12.04.2012	0	17	48	0	0	0	0	0	0	0	0
26.04.2012	0	0	91	15	0	0	0	0	0	0	0
10.05.2012	22	21	92	50	74	10	63	11	0	45	57
24.05.2012	25	23	81	29	0	0	0	2	0	0	0
14.06.2012	29	24	57	55	No data	No data	No data	No data	No data	No data	No data
05.07.2012	0	0	100*	0	0	0	0	0	0	0	0
19.07.2012	80	55	100*	0	75	25	35	38	4	25	62
02.08.2012	0	0	100*	0	0	0	7	0	0	19	0
16.08.2012	0	0	100*	0	0	0	22	0	0	0	0
Average	14	20	66	25	13	3	14	8	5	20	14
Standard dev.	20	25	39	33	26	6	22	15	15	35	23

*100% Removal implies that the outlet concentration was below the LOD of the instrument.

Table A9 Mass removal efficiencies (%) of the selected OMPs in the unplanted HSSFCW (H50) per sampling date. Negative removals are considered as 0% removal.

	Organic micropollutants										
	Neutral compounds				Polar compounds						
Sampling date	**galaxolide**	**tonalide**	**caffeine**	**carbamazepine**	**ibuprofen**	**4-nonylphenols**	**naproxen**	**triclosan**	**bisphenol-A**	**ketoprofen**	**diclofenac**
04.08.2011	22	0	100*	0	0	0	0	0	0	0	39
11.08.2011	15	40	98	21	26	0	41	0	0	42	21
01.09.2011	0	0	0	0	0	0	14	0	0	100*	0
08.09.2011	0	0	89	0	0	0	2	0	0	0	0
23.09.2011	0	0	90	56	0	0	6	0	0	2	32
20.10.2011	0	0	93	37	14	1	42	45	37	0	44
17.11.2011	0	48	72	0	0	0	0	0	0	0	0
01.12.2011	0	33	0	0	0	0	20	0	0	100*	0
19.01.2012	21	15	70	100*	0	0	0	0	0	0	0
15.03.2012	0	0	0	0	0	0	0	0	0	0	0
29.03.2012	0	0	21	100*	22	51	0	0	0	0	0
12.04.2012	0	14	0	0	0	0	0	0	0	0	0
26.04.2012	0	0	74	0	0	0	0	0	0	0	0
10.05.2012	0	0	87	6	61	0	47	0	0	28	24
24.05.2012	6	11	76	18	0	11	0	0	0	0	0
14.06.2012	0	0	49	15	No data	No data	No data	No data	No data	No data	No data
05.07.2012	0	0	100*	0	0	0	0	0	0	0	0
19.07.2012	41	22	100*	0	52	0	14	1	0	1	44
02.08.2012	0	0	100*	0	0	0	45	0	0	0	0
16.08.2012	0	0	100*	0	0	0	0	0	0	0	0
Average	5	9	66	18	9	3	12	2	2	14	11
Standard dev.	11	15	39	32	18	12	18	10	8	32	17

*100% Removal implies that the outlet concentration was below the LOD of the instrument.

Table A10 Monthly average removals a) denotes data from the H50p and b) for the H50. Compounds are alphabetically ordered.

		2011					2012						
Organic micropollutants		**Aug(b)**	**Sep(c)**	**Oct(a)**	**Nov(a)**	**Dec(a)**	**Jan(a)**	**Mar(b)**	**Apr(b)**	**May(b)**	**Jun(a)**	**Jul(b)**	**Aug(b)**
bisphenol A	a)	3	0	63	26	0	0	0	0	0	0	2	0
	b)	0	0	37	0	0	0	0	0	0	0	0	0
caffeine	a)	99	60	0	52	0	89	16	69	86	57	100*	100*
	b)	99	60	93	72	0	70	10	37	81	49	100*	100*
carbamazepine	a)	27	31	14	0	0	100*	50	8	39	55	0	0
	b)	10	19	37	0	0	100*	50	0	12	15	0	0
diclofenac	a)	43	18	17	0	0	0	0	0	28	0	31	0
	b)	30	11	44	0	0	0	0	0	12	0	22	0
galaxolide	a)	22	13	0	0	0	22	7	0	23	29	40	0
	b)	18	0	0	0	0	21	0	0	3	0	21	0
ibuprofen	a)	36	3	0	0	0	0	10	0	37	0	38	0
	b)	13	0	14	0	0	0	11	0	30	0	26	0
ketoprofen	a)	40	67	0	7	0	0	0	0	23	0	12	9
	b)	21	34	0	0	100*	0	0	0	14	0	0	0
naproxen	a)	36	15	29	0	0	0	0	0	32	0	18	14
	b)	21	7	42	0	20	0	0	0	24	0	7	22
4-nonylphenols	a)	6	0	2	0	0	0	0	0	5	0	13	0
	b)	0	0	1	0	0	0	26	0	5	0	0	0
tonalide	a)	32	10	0	64	82	19	0	8	22	24	28	0
	b)	20	0	0	48	33	15	0	7	5	0	11	0
triclosan	a)	21	8	40	0	0	0	0	0	7	0	19	0
	b)	0	0	45	0	0	0	0	0	0	0	1	0

(a)indicates data from one sampling event, (b)the average of two samplings events, (c)the average of three samplings events.

100* means that the outlet concentration was below the limit of detection of the applied instrument. Thus, removal was considered as 100% removal. Increase of load was considered as 0% removal.

Table A11 Concentrations of the selected OMPs (µg L^{-1}) in the planted HSSFCW (H50p) during period I (2011/August-September) at the sampling location 0.125 *FDB*.

Sampling depth	**Organic micropollutants**										
0.13 m	Neutral compounds				Polar compounds						
Sampling date	galaxolide	tonalide	caffeine	carbamazepine	ibuprofen	4-nonylphenols	naproxen	triclosan	bisphenol-A	ketoprofen	diclofenac
04.08.2011	8.92	0.79	41.94	31.26	30.28	7.28	3.11	1.86	2.9	4.36	12.52
11.08.2011	4.86	0.66	30.69	8.49	18.76	3.79	2.02	1.13	2.24	4.38	6.36
01.09.2011	0.47	0.05	1.08	5.74	33.64	4.72	2.48	1.20	10.46	3.37	9.90
08.09.2011	9.09	1.02	32.78	11.08	219.4	33.63	18.94	9.15	13.9	0	67.2
23.09.2011	1.71	0.8	5.14	2.72	11.2	2.3	1.37	1.12	2.04	0	6.21
Median	4.86	0.79	30.69	8.49	30.28	4.72	2.48	1.20	2.90	3.37	9.90
Min	0.47	0.05	1.08	2.72	11.20	2.30	1.37	1.12	2.04	0.00	6.21
Max	9.09	1.02	41.94	31.26	219.40	33.63	18.94	9.15	13.90	4.38	67.20

<LOD = Data was below the limits of detection of the instrument. *FDB* = Fractional Distance within the Bed.

Table A12 Concentrations of the selected OMPs (µg L^{-1}) in the planted HSSFCW (H50p) during period I (2011/August-September) at the sampling location 0.25 *FDB*.

Sampling depth	**Organic micropollutants**										
0.13 m	Neutral compounds				Polar compounds						
Sampling date	galaxolide	tonalide	caffeine	carbamazepine	ibuprofen	4-nonylphenols	naproxen	triclosan	bisphenol-A	ketoprofen	diclofenac
04.08.2011	12.18	1.01	15.99	30.99	35.72	8.8	3.94	2.86	3.68	5.3	20.09
11.08.2011	4.8	0.41	4.87	8.43	5.94	0.94	0	0.38	2.03	0.67	2.71
01.09.2011	4.19	0.30	54.95	7.43	59.12	24.44	7.92	4.57	20.67	0	46.56
08.09.2011	6.88	0.6	3.5	6.69	120.88	17.12	7.87	5.84	5.17	0	34.4
23.09.2011	2.15	0.89	2.8	4.55	6.13	2.49	1.13	0.99	1.67	0	2.62
Median	4.80	0.60	4.87	7.43	35.72	8.80	3.94	2.86	3.68	0.00	20.09
Min	2.15	0.30	2.80	4.55	5.94	0.94	0.00	0.38	1.67	0.00	2.62
Max	12.18	1.01	54.95	30.99	120.88	24.44	7.92	5.84	20.67	5.30	46.56

<LOD = Data was below the limits of detection of the instrument. *FDB* = Fractional Distance within the Bed.

Table A13 Concentrations of the selected OMPs (µg L^{-1}) in the planted HSSFCW (H50p) during period I (2011/August-September) at the sampling location 0.50 *FDB*.

Sampling depth	**Organic micropollutants**										
0.13 m	Neutral compounds				Polar compounds						
Sampling date	galaxolide	tonalide	caffeine	carbamazepine	ibuprofen	4-nonylphenols	naproxen	triclosan	bisphenol-A	ketoprofen	diclofenac
04.08.2011	7.54	0.82	0	30.75	29.57	7.57	3.11	2.23	5.04	3.68	14.56
11.08.2011	4.12	0.35	1.32	6.95	10.87	2.88	1.05	0.86	1.82	1.42	3.64
01.09.2011	0.25	0.04	0	6.1	32.35	6.15	4.02	1.9	11.08	9.7	19.56
08.09.2011	5.49	0.49	1.54	5.54	109.11	13.97	6.09	5.23	4.39	9.93	26.48
23.09.2011	2.17	0.89	2.01	3.18	6.4	1.44	1.03	0.97	1.23	0	2.18
Median	4.12	0.49	1.32	6.10	29.57	6.15	3.11	1.90	4.39	3.68	14.56
Min	0.25	0.04	0.00	3.18	6.40	1.44	1.03	0.86	1.23	0.00	2.18
Max	7.54	0.89	2.01	30.75	109.11	13.97	6.09	5.23	11.08	9.93	26.48

<LOD = Data was below the limits of detection of the instrument. *FDB* = Fractional Distance within the Bed.

Table A14 Concentrations of the selected OMPs (µg L^{-1}) in the planted HSSFCW (H50p) during period I (2011/August-September) at the sampling location 0.75 *FDB*.

Sampling depth	**Organic micropollutants**										
0.13 m	Neutral compounds				Polar compounds						
Sampling date	galaxolide	tonalide	caffeine	carbamazepine	ibuprofen	4-nonylphenols	naproxen	triclosan	bisphenol-A	ketoprofen	diclofenac
04.08.2011	7.6	0.75	0	29.26	15.02	7.15	2.22	3.17	35.94	3.94	7.90
11.08.2011	3.87	0.34	1.27	6.66	10.42	3.23	0.9	0.75	1.93	1.29	3.81
01.09.2011	0.92	0.09	1.04	5.94	15.35	2.04	1.2	0.67	8.46	1.27	3.8
08.09.2011	2.13	0.23	1.19	3.19	61.24	6.25	5.24	3.35	5.65	9.94	11.67
23.09.2011	5.13	1.35	2.33	6.36	26.19	3.52	1.72	1.24	1.6	1.08	5.81
Median	3.87	0.34	1.19	6.36	15.35	3.52	1.72	1.24	5.65	1.29	5.81
Min	0.92	0.09	0.00	3.19	10.42	2.04	0.90	0.67	1.60	1.08	3.80
Max	7.60	1.35	2.33	29.26	61.24	7.15	5.24	3.35	35.94	9.94	11.67

<LOD = Data was below the limits of detection of the instrument. *FDB* = Fractional Distance within the Bed.

Table A15 Concentrations of the selected OMPs (µg L^{-1}) in the unplanted HSSFCW (H50) during period I (2011/August-September) at the sampling location 0.125 *FDB*.

Sampling depth	**Organic micropollutants**										
0.13 m	Neutral compounds				Polar compounds						
Sampling date	galaxolide	tonalide	caffeine	carbamazepine	ibuprofen	4-nonylphenols	naproxen	triclosan	bisphenol-A	ketoprofen	diclofenac
04.08.2011	10.27	0.88	25.76	30.35	33.45	7.66	2.99	2.3	4.26	3.38	17.22
11.08.2011	4.03	0.50	18.98	7.94	9.65	2.13	1.23	0.66	2.38	3.1	4.59
01.09.2011	1.03	0	1.12	6.38	42.64	12.54	4.74	2.86	14.3	0	29.01
08.09.2011	1.17	0.14	7.75	3.22	68.34	4.39	4.64	2.92	2.78	7.63	13.69
23.09.2011	2.01	0.83	7.59	3.07	11.43	2.3	1.22	1.04	1.38	0	4.34
Median	2.01	0.50	7.75	6.38	33.45	4.39	2.99	2.30	2.78	3.10	13.69
Min	1.03	0.00	1.12	3.07	9.65	2.13	1.22	0.66	1.38	0.00	4.34
Max	10.27	0.88	25.76	30.35	68.34	12.54	4.74	2.92	14.30	7.63	29.01

<LOD = Data was below the limits of detection of the instrument. *FDB* = Fractional Distance within the Bed.

Table A16 Concentrations of the selected OMPs (µg L^{-1}) in the unplanted HSSFCW (H50) during period I (2011/August-September) at the sampling location 0.25 *FDB*.

Sampling depth	**Organic micropollutants**										
0.13 m	Neutral compounds				Polar compounds						
Sampling date	galaxolide	tonalide	caffeine	carbamazepine	ibuprofen	4-nonylphenols	naproxen	triclosan	bisphenol-A	ketoprofen	diclofenac
04.08.2011	8.46	0.79	0	28.73	22.93	5.91	0.91	1.93	2.5	0.97	7.75
11.08.2011	5.16	0.45	5.89	9.17	19.88	4.24	3.08	1.15	2.51	6.08	7.60
01.09.2011	1.8	0.15	1.11	6.39	28.82	3.35	1.82	1.23	9.27	2.36	9.49
08.09.2011	4.89	0.44	5.53	7.24	106.53	9.99	4.92	3.87	2.92	0	16.44
23.09.2011	3.75	1.06	7.2	10.32	11.45	1.77	1.26	1.07	1.57	0	3.83
Median	4.89	0.45	5.53	9.17	22.93	4.24	1.82	1.23	2.51	0.97	7.75
Min	1.80	0.15	0.00	6.39	11.45	1.77	0.91	1.07	1.57	0.00	3.83
Max	8.46	1.06	7.20	28.73	106.53	9.99	4.92	3.87	9.27	6.08	16.44

<LOD = Data was below the limits of detection of the instrument. *FDB* = Fractional Distance within the Bed.

Table A17 Concentrations of the selected OMPs (µg L^{-1}) in the unplanted HSSFCW (H50) during period I (2011/August-September) at the sampling location 0.50 *FDB*.

Sampling depth	**Organic micropollutants**										
0.13 m	Neutral compounds				Polar compounds						
Sampling date	galaxolide	tonalide	caffeine	carbamazepine	ibuprofen	4-nonylphenols	naproxen	triclosan	bisphenol-A	ketoprofen	diclofenac
04.08.2011	6.19	0.66	0	29.1	19.97	5.05	1.33	1.46	3.83	1.25	7.12
11.08.2011	3.18	0.28	1.2	6.27	4.89	3.4	0.5	1.05	2.74	0.63	2.7
01.09.2011	0.81	0.09	1.09	6.18	17.6	4.04	1.05	1.64	10.9	2.34	5.3
08.09.2011	0.76	0.12	1.03	2.43	14.33	2.83	2.08	2.15	2.35	0	3.49
23.09.2011	2.62	0.98	2.03	0	10.75	2.53	1.19	1.26	1.58	0	2.81
Median	2.62	0.28	1.09	6.18	14.33	3.40	1.19	1.46	2.74	0.63	3.49
Min	0.76	0.09	0.00	0.00	4.89	2.53	0.50	1.05	1.58	0.00	2.70
Max	6.19	0.98	2.03	29.10	19.97	5.05	2.08	2.15	10.90	2.34	7.12

<LOD = Data was below the limits of detection of the instrument. *FDB* = Fractional Distance within the Bed.

Table A18 Concentrations of the selected OMPs (µg L^{-1}) in the unplanted HSSFCW (H50) during period I (2011/August-September) at the sampling location 0.75 *FDB*.

Sampling depth	**Organic micropollutants**										
0.13 m	Neutral compounds				Polar compounds						
Sampling date	galaxolide	tonalide	caffeine	carbamazepine	ibuprofen	4-nonylphenols	naproxen	triclosan	bisphenol-A	ketoprofen	diclofenac
04.08.2011	5.69	0.66	0	28.69	12.28	6.59	0.75	1.78	3.55	0.94	3.96
11.08.2011	2.2	0.19	1.31	6.73	4.23	2.77	0.34	0.76	3.45	0	2.66
01.09.2011	0.84	0.08	1.1	6.13	24.08	6.1	1.61	1.9	17.46	2.28	7.34
08.09.2011	3.47	0.36	1.58	5.26	30.7	7.82	2.35	2.96	7.21	0	5.6
23.09.2011	4.29	1.29	2.3	5.09	12.8	3.41	1.27	1.28	2.57	0	3.16
Median	3.47	0.36	1.31	6.13	12.80	6.10	1.27	1.78	3.55	0.00	3.96
Min	0.84	0.08	0.00	5.09	4.23	2.77	0.34	0.76	2.57	0.00	2.66
Max	5.69	1.29	2.30	28.69	30.70	7.82	2.35	2.96	17.46	2.28	7.34

<LOD = Data was below the limits of detection of the instrument. *FDB* = Fractional Distance within the Bed.

Table A19 Concentrations of the selected OMPs (µg L^{-1}) in the planted HSSFCW (H50p) during period II (2012/April-May) at the sampling location 0.50 *FDB*. (*Sd* of 0.13 m).

Sampling depth	**Organic micropollutants**										
0.13 m	Neutral compounds				Polar compounds						
Sampling date	galaxolide	tonalide	caffeine	carbamazepine	ibuprofen	4-nonylphenols	naproxen	triclosan	bisphenol-A	ketoprofen	diclofenac
12.04.2012	3.09	1.11	14.21	11.07	100.41	3.28	15.97	1.27	1.87	2.49	40.6
26.04.2012	3.69	1.93	4.4	4.81	119.16	5.05	15.39	0.46	7.41	9.66	77.28
10.05.2012	3.45	1.32	1.7	7.79	41.01	3.19	4.16	1.98	3.1	6.62	18.8
24.05.2012	2.93	1.32	1.95	10.11	72.31	2.74	7.94	1.27	1.93	3	36.53
Median	3.27	1.32	3.18	8.95	86.36	3.24	11.67	1.27	2.52	4.81	38.57
Min	2.93	1.11	1.70	4.81	41.01	2.74	4.16	0.46	1.87	2.49	18.80
Max	3.69	1.93	14.21	11.07	119.16	5.05	15.97	1.98	7.41	9.66	77.28

<LOD = Data was below the limits of detection of the instrument. *FDB* = Fractional Distance within the Bed. *Sd* = Sampling depth.

Table A20 Concentrations of the selected OMPs (µg L^{-1}) in the planted HSSFCW (H50p) during period II (2012/April-May) at the sampling location 0.75 *FDB*. (*Sd* of 0.13 m).

Sampling depth	**Organic micropollutants**										
0.13 m	Neutral compounds				Polar compounds						
Sampling date	galaxolide	tonalide	caffeine	carbamazepine	ibuprofen	4-nonylphenols	naproxen	triclosan	bisphenol-A	ketoprofen	diclofenac
12.04.2012	1.53	0.73	4.19	0	10.35	1.35	0.66	0.85	0.9	2.09	1.14
26.04.2012	3.52	2.05	9.68	4.89	70.09	4.47	9.37	0.32	6.73	7.75	48.59
10.05.2012	3.54	1.45	1.90	7.57	51.68	3.21	3.97	1.94	2.76	6.94	21.01
24.05.2012	2.4	1.1	1.88	4.58	28.79	1.55	2.82	1.14	2.34	1.44	16.74
Median	2.96	1.28	3.05	4.74	40.24	2.38	3.40	1.00	2.55	4.52	18.88
Min	1.53	0.73	1.88	0.00	10.35	1.35	0.66	0.32	0.90	1.44	1.14
Max	3.54	2.05	9.68	7.57	70.09	4.47	9.37	1.94	6.73	7.75	48.59

<LOD = Data was below the limits of detection of the instrument. *FDB* = Fractional Distance within the Bed. *Sd* = Sampling depth.

Table A21 Concentrations of the selected OMPs (µg L^{-1}) in the unplanted HSSFCW (H50) during period II (2012/April-May) at the sampling location 0.50 *FDB*. (*Sd* of 0.13 m).

Sampling depth	**Organic micropollutants**										
0.13 m	Neutral compounds				Polar compounds						
Sampling date	galaxolide	tonalide	caffeine	carbamazepine	ibuprofen	4-nonylphenols	naproxen	triclosan	bisphenol-A	ketoprofen	diclofenac
12.04.2012	4.95	1.24	11.14	9.21	100.80	9.59	9.86	1.27	6.86	2.42	23.48
26.04.2012	3.62	1.74	7.35	5.08	104.14	4.99	13.77	0.58	6.41	8.72	68.8
10.05.2012	4.45	1.61	1.82	12.01	54.4	3.57	5.44	2.24	3.13	7.41	27.56
24.05.2012	2.49	1.15	1.95	6.82	23.29	1.72	3.22	1.2	2.18	2.37	14.18
Median	4.04	1.43	4.65	8.02	77.60	4.28	7.65	1.24	4.77	4.92	25.52
Min	2.49	1.15	1.82	5.08	23.29	1.72	3.22	0.58	2.18	2.37	14.18
Max	4.95	1.74	11.14	12.01	104.14	9.59	13.77	2.24	6.86	8.72	68.80

<LOD = Data was below the limits of detection of the instrument. *FDB* = Fractional Distance within the Bed. *Sd* = Sampling depth.

Table A22 Concentrations of the selected OMPs (µg L^{-1}) in the unplanted HSSFCW (H50) during period II (2012/April-May) at the sampling location 0.75 *FDB*. (*Sd* of 0.13 m).

Sampling depth	**Organic micropollutants**										
0.13 m	Neutral compounds				Polar compounds						
Sampling date	galaxolide	tonalide	caffeine	carbamazepine	ibuprofen	4-nonylphenols	naproxen	triclosan	bisphenol-A	ketoprofen	diclofenac
12.04.2012	3.89	1.03	11.06	8.12	44.19	2.91	4.84	1.26	1.79	2.08	11.94
26.04.2012	4.85	2.30	9.20	4.94	70.63	4.14	11.07	0.46	6.99	5.68	51.39
10.05.2012	4.1	1.66	2.82	16.2	43.86	3.64	4.69	2.17	3.23	7.24	23.46
24.05.2012	3.48	1.59	2.12	11.6	65.84	4.75	6.48	1.64	11.18	6.62	42.63
Median	4.00	1.63	6.01	9.86	55.02	3.89	5.66	1.45	5.11	6.15	33.05
Min	3.48	1.03	2.12	4.94	43.86	2.91	4.69	0.46	1.79	2.08	11.94
Max	4.85	2.30	11.06	16.20	70.63	4.75	11.07	2.17	11.18	7.24	51.39

<LOD = Data was below the limits of detection of the instrument. *FDB* = Fractional Distance within the Bed. *Sd* = Sampling depth.

Table A23 Concentrations of the selected OMPs (µg L^{-1}) in the planted HSSFCW (H50p) during period II (2012/April-May) at the sampling location 0.50 *FDB*. (*Sd* of 0.4 m).

Sampling depth	**Organic micropollutants**										
0.4 m	Neutral compounds				Polar compounds						
Sampling date	galaxolide	tonalide	caffeine	carbamazepine	ibuprofen	4-nonylphenols	naproxen	triclosan	bisphenol-A	ketoprofen	diclofenac
12.04.2012	3.27	1.18	16.35	15.31	139.92	7.49	22.12	1.25	14.21	2.97	45.01
26.04.2012	3.14	1.69	3.61	6.07	123.9	5.59	17.07	0.51	29.77	10.85	75.35
10.05.2012	4.6	1.76	1.82	19	87.64	3.78	8.08	2.1	4.08	9.44	38.05
24.05.2012	2.44	1.05	1.84	5.8	34.45	2.8	4.09	1.18	4.67	2.6	18.27
Median	3.21	1.44	2.73	10.69	105.77	4.69	12.58	1.22	9.44	6.21	41.53
Min	2.44	1.05	1.82	5.80	34.45	2.80	4.09	0.51	4.08	2.60	18.27
Max	4.60	1.76	16.35	19.00	139.92	7.49	22.12	2.10	29.77	10.85	75.35

<LOD = Data was below the limits of detection of the instrument. *FDB* = Fractional Distance within the Bed. *Sd* = Sampling depth.

Table A24 Concentrations of the selected OMPs (µg L^{-1}) in the planted HSSFCW (H50p) during period II (2012/April-May) at the sampling location 0.75 *FDB*. (*Sd* of 0.4 m).

Sampling depth	**Organic micropollutants**										
0.4 m	Neutral compounds				Polar compounds						
Sampling date	galaxolide	tonalide	caffeine	carbamazepine	ibuprofen	4-nonylphenols	naproxen	triclosan	bisphenol-A	ketoprofen	diclofenac
12.04.2012	3.59	0.99	12.04	8.39	69.56	2.5	6.93	1.2	2.94	2.34	13.47
26.04.2012	3.25	1.61	2.54	4.42	97.66	4.31	14.75	0.4	9.51	14.79	72.34
10.05.2012	3.89	1.61	1.57	7.32	57.66	3.4	4.34	2	3.1	6.95	26.1
24.05.2012	3.03	1.25	1.83	5.35	69.54	3.13	5.28	1.38	2.26	2.54	40.42
Median	3.42	1.43	2.19	6.34	69.55	3.27	6.11	1.29	3.02	4.75	33.26
Min	3.03	0.99	1.57	4.42	57.66	2.50	4.34	0.40	2.26	2.34	13.47
Max	3.89	1.61	12.04	8.39	97.66	4.31	14.75	2.00	9.51	14.79	72.34

<LOD = Data was below the limits of detection of the instrument. *FDB* = Fractional Distance within the Bed. *Sd* = Sampling depth.

Table A25 Concentrations of the selected OMPs (µg L^{-1}) in the unplanted HSSFCW (H50) during period II (2012/April-May) at the sampling location 0.50 *FDB*. (*Sd* of 0.4 m).

Sampling depth	**Organic micropollutants**										
0.4 m	Neutral compounds				Polar compounds						
Sampling date	galaxolide	tonalide	caffeine	carbamazepine	ibuprofen	4-nonylphenols	naproxen	triclosan	bisphenol-A	ketoprofen	diclofenac
12.04.2012	2.61	1.04	13.55	8.28	45.28	1.91	4.96	1.06	1.07	2.08	11.99
26.04.2012	3.73	1.75	8.57	5.28	104.81	4.53	13.92	0.52	9.05	7.65	61.94
10.05.2012	3.78	1.52	1.81	10.08	53.41	3.41	5.62	2.05	3.71	8.09	22.21
24.05.2012	2.76	1.12	1.93	5.6	35.98	1.93	3.76	1.22	2.95	2.45	16.27
Median	3.25	1.32	5.25	6.94	49.35	2.67	5.29	1.14	3.33	5.05	19.24
Min	2.61	1.04	1.81	5.28	35.98	1.91	3.76	0.52	1.07	2.08	11.99
Max	3.78	1.75	13.55	10.08	104.81	4.53	13.92	2.05	9.05	8.09	61.94

<LOD = Data was below the limits of detection of the instrument. *FDB* = Fractional Distance within the Bed. *Sd* = Sampling depth.

Table A26 Concentrations of the selected OMPs (μg L^{-1}) in the unplanted HSSFCW (H50) during period II (2012/April-May) at the sampling location 0.75 *FDB*. (*Sd* of 0.4 m).

Sampling depth	**Organic micropollutants**										
0.4 m	Neutral compounds				Polar compounds						
Sampling date	galaxolide	tonalide	caffeine	carbamazepine	ibuprofen	4-nonylphenols	naproxen	triclosan	bisphenol A	ketoprofen	diclofenac
12.04.2012	2.6	0.9	9.03	0	44.19	1.65	3.17	1.07	1.39	2.07	8.94
26.04.2012	2.96	0	8.83	5.52	116.24	5.28	14.65	0.55	8.48	11.74	61.43
10.05.2012	3.41	1.31	1.72	9.35	72.48	3.65	5.66	2.17	3.08	8.55	31.91
24.05.2012	2.68	1.12	1.92	4.59	41.48	2.41	4.16	1.26	2.39	2.69	17.05
Median	2.82	1.01	5.38	5.06	58.34	3.03	4.91	1.17	2.74	5.62	24.48
Min	2.60	0.00	1.72	0.00	41.48	1.65	3.17	0.55	1.39	2.07	8.94
Max	3.41	1.31	9.03	9.35	116.24	5.28	14.65	2.17	8.48	11.74	61.43

<LOD = Data was below the limits of detection of the instrument. *FDB* = Fractional Distance within the Bed. *Sd* = Sampling depth.

Table A27 Concentrations of the selected OMPs (μg L^{-1}) in the planted HSSFCW (H50p) during period III (2012/July-Aug.) at the sampling location 0.50 *FDB*. (*Sd* of 0.13 m).

Sampling depth	**Organic micropollutants**										
0.13 m	Neutral compounds				Polar compounds						
Sampling date	galaxolide	tonalide	caffeine	carbamazepine	ibuprofen	4-nonylphenols	naproxen	triclosan	bisphenol-A	ketoprofen	diclofenac
12.04.2012	3.27	1.18	16.35	15.31	139.92	7.49	22.12	1.25	14.21	2.97	45.01
26.04.2012	3.14	1.69	3.61	6.07	123.9	5.59	17.07	0.51	29.77	10.85	75.35
10.05.2012	4.6	1.76	1.82	19	87.64	3.78	8.08	2.1	4.08	9.44	38.05
24.05.2012	2.44	1.05	1.84	5.8	34.45	2.8	4.09	1.18	4.67	2.6	18.27
Median	3.21	1.44	2.73	10.69	105.77	4.69	12.58	1.22	9.44	6.21	41.53
Min	2.44	1.05	1.82	5.80	34.45	2.80	4.09	0.51	4.08	2.60	18.27
Max	4.60	1.76	16.35	19.00	139.92	7.49	22.12	2.10	29.77	10.85	75.35

<LOD = Data was below the limits of detection of the instrument. *FDB* = Fractional Distance within the Bed. *Sd* = Sampling depth.

Table A28 Concentrations of the selected OMPs (μg L^{-1}) in the planted HSSFCW (H50p) during period III (2012/July-Aug.) at the sampling location 0.75 *FDB*. (*Sd* of 0.13 m).

Sampling depth	**Organic micropollutants**										
0.13 m	Neutral compounds				Polar compounds						
Sampling date	galaxolide	tonalide	caffeine	carbamazepine	ibuprofen	4-nonylphenols	naproxen	triclosan	bisphenol-A	ketoprofen	diclofenac
12.04.2012	3.59	0.99	12.04	8.39	69.56	2.5	6.93	1.2	2.94	2.34	13.47
26.04.2012	3.25	1.61	2.54	4.42	97.66	4.31	14.75	0.4	9.51	14.79	72.34
10.05.2012	3.89	1.61	1.57	7.32	57.66	3.4	4.34	2	3.1	6.95	26.1
24.05.2012	3.03	1.25	1.83	5.35	69.54	3.13	5.28	1.38	2.26	2.54	40.42
Median	3.42	1.43	2.19	6.34	69.55	3.27	6.11	1.29	3.02	4.75	33.26
Min	3.03	0.99	1.57	4.42	57.66	2.50	4.34	0.40	2.26	2.34	13.47
Max	3.89	1.61	12.04	8.39	97.66	4.31	14.75	2.00	9.51	14.79	72.34

<LOD = Data was below the limits of detection of the instrument. *FDB* = Fractional Distance within the Bed. *Sd* = Sampling depth.

Table A29 Concentrations of the selected OMPs (µg L^{-1}) in the unplanted HSSFCW (H50) during period III (2012/July-Aug.) at the sampling location 0.50 *FDB*. (*Sd* of 0.13 m).

Sampling depth	**Organic micropollutants**										
0.13 m	Neutral compounds				Polar compounds						
Sampling date	galaxolide	tonalide	caffeine	carbamazepine	ibuprofen	4-nonylphenols	naproxen	triclosan	bisphenol-A	ketoprofen	diclofenac
12.04.2012	2.61	1.04	13.55	8.28	45.28	1.91	4.96	1.06	1.07	2.08	11.99
26.04.2012	3.73	1.75	8.57	5.28	104.81	4.53	13.92	0.52	9.05	7.65	61.94
10.05.2012	3.78	1.52	1.81	10.08	53.41	3.41	5.62	2.05	3.71	8.09	22.21
24.05.2012	2.76	1.12	1.93	5.6	35.98	1.93	3.76	1.22	2.95	2.45	16.27
Median	3.25	1.32	5.25	6.94	49.35	2.67	5.29	1.14	3.33	5.05	19.24
Min	2.61	1.04	1.81	5.28	35.98	1.91	3.76	0.52	1.07	2.08	11.99
Max	3.78	1.75	13.55	10.08	104.81	4.53	13.92	2.05	9.05	8.09	61.94

<LOD = Data was below the limits of detection of the instrument. *FDB* = Fractional Distance within the Bed. *Sd* = Sampling depth.

Table A30 Concentrations of the selected OMPs (µg L^{-1}) in the unplanted HSSFCW (H50) during period III (2012/July-Aug.) at the sampling location 0.75 *FDB*. (*Sd* of 0.13 m).

Sampling date	**Organic micropollutants**										
0.13 m	Neutral compounds				Polar compounds						
Sampling date	galaxolide	tonalide	caffeine	carbamazepine	ibuprofen	4-nonylphenols	naproxen	triclosan	bisphenol-A	ketoprofen	diclofenac
12.04.2012	2.6	0.9	9.03	0	44.19	1.65	3.17	1.07	1.39	2.07	8.94
26.04.2012	2.96	0	8.83	5.52	116.24	5.28	14.65	0.55	8.48	11.74	61.43
10.05.2012	3.41	1.31	1.72	9.35	72.48	3.65	5.66	2.17	3.08	8.55	31.91
24.05.2012	2.68	1.12	1.92	4.59	41.48	2.41	4.16	1.26	2.39	2.69	17.05
Median	2.82	1.01	5.38	5.06	58.34	3.03	4.91	1.17	2.74	5.62	24.48
Min	2.60	0.00	1.72	0.00	41.48	1.65	3.17	0.55	1.39	2.07	8.94
Max	3.41	1.31	9.03	9.35	116.24	5.28	14.65	2.17	8.48	11.74	61.43

<LOD = Data was below the limits of detection of the instrument. *FDB* = Fractional Distance within the Bed. *Sd* = Sampling depth.

Table A31 Concentrations of the selected OMPs (µg L^{-1}) in the planted HSSFCW (H50p) during period III (2012/July-Aug.) at the sampling location 0.50 *FDB*. (*Sd* of 0.4 m).

Sampling date	**Organic micropollutants**										
0.4 m	Neutral compounds				Polar compounds						
Sampling date	galaxolide	tonalide	caffeine	carbamazepine	ibuprofen	4-nonylphenols	naproxen	triclosan	bisphenol-A	ketoprofen	diclofenac
12.04.2012	1.68	1.69	0	0	55.58	4.23	7.32	0.92	1.07	10.47	21.96
26.04.2012	1.78	1.12	0	0	26.3	4.98	3.12	0.86	1.06	0.97	9.23
10.05.2012	1.46	0.35	0	0	50.93	3.05	4.5	0.87	0.29	0.94	17.22
24.05.2012	1.67	0.43	0	6.17	51.04	5.64	4.83	0.79	1.57	5.5	30.59
Median	1.68	0.78	0.00	0.00	50.99	4.61	4.67	0.87	1.07	3.24	19.59
Min	1.46	0.35	0.00	0.00	26.30	3.05	3.12	0.79	0.29	0.94	9.23
Max	1.78	1.69	0.00	6.17	55.58	5.64	7.32	0.92	1.57	10.47	30.59

<LOD = Data was below the limits of detection of the instrument. *FDB* = Fractional Distance within the Bed. *Sd* = Sampling depth.

Table A32 Concentrations of the selected OMPs (µg L^{-1}) in the planted HSSFCW (H50p) during period III (2012/July-Aug.) at the sampling location 0.75 *FDB*. (*Sd* of 0.4 m).

Sampling date	**Organic micropollutants**										
0.4 m	Neutral compounds				Polar compounds						
Sampling date	galaxolide	tonalide	caffeine	carbamazepine	ibuprofen	4-nonylphenols	naproxen	triclosan	bisphenol-A	ketoprofen	diclofenac
12.04.2012	1.34	1.6	0	0	6.06	3.78	2.66	1.13	4.27	8.72	5.35
26.04.2012	2.03	1.13	0	0	21.09	4.02	2.96	0.84	0.92	0.96	7.28
10.05.2012	1.65	0.43	0	0	31.71	4.31	2.41	0.93	0.36	0.94	9.75
24.05.2012	1.06	0.28	0	0	42.93	39.6	3.21	0.61	1.86	4.65	29.14
Median	1.50	0.78	0.00	0.00	26.40	4.17	2.81	0.89	1.39	2.81	8.52
Min	1.06	0.28	0.00	0.00	6.06	3.78	2.41	0.61	0.36	0.94	5.35
Max	2.03	1.60	0.00	0.00	42.93	39.60	3.21	1.13	4.27	8.72	29.14

<LOD = Data was below the limits of detection of the instrument. *FDB* = Fractional Distance within the Bed. *Sd* = Sampling depth.

Table A33 Concentrations of the selected OMPs (µg L^{-1}) in the unplanted HSSFCW (H50) during period III (2012/July-Aug.) at the sampling location 0.50 *FDB*. (*Sd* of 0.4 m).

Sampling date	**Organic micropollutants**										
0.4 m	Neutral compounds				Polar compounds						
Sampling date	galaxolide	tonalide	caffeine	carbamazepine	ibuprofen	4-nonylphenols	naproxen	triclosan	bisphenol-A	ketoprofen	diclofenac
12.04.2012	2.37	2.24	0	0	9.6	3.77	2.59	1.44	4.04	0	6.13
26.04.2012	1.27	1	0	0	5.37	3.19	2.38	1.16	2.46	0.95	4.23
10.05.2012	1.57	0.35	0	0	15.46	2.19	1.86	1.02	0.48	0	7.96
24.05.2012	1.47	0.38	0	0	16.8	7.47	1.81	0.78	1.1	0	10.38
Median	1.52	0.69	0.00	0.00	12.53	3.48	2.12	1.09	1.78	0.00	7.05
Min	1.27	0.35	0.00	0.00	5.37	2.19	1.81	0.78	0.48	0.00	4.23
Max	2.37	2.24	0.00	0.00	16.80	7.47	2.59	1.44	4.04	0.95	10.38

<LOD = Data was below the limits of detection of the instrument. *FDB* = Fractional Distance within the Bed. *Sd* = Sampling depth.

Table A34 Concentrations of the selected OMPs (µg L^{-1}) in the unplanted HSSFCW (H50) during period III (2012/July-Aug.) at the sampling location 0.75 *FDB*. (*Sd* of 0.4 m).

Sampling date	**Organic micropollutants**										
0.4 m	Neutral compounds				Polar compounds						
Sampling date	galaxolide	tonalide	caffeine	carbamazepine	ibuprofen	4-nonylphenols	naproxen	triclosan	bisphenol-A	ketoprofen	diclofenac
12.04.2012	1.15	1.36	0	0	39.48	2.61	4.89	0.8	0.91	8.5	18.18
26.04.2012	0.91	0.9	0	0	2.7	2.1	2.11	0.76	1.97	0	2.82
10.05.2012	1.43	0.33	0	0	19.79	3.55	2.76	1.08	7.37	0	13.47
24.05.2012	0.94	0.29	0	0	29.65	82.11	3.22	0.64	7.25	5.55	24.29
Median	1.05	0.62	0.00	0.00	24.72	3.08	2.99	0.78	4.61	2.78	15.83
Min	0.91	0.29	0.00	0.00	2.70	2.10	2.11	0.64	0.91	0.00	2.82
Max	1.43	1.36	0.00	0.00	39.48	82.11	4.89	1.08	7.37	8.50	24.29

<LOD = Data was below the limits of detection of the instrument. *FDB* = Fractional Distance within the Bed. *Sd* = Sampling depth.

Acknowledgments

I express my deep gratitude to my university supervisor from the Martin-Luther-Universität Halle-Wittenberg, Prof. Dr.-Ing. habil. Heinz Köser who supervised my PhD thesis. I am very gratefull with Dr. Monika Möder and Dr. Peter Kuschk for their guidance, support and the opportunity to do my PhD thesis at the UFZ. I also want to thank former colleagues who supported me to start my studies in Europe, Prof. Dr. Andrew Barry, Dr. Alessandro Brovelli and Dr. Luca Rossi, from EPFL in Switzerland.

This work was possible thank to the collaboration with several colleagues from different departments of the UFZ. My profound gratitude to the LRB-Team: L. Schultze-Nobre, D. Wang, S. Müller, C. Bartsch, A.-S. Al-Dahoodi, F. Schulz and V. Castillo-Taleno. Moreover, I thank Dr. Jaime Nivala and Katy Bernhard (UBZ) for their support at the research facility "Langenreichenbach" and Petra Keil (ANA) for the invaluable support in the laboratory.

I sincerely thank Pablo Ristori, Luis Panichelli (Sonido Z), Juan David Villegas, Seifeddine Jomaa, Nadine Czekalski, Diego Paredes, Carlos Arias, Hector Orozco, Isaraín Chávez, Mauricio Duarte, Karolina Nowak, Andreas Mussolf, Agustin Breña, Varun Vats, Camelia Algora, Oswaldo Yee, Christin Heber, Paula Martinez, Cristobal Girardi, Enrique Sulbaran, Eduardo Velazquez, Miriam Müller, Ailette Prieto, Uriel Arroyo, Natalia Reyes, Florencia Talavera, Aashirbad Mohanty, Helga Fazekas, Oleksa Shved, Gabriel Hernandez, Alejandra Galiote, Joaquin Olivares, Gerardo Lerma, Rafael Pedregal (Mariachi Los Pachukoß), Maribel Hernandez, die SG LVB Fussball Zweite Mannschaft and my colleagues from UFZ (ANA, UBT), EPFL, Faculty of enginerring UAS, UNAM and the Goethe Institute for helped me, supported me, encourage me or inspired me during my PhD studies.

I thank my family for their enormous support during my PhD studies, Maria de la Luz Díaz Cuevas, Mario Carranza Aguilar, Fernando, Josefina, Berenice, Maria, Ernesto, Salud, Carolina, Pepe, Cesar, Rodrigo, Cecilia, Araceli, Veronica, Rafael. This work would have not been possible if not for the scholarship granted by CONACYT, Mexico (exp. No. 200110) and the DAAD, Germany (Kennziffer A/10/72795). Moreover, I thank UAS (Mexico) for their support within the program of young doctors in strategic areas and the Helmholtz Interdisciplinary Graduate School for Environmental Research (HIGRADE) of the UFZ.

Curriculum vitae

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List of publications

- **Carranza-Diaz, O.**, Schultze-Nobre L., Moeder, M., Nivala, J., Kuschk, P., Koeser, H. **2014** Removal of selected organic micropollutants in planted and unplanted pilot-scale horizontal flow constructed wetlands under conditions of high organic load. *Ecological Engineering*, 71, 234-245.
- Ahumada-Santos Y.P., Báez-Flores M.E., Díaz-Camacho S.P., Uribe-Beltrán M.J., López-Angulo G., Vega-Aviña R., Chávez-Duran F.A., Montes-Avila J., **Carranza-Díaz O.**, Möder M., Kuschk P., Delgado-Vargas F. **2014**. Spatiotemporal distribution of the bacterial contamination of agricultural and domestic wastewater discharged to a drainage ditch (Sinaloa, Mexico). *Ciencias Marinas*, 40(4): 277-289.
- Girardi, C., Nowak, K.M., **Carranza-Diaz, O.**, Lewkow, B., Miltner, A., Gehre, M., Schäffer, A., Kästner, M. **2013**. Microbial degradation of the pharmaceutical ibuprofen and the herbicide 2,4-D in water and soil - Use and limits of data obtained from aqueous systems for predicting their fate in soil. *Science of the Total Environment*, 444, 32-42.
- Brovelli, A., **Carranza-Diaz, O.**, Rossi, L., Barry, D.A. **2011**. Design methodology accounting for the effects of porous medium heterogeneity on hydraulic residence time and biodegradation in horizontal subsurface flow constructed wetlands. *Ecological Engineering*, 37(5), 758-770.

Oral presentations and posters at international conferences

- **Carranza-Diaz, O.**, Keil, P., Möder, M., Schultze-Nobre, L., Kuschk, P., Nivala, J., Köser, H., **2013**. Removal of polar organic micropollutants in constructed wetlands. 9th international conference daRostim. Phytohormones, humic and other biologically active compounds for agriculture, human health and environmental protection. 7-10 October 2013, Lviv, Ukraine. (Oral presentation).
- **Carranza-Diaz, O.**, Möder, M., Kuschk, P., Keil, P., Schultze-Nobre, L., Köser, H., Nivala, J., Reemtsma, T., **2012**. Spatial and temporal elimination of selected micropollutants in different types of horizontal flow constructed wetlands. 13th International Conference on Wetland Systems for Water Pollution Control. 25-29 November 2012, Perth, Australia. (Oral presentation).

- **Carranza-Diaz, O.**, Möder, M., Kuschk, P., Keil, P., Schultze-Nobre, L., Koeser, H., **2012**. Pharmaceutical active compound removal in a pilot-scale horizontal flow constructed wetland system: A first screening. 1st Panamerican Conference on Wetland Systems for water quality improvement, management and treatment. 26 February - 1 March 2012, Pereira, Colombia. (Poster).
- **Carranza-Diaz, O.**, Schultze-Nobre, L., Nivala, J., Kuschk, P., Möder, M., Köser, H., **2012**. Water balance in different types of horizontal subsurface-flow constructed wetlands in moderate climates. 13th International Conference on Wetland Systems for Water Pollution Control. 25-29 November 2012, Perth, Australia. (Poster).

Participation at international academic exchange programs

- **UFZ-UAS**. Spatial and temporal occurrence of selected organic micropollutants in planted drainage systems of a highly agricultural productive area of Sinaloa, Mexico: Implications for aquaculture. Financed by CONACYT-Mexico/DAAD-Germany (**2012-2014**).
- **UFZ-UTP**. Floating treatment wetlands-fundamental research and technology development. Financed by COLCIENCIAS-Colombia/DAAD-Germany (**2011-2013**).